THE CONTROL OF SEASONAL CHANGES IN REPRODUCTION AND FOOD INTAKE IN GRAZING RED DEER HINDS (*Cervus elaphus*)

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ABSTRACT

Red deer exhibit seasonal rhythms of metabolism, reproduction and pelage which have evolved in response to the variation in climate and food resources characterizing temperate zone habitats. The aim of this study was to investigate the interaction between herbage availability and the endogenous seasonal rhythms controlling seasonality.

Advancing the phase of seasonal rhythms by administering melatonin between July and October was associated with a reduction in the level of herbage intake by non-lactating hinds grazing a high herbage availability pasture in autumn. This is the first direct evidence that the seasonal appetite cycle demonstrated in enhoused deer fed an *ad libitum* diet, can influence the food intake of grazing deer. However, expression of seasonal and lactational appetite changes were dependent on availability of herbage resources. The ability of hinds to compensate for reductions in herbage abundance by modifying grazing strategies was limited. This appeared to be primarily due to a ceiling on the duration of daily grazing activity of about 12 hours.

The timing of the onset of the breeding season was unaffected by low herbage resources or lactation. The principal influence of these factors on reproduction was to reduce the proportion of hinds exhibiting oestrous cyclicity. Lactation only suppressed oestrous cycles if it was associated with a loss of body condition and thus its impact was related to prevailing herbage availability.

The timing of seasonal changes in coat growth were associated with changes in plasma prolactin concentrations. Low herbage availability and lactation delayed the timing of winter primary fibre growth, and reduced the density of coat fibres.

This study also examined the influence of the breeding season on the timing of seasonal changes in VFI, live weight, coat growth and prolactin secretion. Only seasonal changes in pelage exhibited a significant phase delay in mature compared to pre-pubertal females.

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ABBREVIATIONS

B	c.p.m. of labelled hormone bound to antiserum in tubes containing plasma sample or standard hormone.
Bo	c.p.m. of labelled hormone bound to antiserum for maximum binding tubes (i.e. no unlabelled hormone present)
BMR	basal metabolic rate
c.	*circa*
CL	corpus luteum
cm	centimetre
c.p.m.	counts per minute (of I^{125} labelled hormone).
DARS	donkey anti-rabbit serum
DM	dry matter
FMR	fasting metabolic rate (BMR plus activity in respiration chamber)
FSH	follicle stimulating hormone
GnRH	gonadotrophic releasing hormone
ha	hectare
h	hour
I^{125}	iodine^{125}
kg	kilogramme
l	litre
LD	light/dark cycle
LH	luteinizing hormone
m	metre
µl	microlitre
mg	milligramme
min	minute
ml	millilitre
ng	nanogramme
NRS	normal rabbit serum
NSB	non specific binding (c.p.m. of labelled hormone 'bound' in tubes containing no antiserum).
PRL	prolactin
OM	organic matter
QC	quality control pooled plasma
RIA	radioimmunoassay
SD	standard deviation
SCN	superior chiasmatic nuclei
s.e.m.	standard error of the mean
T	Total c.p.m. of labelled hormone added to each tube.
VFI	voluntary food intake

CHAPTER 1

1.1. INTRODUCTION

The temperate climatic zones are characterised by a pattern of generally predictable seasonal changes in daylength and temperature. These phenomena exert a strong selective pressure both directly and indirectly via their influence on plant photosynthesis. This has resulted in the evolution of highly seasonal life-cycles in temperate zone animals.

The adaptations of large mammalian herbivores like the red deer, the subject of this study, are of particular interest. These species are long-lived and thus require strategies able to measure time and synchronise changes over prolonged periods. Also, with the exception of the North American caribou *(Rangifer tarandus)*, deer do not avoid adverse environmental conditions by long distance migration, and thus require adaptations that permit survival throughout the year in the same habitat.

Evidence, mainly from domestic ungulates suggests endogenous rhythms, entrained primarily by daylength, are used to synchronise seasonal adaptations with appropriate changes in food resources and climate. These rhythms influence a variety of parameters including the timing of reproductive events, pelage and metabolism. The character of these has been well documented in a variety of species. However, comparatively little is known about the interaction between endogenously driven changes and exogenous factors like food availability. In general the seasonal rhythms successfully synchronise requirements with resources. However, there can be substantial between year variation, especially locally, in food abundance. This is due to climate and biological factors like density of competitors, predators or parasites. These fluctuations in resources are believed to exert a substantial influence on grazing behaviour and reproductive success, but as yet their effects on expression of seasonal changes remains largely unquantified. The objective of this study was to investigate two aspects of this interaction. Firstly, to determine how seasonal metabolic status interacts with current food availability to control food intake and

grazing behaviour, and secondly how food intake is determined by and influences reproduction.

The European red deer (*Cervus elaphus elaphus* L.) is a member of a sub-species complex distributed extensively throughout the northern temperate zone. Populations range between latitudes 30^0 - 65^{0}N and discontinuously from western Europe, through central Asia to north America (for details see: Clutton-Brock *et al.*, 1982a; Kay and Staines, 1981; Mitchell *et al.*, 1977). Typical of temperate zone inhabitants, this species exhibits profound seasonality (major seasonal changes in the female are summarised in Figure 1.1).

Most matings in British populations occur during a brief rutting period in September and October (Fraser Darling 1937; Zuckerman, 1952). However, both stags (males) and hinds (females) remain fertile for several months after the normal rut. In the former, spermatogenesis continues throughout the period of 'hard antler' from August until the following March (Lincoln, 1971). In the hind, whilst most individuals conceive at their first oestrus (84 %: Adam *et al.*, 1985), if prevented from mating, they exhibit repeated oestrous cycles until the following March (Guinness *et al.*, 1971; Loudon *et al,.* 1989; Adam *et al.*, 1989a). The average oestrous cycle length has been reported as between 18-21 days (18.3 days: Guinness *et al.*, 1971; 21 days: Adam *et al.*, 1985: 18.2 days: Kelly *et al.*, 1985) with oestrous behaviour lasting up to 24 hours. During this time hinds may be mated several times by the same or different stags. A single offspring, or occassionally twins (Guinness *et al.*, 1971; Adam *et al.*, 1985), are born during late May or early June after a gestation period of 231-236 days (234: Prell, 1938; 231: Guinness *et al.*, 1971; 236: Adam *et al.*, 1985).

Pelage and metabolism also exhibit distinct seasonality. Red deer moult twice each year, once during late summer into a dense winter coat containing wool fibres, and then in late spring, into the shorter reddish brown coat from which the animal gained its name (Ryder and Kay, 1973; Ryder, 1977). The dietary niche of this species is regarded as intermediate between grazers and browsers (Hofmann, 1973) showing both local and seasonal flexibility (Clutton-Brock *et al.*, 1982a; Collins and Urness, 1983; Kay and

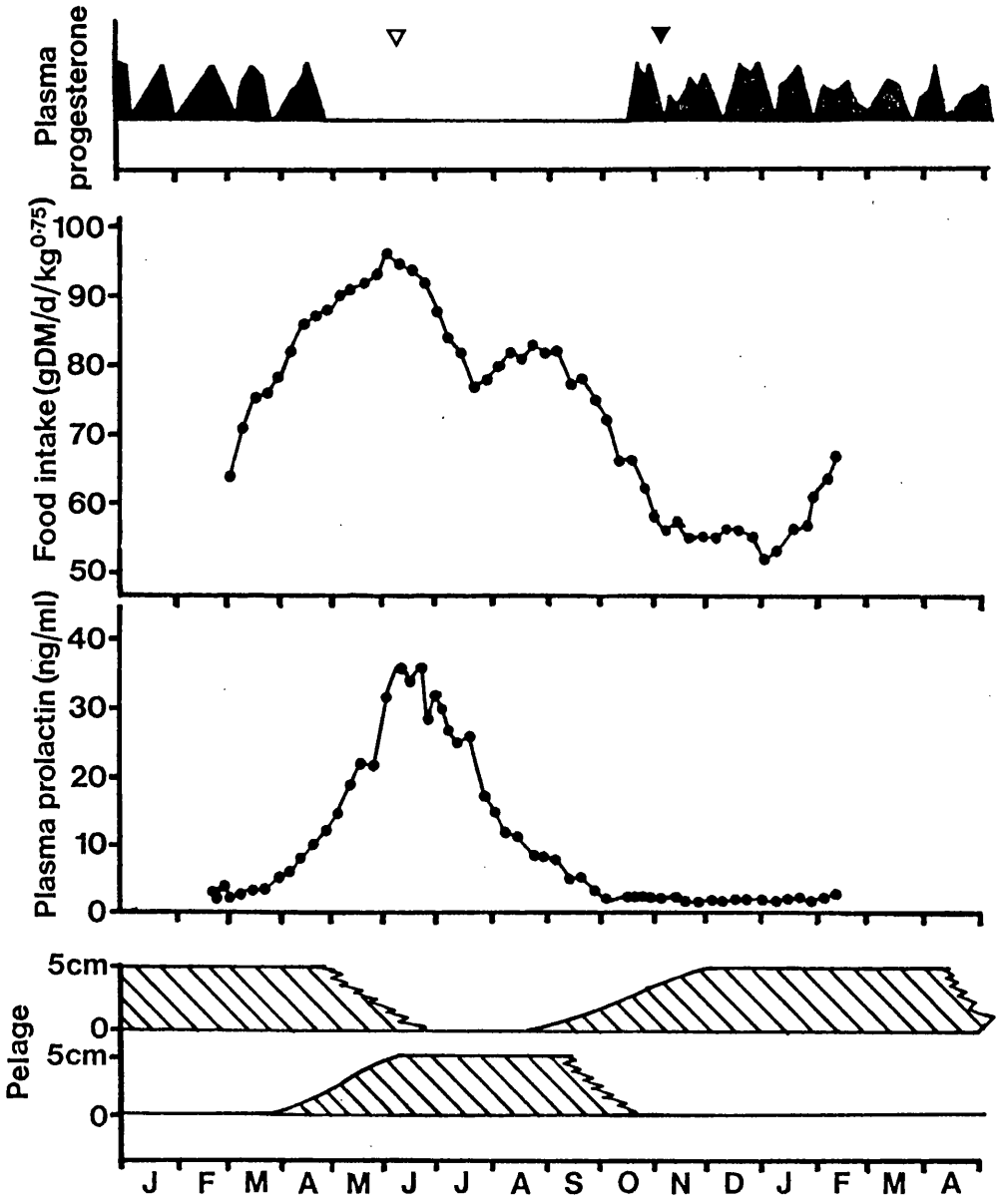
Staines, 1981; van de Veen, 1979). Studies of animals maintained on *ad libitum* diets (Kay, 1979; Loudon *et al.*, 1989) have demonstrated that there is a clear annual pattern of appetite which reflects seasonal changes in herbage availability (i.e. food intake is highest during summer and lowest during winter - see Figure 1.1).

Red deer do not attain sexual maturity until between 17-40 months of age (Clutton-Brock *et al.*, 1982a; Loudon *et al.*, 1989). Life-expectancy is however, long. Captive females live until at least 15 years old (M.L.U.R.I., Annual Report, 1987). In the wild, life expectancy of Scottish populations is normally about 12 years for hinds and 9 years for males (Clutton-Brock *et al.*, 1982a). Fertility (of hinds) increases with age until about 4 years old, but thereafter remains relatively constant throughout life (Guinness *et al.*, 1978). Age related differences are apparently due to age-specific variation in body weight, jaw size and reproductive history (Albon *et al.*, 1983).

The profound seasonality, and extensive range of this species make it an ideal model in which to investigate aspects of the interaction between seasonal adaptations and food availability.

Experimentally, there are practical benefits associated with studying red deer. In captivity, individuals tame easily and are relatively tolerant of intensive handling. Also the recent expansion of the British deer farming industry provides easy access to captive stock as well as management expertise. The decision to study hinds rather than stags was due to the fact that hand-reared or intensively handled males become dangerous to humans during the rut. This prohibits the type of intensive measurements anticipated during this study.

FIGURE 1.1: A summary of seasonal changes in non-pregnant red deer hinds maintained on an *ad libitum* diet. Based on data from Loudon *et al.* (1989). Typical conception (▼) and calving (▽) dates have been included. The duration of the potential breeding season is represented by the period of cyclical changes in plasma progesterone.



1.2. LITERATURE REVIEW

1.2.1. THE PHENOMENON OF SEASONALITY

The environments inhabited by most animals experience seasonal fluctuations in a variety of physical parameters, such as daylength, temperature and rainfall. These changes influence plant photosynthesis and, consequently, the availability of food. To overcome both adverse climatic conditions and variation in resources, animals undergo seasonal changes in their behaviour and physiology. In general, activites like reproduction become synchronized to the period of the year during which they are most likely to succeed. In temperate and arctic habitats, temperature and daylength restrict reproduction to a progressively briefer period in spring or summer as latitude increases. This is well illustrated by changes in the duration of the breeding season of deer in the genus *Odocoileus* (reviewed by Bronson, 1985). Briefly, at the southern extreme of their range (close to the equator) not only does mating occur in every month but females exhibit a post-partum oestrus and thus are able to breed more than once each year. Further north in the Florida Everglades, breeding is restricted to once a year. However, matings still occur sporadically in almost every month, with a marked peak in September. Above latitude 30°N matings only occur during a short and intense rutting period in autumn and early winter.

The development of seasonality is complicated by the fact that a variety of different biological processes are subject to selective pressures for seasonality. Some of these are related to each other (e.g. antler growth and increases in neck girth require high food intake) whilst others (e.g. fat deposition and rutting behaviour) are mutually incompatible. This has resulted in complex seasonal responses that are not simply the reflection of environmental changes. These presumably represent compromises taking account of the priorites of other dependent and competitive biological processes (e.g. in migrating birds moult takes place after reproduction to avoid competition for food resources, but before migration: Gwinner and Dorka, 1976).

1.2.1.1. Role of ultimate factors in control of seasonality

It is assumed that seasonality evolved by natural selection favouring the inheritable traits of those individuals which carried out particular activities at the time of optimum environmental conditions. The environmental variables exerting the selection pressure, which over the course of time have led to the synchronization of events within a population, are known as '*ultimate causes*' or '*factors*' (Baker, 1938; Thomson, 1950). These factors vary depending on both species and the biological processes they influence. In addition, several may interact to determine a particular seasonal activity. In most mammals and birds the major ultimate factor determining reproductive events is food availability. Typically the birth of offspring coincides with the period of greatest food abundance. This is illustrated by the distribution of mating (and hence calving) in the genus *Odocoileus* (descibed above). This becomes progressively restricted as seasonality of plant production becomes more marked with increasing latitude. The extent to which species from different locations have adapted to their own particular set of environmental variables, is demonstrated by the seasonal pattern of births in deer maintained at the same latitude (51°30'N: Lincoln, 1985). Despite exposure to the same environmental conditions, the distribution of births reflected the optimum strategy for the habitat of each species origin. Thus, calving was most resticted in deer from arctic habitats (i.e. reindeer) but evenly distributed in those from tropical regions (i.e. hog, axis and sambar deer).

1.2.1.2. Role of proximate factors in control of seasonality

Most seasonal processes cannot be initiated instantaneously when the ultimate factors reach a critical state. For example, red deer have a gestation length of 234 days (Prell, 1938). This means that mating must take place during autumn when food availability and climate are deteriorating for calving to occur at the optimum period in late spring. In situations like this, the critical state of the ultimate factor (to which the seasonal activity is timed) occurs too

late to be suitable for the acute control of seasonality. As a result, natural selection has favoured those genes conferring the ability to anticipate environmental changes. This has led to the evolution of seasonal strategies which utilize reliable fore-warning environmental signals (temporally related to the ultimate factors) to time seasonal activities. These cues are referred to as *'proximate causes' or 'factors'* (Baker, 1938; Thomson, 1950).

Environmental variables are therefore important to seasonal animals in two distinct ways. Firstly they provide the selection pressure resulting in the evolution of seasonal strategies (i.e. as ultimate factors) and secondly, they are responsible for the immediate control of the physiological processes of seasonality (i.e. as proximate factors).

In certain situations, particularly where seasonal activities can be initiated relatively promptly, the proximate factors are the same as ultimate ones. For example, both the success and timing of swarming behaviour and colony foundation in termites is determined by rainfall softening the soil (Owen, 1961). Usually, however, biological processes need to be initiated long before ultimate factors become critical. This means other environmental variables are normally utilized as proximate factors (although they must exhibit a close temporal relationship with the former). The most consistent and reliable environmental variable in temperate and arctic habitats is photoperiod (Baker, 1938). This is the length (hours) of the light phase in a light-dark cycle. It has been implicated in the control of seasonality in many animal groups, and is regarded as the dominant proximate factor for vertebrate endotherms like mammals and birds (Gwinner, 1981a,b). The most convincing evidence for the role of photoperiod is provided by experiments involving its manipulation. For example, the phase of seasonality can be altered if the pattern of annual daylength changes is artifically simulated over a period differing from the normal 365 days. By using 'annual' cycles of less than this duration, it has been possible to compress seasonal changes in antler growth (sika deer: Goss *et al.*, 1974; red deer: Simpson *et al.*, 1983/4; Suttie and Simpson, 1985); appetite (red deer: Kay, 1979, Brown *et al.*, 1979; Suttie and Simpson, 1985) and reproductive

status (red deer: Simpson *et al.*, 1983/4; Suttie *et al.*, 1989) into periods ranging from 4-6 months. The antler cycle of yearling (but not adult) sika stags has also been extended to 24 months (Goss *et al.*, 1974). Further evidence is provided by exposing animals to short photperiods (i.e. similar to those of autumn) from early summer resulting in an advance in the onset of the breeding season which normally occcurs in autumn. (red deer: Webster and Barrell, 1985; sheep: English *et al.*, 1986).

The annual pattern of environmental temperature changes also exhibits the predictable oscillation necessary to act as a proximate factor. There is little evidence to suggest, however, that these changes are important in controlling seasonality in ungulates (exposed to natural photoperiod changes). This was investigated in an experiment in which Southdown and Pippin Merino ewes were maintained on a constant equatorial photoperiod but subject to reversed thermal seasons for 2.5 years (Wodzicka-Tomaszewska *et al.*, 1967). The resulting pattern of reproductive activity did not re-entrain to the manipulated thermal season. Instead it resembled that of ewes maintained under simulated natural conditions. In contrast, the breeding season of ewes subject to a normal temperatures but reversed photoperiodic seasons did re-entrain to the prevailing photoperiods (Wodzicka-Tomaszewska *et al.*, 1967). One criticism of the design of this experiment was the use of rams normally maintained under natural conditions to detect oestrus in ewes. Evidence (discussed in section 1.2.11.2) indicates that social contact can result in the transmission of photoperiodic information. This could explain why reproductive activity remained in phase with natural photoperiod changes, rather than the reversed temperatures. Clearly though, any influence that temperature may exert is subordinate to that of photoperiod.

As discussed above, seasonal variation in food availability is an important ultimate factor. Evidence that it also acts as a proximate factor in ungulates is limited. Lincoln *et al.* (1989) blocked photoperiodic perception (by superior cervical ganglionectomy) in rams of the semi-domesticated Soay breed, and then maintained them on either an *ad libitum* constant quality diet or under natural grazing conditions for 4 years. All those

experiencing seasonal changes in nutrition exhibited well defined annual cycles in testicular size, plasma prolactin and body weight that were similar to intact controls. In contrast, animals kept on a constant maintenance diet failed to show marked cyclicial changes in testes size or prolactin (body weight not described). This suggests seasonal changes in nutrition can act as a proximate factor. However, the presence of a two month phase advance in the reproductive cycle of treated rams, indicates that under normal conditions it is photoperiod that primarily controls seasonality.

1.2.2. PHOTOPERIODIC TIME MEASUREMENT

To utilize seasonal changes in daylength to time biological processes, it is necessary for the animal to assess daily changes in the duration of the light and/or dark phases. Theories concerning the mechanisms involved fall into 2 distinct categories, both of which have been demonstrated in certain groups of animals.

1.2.2.1. The "hour glass" or "interval timer" model:

In this photoperiodic time measurement (PTM) is regarded as involving a passive process which is forced to oscillate by the light-dark cycle. Briefly, it is proposed the model involves some photochemical process which occurs during either the light or dark phase, and which is reversed by the other portion of the light-dark cycle. If the duration of daylight (or darkness) is sufficient for the level of the hypothetical reaction product to exceed a certain threshold, then the appropriate seasonal activity is initiated. Thus, the response depends on the absolute length of the stimulus in each 24 hour period. Although convincingly demonstrated in few species, Beck (1968) has suggested that this timing mechanism may be widespread amongst insects. For example, in the aphid *Megoura*, the timer measures night-length and is re-set by every light period. The critical threshold photoperiod is 9.5 hours darkness. With 9 hours the whole population produces parthenogenetic offspring, but with 10 hours dark (typical of autumn) all adults produce sexual offspring (Lees and Hardie, 1981). The only vertebrate in which there is persuasive evidence of the involvement of an interval timer is the

lizard *Anolis carolinenis* (Underwood, 1981). In this, the timer uses the absolute duration of the light period to determine testicular function.

1.2.2.2. The circadian models:

Bunning (1936) suggested circadian rhythms could be involved in the determination of photoperiodic time measurement. A universal characteristic of eukaryotic life, these are endogenous oscillations which, in the absence of environmental cues, exhibit a periodicity (known as the 'free running period') similar to that of the solar day (i.e. 24 hours). For example, in hamsters maintained in constant darkness and at a constant temperature, the phase of their daily activity pattern becomes temporally disassociated from that of animals kept under a natural light regime (Elliot and Goldman, 1981). The major role of the environment is not to generate the rhythm, but instead to synchronise (or entrain) an internal pacemaker such that the period and phase of the rhythm maintain a close temporal relationship with the environmental periodicity. Environmental cues used to entrain rhythms are known as zeitgebers (Pittendrigh and Minis, 1964). The endogenous pacemaker for circadian rhythms in mammals is believed to be located in the hypothalamus, probably within the suprachiasmatic nuclei (SCN)(Rusak and Zucker, 1979). This has been demonstrated by lesioning the SCN which eliminates or disrupts many observed circadian rhythms (e.g. the activity rhythms of ground squirrels: Zucker *et al.*, 1983).

Bunning's hypothesis, refined by Pittendrigh and Minis, (1964) and now refered to as the '*external coincidence*' model postulates that light has two distinct roles in PTM. Its primary action is to entrain the circadian rhythms of the organism including a rhythm of photosensitivity (i.e. during part of the cycle the animal is responsive to light but during the remainder it is not). Light's secondary role is to act as inducer of the photoperiodic response when illumination coincides with the period of photosensitivity. The temporal coincidence of sensitivity and light depends on how the circadian rhythm has been entrained to the light-dark cycle (LD cycle) and not simply on the duration of the photoperiod.

There is evidence supporting this model in a number of mammal and bird species, obtained from studies where the LD cycle has been manipulated to reveal which feature is important for PTM. The experiments discussed below were carried out in hamsters. In these, mating activity (unlike in deer and sheep) is associated with increasing daylengths.

Studies have included 'resonance' experiments. During these animals are subject to a fixed short photoperiod coupled to intervals of darkness of different duration (i.e. L6:D18, L6:D24, etc.). As a result the light stimulus falls at different phases of the circadian day depending on the resonance cycle used. If, as proposed by the external coincidence model it is coincidence of illumination with a photosensitive period that is important (rather than a critical duration of light as suggested by the interval timer model), then certain resonance cycles should be interpreted as stimulatory photoperiods but not others. This appears to be the case. In male hamsters raised on a stimulatory photoperiod (L14:D10) exposure to resonance cycles of L6:D30 and L6:D54 maintained large testes but under L6:D18 and L6:D42 regimes testes regressed (Elliot and Goldman, 1981). These studies demonstrate that the photoperiodic signal needs to occur in multiples of 24 hours to be effective.

This has been further investigated in so called 'T-cycle' experiments, during which animals are kept on LD cycles close to 24 hours in duration. In hamsters exhibiting 'free-running' activity rhythms in constant darkness, subsequent exposure to a 24 hour LD cycle resulted in entrainment of the rhythm (Elliot, 1976). However, animals transferred to LD cycles with a period of 23.34 hours and 24.67 hours failed to entrain. Thus, there appears to be a narrow range of entrainment.

An additional demonstration of circadian involvement in PTM has been provided by an experiment in which a single 'skeleton' photoperiod (2 light pulses used to simulate dawn and dusk effects of a longer continuous photoperiod) has been shown to be either photo-inductive or non-inductive in different individuals (Elliot and Goldman, 1981). Male hamsters were initially maintained in total darkness permitting daily activity rhythms to free-run. They were then exposed to a skeleton photoperiod regime consisting of two 15

minute light pulses separated by intervals of darkness lasting 13.5 hours and 10 hours (0.25L : 13.5D : 0.25L : 10D). It was observed that rhythm entrainment to this photoperiod regime resulted in a phase-advance of activity in some individuals but a delay in others. Associated with this was gonadal regression in animals in which activity occured during the 13.5 hour dark period but not the 10 hour period. As the duration of the free-running period varied between individuals during the constant dark period (23.95 - 24.19 hour), the phase of the circadian activity rhythm had become dissociated between different hamsters. Thus, light pulses coincided with different phases of circadian activity rhythm (and assumed photosensitivity) in different animals. Consequently, it is suggested that each hamster interpreted the photoperiod regime differently depending on when the light pulses fell with respect phase of their rhythm. Animals exhibiting gonadal regression interpreted the skeleton photoperiod as non-inductive (i.e. a short-day) whereas in the remainder it was inductive, maintaining testes size.

More direct evidence for the involvement of circadian rhythms comes from SCN lesion experiments. After lesions male hamsters retained testicular activity despite exposure to photoperiodic regimes (e.g. short days: Lehman *et al.*, 1984) which would normally result in gonadal regression.

Thus, the LD cycle has two distinct roles within this model. Firstly, they entrain a circadian rhythm which would otherwise free-run, and secondly they stimulate reproduction (etc.) if light falls at the photosensitive phase.

An alternative model for the role of circadian rhythms in PTM was proposed by Pittendrigh (1972) and is refered to as the '*internal coincidence*' model. This derives from the concept that organisms possess a variety of functionally associated oscillators (Pittendrigh, 1960). It proposes that the role of light is to simply entrain different endogenous rhythms and that photo-induction is a result of the internal phase-relationships of at least two of these (Pittendrigh, 1972). An advantage of this model is that factors other than light (e.g. temperature) might entrain some of these

internal rhythms. Meier (1976) suggested that in the white-throated sparrow changes in the phase relationship of the circadian rhythms of prolactin and corticosterone are responsible for seasonal changes in testicular activity and migratory behaviour. However, which of the two circadian models most accurately describes PTM has yet to be determined.

The exact role of circadian rhythms in determining PTM has yet to be elucidated. However, the bulk of evidence indicates that rhythms entrained by photoperiod, rather than an interval timer, are utilised by mammals to synchronise seasonal changes with environmental periodicity. The next section of this review will consider evidence for the mechanism by which the photoperiodic stimulus is transformed into an endocrine signal.

1.2.3. MELATONIN AS THE PHOTOPERIODIC MESSENGER

In 1964 Czyba *et al.* and in 1965 Hoffman and Reiter demonstrated that in Syrian hamsters (*Mesocricetus auratus*) pinealectomy prevented the gonadal regression normally initiated by blinding or short-day photoperiods. This was the first direct evidence for the involvement of this endocrine gland in the transmission of the photoperiodic response. Since then it has been revealed that the pineal hormone melatonin (5-methoxy-N-acetyl-tryptamine) isolated by Lerner *et al.* (1958) is responsible for the mediation of the effects of the pineal. Current evidence (detailed below) suggests that photoperiodic information is transformed into an endocrine signal by light determining the circadian pattern of secretion of melatonin. Light has two functions: firstly to suppress the release of melatonin, and secondly, to entrain the circadian rhythm generators in the SCN which regulate the endogenous melatonin rhythm. As a result the daily secretory profile alters in response to changes in the photoperiod, thereby relaying information about seasonal changes in daylength (Hastings *et al.*, 1985; Klein, 1985; Lincoln *et al.*, 1985; Underwood and Goldman, 1987; Arendt *et al.*, 1988).

1.2.3.1. Reception of photoperiodic information

In mammals, photoperiodic information is received by the eyes. This has been demonstrated by blinding ewes which had been maintained under a photoperiodic regime of alternating 90 day periods of long or short daylengths. Prior to blinding long-days initiated anoestrus and short-days oestrous cyclicity. Following bilateral orbital enucleation this photoperiodic control was lost (Legan and Karsch, 1983).

The photoperiod information is then relayed by a neural pathway from the retina to the SCN in the anterior hypothalamus (Moore and Lenn, 1972).

1.2.3.2. The suprachiasmatic nuclei (SCN)

The SCN are the major, if not sole, circadian pacemakers in mammals (Underwood and Goldman, 1987). They are responsible for generating the daily rhythm controlling pineal synthesis and release of melatonin. Destruction of SCN function leads to the loss of circadian rhythmicity in pineal melatonin release, with levels of the hormone remaining low at all times (Rusak and Zucker, 1979; Klein *et al.*, 1983). Although the circadian pacemaker in the SCN is capable of generating rhythms autonomously (melatonin levels continue to oscillate on a 24 hour basis in animals kept in constant darkness: reindeer: Eloranta *et al., in press*; sheep: Lincoln *et al.*,1985) normally it is strongly influenced by light. This has two distinct roles. Firstly it entrains the SCN pacemaker(s). This involves synchronizing the endogenous rhythm to the light-dark cycle of the environment so that the melatonin stimulatory period coincides with darkness. This has been demonstrated experimentally in sheep by an abrupt 8 hour advance in the time of lights-out (a switch from 16L:8D to 8L:16D). The result was a gradual phase-advancement in the onset of the daily melatonin peak leading to a coincidence between this and the onset of darkness (Bittman *et al.*, 1983). The second function of light is to terminate neural stimulation of the pineal gland (Klein and Weller, 1972) resulting in a rapid decline in melatonin production (sheep: Rollag and

Niswender, 1976). In reindeer, for example, during the arctic mid-summer (≈ constant light) melatonin secretion is completely suppressed (Eloranta *et al., in press*). The relative significance of these two functions in determining the daily pattern of melatonin secretion remains the subject of debate. Two models that have been proposed are outlined below.

Illnerova and Vanecek (1982, 1988) working on the rat pineal N-acetyltransferase rhythm (NAT, a key enzyme involved in melatonin synthesis) proposed that under natural photoperiods the melatonin rhythm is determined by the entraining effect of light on the pacemaker and not its suppressant effect. In rats exposed to a 1 minute light pulse before midnight (and then maintained in constant darkness) the following evening rise in NAT levels and the morning decline were phase-delayed by almost the same extent. In contrast if the pulse was given after midnight then the following evening rise in NAT was unaffected but the subsequent morning decline was phase-advanced. Illnerova and Vanecek (1982, 1988) suggested this was consistent with the hypothesis that the evening increase in NAT and the morning decline are controlled by separate circadian oscillators. In this model the role of light is to entrain the phase of each, and thus the duration of melatonin secretion is therefore the product of their phase relationship. During long days, the oscillator controlling onset of secretion becomes delayed while that controlling termination is advanced, resulting in a relatively brief secretory period (the reverse occurring during short photoperiods). An advantage of this model is it can explain how the duration of melatonin secretion during certain short-day photoperiods can exceed that observed under constant darkness, when secretion is presumed to be determined solely by the endognous rhythm. For example, peak duration under 8L:16D may reach 16 hours, but under constant darkness only 12-14 hours (sheep: Lincoln *et al.*, 1985). If a dual-oscillator system exists, it is predicted that under persistent darkness the oscillators should drift apart. Evidence from rams kept in constant darkness for 8 weeks suggests this may happen as melatonin rhythms became poorly defined and variable (Lincoln et al., 1985).

An alternative model has been proposed by Lincoln *et al.* (1985). They observed that in sheep a 1 hr light pulse every 24 hours after a period of constant darkness could entrain the melatonin rhythm such that the onset of peak levels occurred shortly after the light period. From this they concluded that the light-dark cycle entrains the rhythm with the end of the light phase providing the signal for pineal melatonin release. The termination of pineal stimulation is, however, controlled by light acting directly to inhibit melatonin secretion. This they concluded from the observation that the period of endogenous melatonin secretion in constant darkness was 12-14 hours but under a long-day regime it was less then 10 hours.

Whilst it remains unclear exactly how light modifies the circadian rhythm generated by the SCN, the photo-manipulated rhythm produces a signal relaying information about daylength. This is transmitted from the SCN to the pineal via the sympathetic nervous system (Moore, 1977).

1.2.3.3. Pineal gland

The importance of the pineal gland in expression of the photoperiodic response has been demonstrated by pinealectomy. Following this procedure artifical photoperiodic challenges lose their ability to influence testicular function, ovarian cyclicity and steroid feedback responsiveness in sheep (Lincoln and Short, 1980; Bittman *et al.*, 1983) and hamsters (reviewed by Reiter, 1980; Pevet, 1988). For example, in ovariectomized ewes implanted with oestradiol, long-days cease to suppress LH secretion and short-days fail to initiate a rise in LH concentrations (Bittman *et al.*, 1983). It appears that the primary role of the pineal is as a neuroendocrine transducer, converting the neurally transmitted signal from the SCN into an endocrine signal. This signal is conveyed via the release of the pineal hormone, melatonin. The synthesis and secretory profile of this hormone is characterised by peak levels during night and low, barely detectable levels during the day (sheep: Rollag and Niswender, 1976; Bittman and Karsch, 1984). Predictably, pinealectomy results in the elimination of the

night-time rise in melatonin, concentrations remaining at levels typical of day-time (Kennaway *et al.*, 1983; Bittman and Karsch, 1984).

Confirmation that the loss of photoperiodic response in pinealectomised animals is due to loss of the melatonin rhythm, has been provided by melatonin replacement studies. Bittman and Karsch (1984) demonstrated that infusion of melatonin for a period mimicking that experienced during short days (16 hr infusion = 8L:16D) and long days (8 hour infusion = 16L:8D) for 70 days six months apart, synchronised reproductive function in ewes with that of pineal-intact animals (those without the infusion remained asynchronous with respect to each other and the pineal-intact animals). In more elaborate experiments (reviewed by Bittman, 1985) melatonin infusions were given to restore the normal nocturnal profile or to provide profiles that differed from those normally present under prevailing photoperiods. The objective was to determine whether reproductive responses (assessed by measuring seasonal changes in LH) reflected the character of the melatonin signal or the ambient photoperiod regime. In one such study, pinealectomized ewes were subjected to a short day photoperiod but infused with a long-day melatonin profile (Bittman and Karsch, 1984). This resulted in a fall in LH levels similar to that observed in pineal-intact ewes kept on long days. In another study, pinealectomized ewes maintained under long days received a short-day melatonin signal (16 hr infusion). Despite the 'inhibitory' photoperiod, infusions led to an unambiguous reproductive induction during the anoestrous season (Yellon *et al.*, 1985). These results indicate that melatonin is a critical component in the transmission of the photoperiodic signal. They also suggest that rather than having a permissive role (i.e. simply permitting expression of daylength effects on seasonality) melatonin 'drives' responses to both stimulatory and inhibitory daylengths (Bittman, 1985).

Evidence from some studies has disputed the role of the pineal as the transducer of photoperiodic information. Disruption of pineal function by superior cervical ganglionectomy (removal of pineal sympatheic innervation) failed to prevent stags (Lincoln, 1985) or sheep (Argo, 1986) responding to photoperiodic manipulation as

predicted. It is significant that in both studies, ablated and intact animals were maintained in close contact. Recent evidence suggests that social cues have a timekeeping role, at least in the absence of photoperiodic signals. Wayne *et al.* (1989) observed that pinealectomized ewes maintained with intact animals under a natural photoperiod exhibited a synchronous onset of breeding season (determined by the seasonal rise in LH) with the latter, but that those kept in isolation showed a 2.5 month delay.

As the period of SCN stimulation of melatonin release reflects the ambient photoperiod the resulting hormone profile provides an endocrine signal that can be used for the synchronisation of physiological changes with environmental seasonality.

1.2.3.4. Characteristics of the melatonin signal providing photoperiodic information

The bulk of experimental evidence is consistent with the '*duration*' hypothesis. This proposes that the length of melatonin elevation during each 24 hour period is proportional to the length of night, and seasonal responses depend on this interval. Evidence in both hamsters (Goldman, 1983) and sheep (Bittman *et al.*, 1983) confirms that the duration of the nocturnal rise in melatonin (pineal content and plasma levels respectively) is related to nightlength (i.e. shorter during long days than short days). Experimental evidence that duration influences photoperiodic response has been provided by replacing the endogenous melatonin profile (abolished by pinealectomy) with timed melatonin infusions designed to mimic nightime levels of different duration. In Djungarian hamsters 12 hour daily infusions, but not 4 or 6 hour infusions blocked gonadal development of juvenile animals (Goldman, 1983). The effect of the 12 hour infusion was similar to the response to short daylengths (irrespective of when infusion occurred relatve to the LD cycle) suggesting that the duration and not timing of the melatonin signal was critical in relaying photoperiodic information.

A recent modification of the duration hypothesis has been proposed by Maywood *et al.* (1990). They observed that the length of

interval between successive fixed duration melatonin infusions was significantly correlated with the photoperiodic response of Syrian hamsters, as assessed by testicular regression. In this case the longer the interval the greater the degree of gonadal inhibition (range 10-18 hours). As a result of this and other evidence (reviewed by Maywood *et al.*, 1990) it has been proposed that the Syrian hamster uses two components of the melatonin signal. The duration of the signal (signifying a 'quantum' of the photoperiodic information) and the frequency with which these signals arrive (i.e. the interval free of melatonin). It is significant that in the hamster, blocking the melatonin free interval by implanting melatonin (subcutaneously) prevents short-days from inducing gonadal regression (Reiter, 1980). As a result gonads remain permanently active. Thus, repeated daily cycles of melatonin are important for normal cueing of physiological function. The absence of response to melatonin implants (i.e. hamsters behave as if on constant long-days and testes remain enlarged) is in marked contrast to the effect of melatonin in ruminants (see section 1.2.3.5.).

Thus, the circadian system (operating via the SCN in mammals) drives the pineal to produce its daily melatonin signal. Normally, the phase relationship between circadian rhythms (i.e. activity, body temperature, etc.) and melatonin is very tight and they appear 'phase-locked'. However, the melatonin signal which is read by the brain does not need to arrive in discrete 24 hour pulses. It just happens that it does so, because it is driven by a circadian oscillator (the SCN).

1.2.3.5. Manipulation of photoperiodic response using exogenous melatonin

In the studies described so far, exogenous melatonin has been administered to replace the endogenous signal following, for example, pinealectomy. An alternative method of manipulating the melatonin signal is to extend the endogenous nocturnal profile using an exogenous source. This procedure has been used in a number of studies reviewed later in this chapter, as well as in two experiments

during this thesis. These manipulations take two basic forms. One way is to administer the hormone prior to the onset of the nightime rise and thereby extend the period of elevated melatonin concentrations. In deer this is usually carried out by injection (e.g. Webster and Barrell, 1985), oral dosing (e.g. Milne *et al.*, 1990) or addition to foodstuffs (e.g. Adam *et al.*, 1986). The time of application varies but normally occurs during late afternoon (e.g. 16.00 hr, Adam *et al.*, 1986). Extending the duration of elevated melatonin should mimic the hormone profile of a shorter daylength. The response of deer and sheep to prolonged daily administration in this fashion is generally consistent with the hypothesis that they interpret the extended profile as a short-day signal. For example, exposure to artifical short-days or daily melatonin treatment from mid-summer, both result in a similar advance in the timing of reproductive activity in autumn (deer, Webster and Barrell, 1985; sheep, English *et al.*, 1986).

The alternative method of manipulating the melatonin signal is by using continuous slow release implants (e.g. Fisher *et al.*, 1988,1990). This form of treatment results in raised levels of the hormone during both night and day (sheep: Lincoln and Ebling, 1985). Although the profile does not resemble the pattern of secretion under any natural photoperiod, the effect of implants appears to be similar to a short-day response in sheep and deer. The only direct comparison found no difference in the reproductive response of ewes subject to either artifical short-days, daily melatonin or slow release implants during summer (English *et al.*, 1986). Whilst this does not prove that continuously elevated levels are perceived as a short day photoperiod, the observed response is certainly similar. It is possible that the animal interprets the signal as a 'super-short' day (Lincoln and Ebling, 1985) if it is the duration of elevated melatonin that provides the critical photoperiodic information (see previous section). However, this is inconsistent with the assertion that a melatonin-free interval is important in rodents (Maywood *et al.*, 1990). In the Syrian hamster the responses to short photoperiods and melatonin implants differ. Normally in this species a switch from long- to short-days results in inhibition of reproductive activity (see section 1.2.4). However, melatonin

implants administered to animals under long-days not only had no effect, but prevented a subsequent response to short-days (Reiter, 1980). The response is similar to that observed following pinealectomy in this species (i.e. photoperiodic response abolished). This is significant to understanding the action of implants in deer and sheep. Possibly, continuous release melatonin acts by blocking the inhibitory long-day photoperiod signal. There is evidence that this could have caused the observed advance in reproduction associated with melatonin treatment. Removal of the photoperiodic signal by pinealectomy of ewes at the time of the summer solstice also results in an advanced breeding season (Wayne *et al.*, 1988). Thus, the similarity between the response to short photoperiods, daily or continuous melatonin treatments and pinealectomy, during seasonal anoestrus could have occurred if the advance of the breeding season was primarily due to removal of long-day inhibition of reproduction. Even if interpreted differently, these treatments would have resulted in the blockade of this signal. Whether or not this is true, melatonin implants provide (in ruminants) a convenient and cheap way of manipulating seasonality.

1.2.4. PHOTOPERIODIC SIGNALS CONTROLLING SEASONALITY

1.2.4.1. Direction of photoperiodic changes

Under natural environmental conditions the same daylength occurs twice a year (before and after each solstice) yet most medium and large sized mammals in temperate environments breed only once. Also, reproductive events (in all mammals) are synchronised so that birth of offspring occurs during the same period each year. If as proposed in the preceding sections, daylength is used to time seasonal changes, then animals must be able to distinguish between the same photoperiod when it occurs at different times of the year. One way in which this could be achieved is if the seasonal response to a given daylength depends upon previous photoperiodic experience. This was investigated by Robinson and Karsch (1987) using ovarectomized ewes treated with oestradiol implants (see section 1.2.5.3.). Pineal-intact ewes were transferred to a common 13L:11D

photoperiod following prior exposure to either 16L:8D or 10L:14D photoperiods, groups thereby experiencing either a 3 hour increase or 3 hour decrease in daylength. The resulting response, determined by changes in serum LH concentrations, was significantly related to previous treatment. LH levels fell in the groups of ewes which experienced an increase in daylength and increased in those which experienced a decrease in daylength. Since the final photoperiodic history was the same (13L:11D) it is clear that prior photoperiodic history was a major determinant of the response. This differential reaction to the direction of photoperiodic change could occur due to either an effect on the pineal melatonin signal or, due to differences in target sensitivity to melatonin. Measurement of the melatonin profile during the study revealed that the latter always conformed to prevailing daylength. A similar observation was reported in ewes maintained on a 12L:12D photoperiod (Jackson *et al.*, 1990). Although their melatonin profiles were similar, there was extreme variation in the character of their response to this photoperiod (i.e. continuous oestrous cyclicity in some, random cycling in others and in one none at all). These results suggest that the mechanism by which different responses are elicited by the same photoperiod, must be at or below the level of processing of the melatonin signal.

Thus, it appears that seasonal mammals are able to assess correctly the time of year by a combination of the melatonin signal, which relays information about prevailing daylength, and variation in target sensitivity to this signal, resulting from previous exposure to that signal. The importance of changes in target sensitivity to melatonin in determining the photoperiodic response has been further illustrated by investigation of which photoperiods are responsible for initiating particular seasonal changes.

1.2.4.2. Control of seasonality in rodents

In small, short-lived mammals (e.g. rodents), with relatively brief gestation periods (15-25 days: Reiter, 1980), breeding activity commences in spring whilst exposed to increasing daylengths and ends in late summer when daylength declines. The termination of

reproductive activity appears dependent on exposure to short photoperiods (less than 12.5 hr of light results in gonadal regression) as long days maintain reproductive competance (Reiter, 1980). In contrast, gonadal regrowth in spring appears to be an endogenously driven event. This has been demonstrated by the spontaneous regeneration of the testes observed in hamsters kept on short daylengths (Reiter, 1980). The term introduced to describe the loss of responsiveness to an inhibitory (or stimulatory) photoperiodic stimulus, as a result or previous exposure to it, is photorefractoriness (Reiter, 1972). Increasing daylengths do however have an important role. After becoming refractory to short days hamsters remain in reproductive condition indefinitely (Reiter, 1980). The ability to exhibit regression of the reproductive system is dependent on exposure to a sufficient period of long daylengths whereupon short-days can once again cause regression. The duration of long-days required was demonstrated to be between 10-22 weeks. Changes in photo-sensitivity that occur during refractoriness maybe the result of a change in sensitivity to the melatonin signal rather than a change in the signal itself. In Syrian hamsters, rhythms of pineal melatonin content did not alter as testes regressed and spontaneously recrudesced under prolonged short days, unless hibernation occurred (Vanecek *et al.*, 1984). In addition, injection of melatonin into hamsters following spontaneous recrudescence could not induce a second regression unless those animals had been exposed to a prolonged period of long days (Bittman, 1984). Thus, in the hamster (and probably other rodents) seasonal changes are driven by a combination of a short-day photoperiodic signal terminating reproductive activity in late summer and refractoriness to this signal initiating its resumption in spring. The role of long days appears primarily to be to break the refractoriness permitting future regression. In the absence of a photoperiodic signal (e.g. following pinealectomy) seasonality is lost, the reproductive system regenerates (if it had been regressed) and animals remain in a constant state of reproductive competence (Reiter, 1980). Utilization of a refractory response, rather than direct use of daylength, to time seasonal gonadal regrowth in spring may have

evolved because hibernating mammals like the hamster receive little if any photoperiodic information during winter.

1.2.4.3. The control of seasonality in ungulates

In larger, long lived mammals like sheep and deer, prolonged gestation lengths (150 and 234 days respectively) ensure that birth of offspring coincide with increasing plant growth in spring, and mating must occur during autumn. In contrast to the hamster, daylength is declining by the onset of the breeding season and increasing at its termination. This suggests that the photoperiodic stimuli used by female sheep and deer to time particular seasonal events differ from those use by most rodents.

The ability of photoperiod to drive seasonal changes in the ewe has been demonstrated by subjecting animals to artifical short-day (8L:16D) and long-day (16L:8D) photoperiods which were alternated every 90 days (Legan and Karsch, 1980). Under these conditions ewes underwent two breeding and anoestrous seasons in one year. Oestrous cyclicity was associated with exposure to short-day photoperiods and anoestrus with long days. Similar results were obtained in animals subject to 120 day alternations between daylengths (except that the period of each cycle was greater). This implies photoperiodic changes can directly control seasonal responses in the ewe. However, evidence from a number of studies indicates that this species can exhibit photofractoriness to both initially inductive and suppressive photoperiod regimes. The role of photorefractoriness in the control of seasonality in the ewe, is discussed in the following section with respect to reproductive activity.

1.2.5. PHOTOPERIODIC CONTROL OF REPRODUCTION

1.2.5.1. The onset of reproductive activity

If ewes are maintained on a summer solstice photoperiod from this solstice (depriving them of exposure to a declining daylength) then the onset of reproductive activity still occurs at the appropriate time in autumn (Robinson *et al.*, 1985; Worthy *et al.*,

1985; Malpaux *et al.*, 1989; O'Callaghan *et al.*, 1989). This indicates that a declining photoperiod stimulus is not required to terminate anoestrus. It suggests instead that under natural daylengths this event is determined by an endogenous process resulting in photorefractoriness to long-day inhibition (this is analogous to the spontaneous re-growth of the hamster testis under short photoperiods). Normal timing of reproductive activity only occurs, however, if ewes have been exposed to increasing daylengths during spring and summer. For example, when ewes were held on winter solstice photoperiods from this solstice, then the onset of reproductive activity was substantially delayed (at least 2 months in 4/5 ewes, Malpaux *et al.*, 1989; at least 3 months in 7/9 ewes Sunderland *et al.*, 1990; one month, O'Callaghan *et al.*, 1990). In addition, during photoperiod controlled experiments when daylength increases occured earlier or later than normal, then the onset was respectively advanced or delayed (Malpaux *et al.*, 1989). Further evidence of the importance of spring/summer photoperiods has been provided by infusing pinealectomized ewes with melatonin profiles characteristic of either summer, autumn, winter or spring, at the appropriate time of year over several years. This has revealed that only infusions designed to mimic profiles normally experienced during spring and summer were capable of synchronizing the onset of the breeding season with pineal-intact controls (Woodfill *et al.*, 1990). In an experiment in which ewes were maintained on a winter solstice photoperiod from the solstice O'Callagher *et al.* (1990) have shown that as little as 10 days exposure to long days (18L:6D) during April can synchronize reproduction of treated ewes with animals under a simulated natural photoperiod. Considered together, this evidence indicates that exposure to long days is critical in synchronizing the long-term endogenous processes which result in the termination of long-day photorefractoriness and hence a normal onset to the breeding season many months later.

Predictably, pinealectomy of ewes at the spring equinox leads to a late and non-synchronous breeding season due to the loss of a long-day signal (Wayne *et al.*, 1988). However, when performed at the summer solstice not only were ewes synchronous but its onset was advanced (Wayne *et al.*, 1988). This implies that exposure to spring

photoperiods would lead to an earlier breeding season, nearer the summer solstice, but that normally long days actively suppress reproduction at this time.

1.2.5.2. The onset of seasonal anoestrus

Exposure to artifical long-days (16L:8D) from mid-January advances the onset of seasonal anoestrus in late winter (Worthy and Haresign, 1983). However, even in the absence of increasing daylength the breeding season ends at about the normal time of the year (Worthy and Haresign, 1983; Robinson and Karsch, 1984; Malpaux *et al.*, 1988a). In addition, exposure to abrupt artifical reductions in daylength or increasingly shortened photoperiods after the winter solstice extends the duration of reproductive activity but does not prevent seasonal anoestrus (Malpaux *et al.*, 1988a,b). This implies that under natural conditions the initiation of anoestrus is primarily determined by refractoriness to an inductive short photoperiod signal.

Karsch *et al.* (1986) carried out two experiments to examine whether photorefractoriness was due to pre- or post-pineal processing of the melatonin signal. In the first, the circadian pattern of melatonin secretion was monitored in pineal-intact ewes subject to constant photoperiods that had been initially inductive (short days) or inhibitory (long days). They observed that despite eventual loss of photoperiodic response there were no associated changes in the circadian melatonin pattern. In the second experiment, pinealectomized ewes were infused with a circadian pattern of melatonin similar to that present during short days. This initially provoked reproductive induction (determined by elevated LH concentrations), but the response then lessened and LH levels fell over a similar time period as seen in pineal intact animals. These results are consistent with the hypothesis that refractoriness and termination of the breeding season is due to post-pineal processing of the melatonin signal (i.e. represents a loss of target sensitivity to this signal).

Most evidence in the ewe suggests under normal environmental conditions, the sequence of reproductive events is determined by the development of refractoriness to ambient photoperiodic signals. The onset of the breeding season results from refractoriness to long-day inhibition and direct suppression of reproductive activity around the summer solstice. Its termination results from refractoriness to stimulatory short-day photoperiods.

Studies on the ewe provide the only current data in ruminants examining the interaction of the photoperiodically driven melatonin signal with internal endogenous rhythms. Whether the ewe provides an accurate model of seasonality in the red deer hind remains unclear. However, red deer hinds do exhibit long-day photorefractoriness. This has been shown by subjecting pre-pubertal hinds to summer solstical photoperiods from their first winter solstice (Loudon and Brinklow, 1990). These animals not only commenced oestrous cyclicity the following autumn whilst still exposed to long days from the previous winter solstice, but they also started cycling earlier than controls. This result is similar to the response of adult ewes transferred to long-days in mid-winter (Malpaux *et al.*, 1989). The major inconsistency between observations in ewes and red deer hinds relates to the mechanism controlling the termination of the breeding season. When a group of hinds were treated daily from February with exogenous melatonin administered in feed to mimic short-day profiles of the hormone (see section 1.2.3.5.) animals failed to exhibit seasonal anoestrus until treatment ceased 16 months later (Adam *et al.*, 1989). This contrasts with a comparable experiment in ewes where melatonin treatment only prolonged reproductive activity by 6 weeks (Nett and Niswender, 1982). In addition, continuous release melatonin implants administered to Pere David's deer in mid-winter, only extended the breeding season by 12.3 days, less in fact than the length of a single oestrous cycle (c. 18 days)(C.M. Argo, A.S.I. Loudon and B.R. Brinklow, unpublished data). The failure of red deer hinds exposed to a short-day melatonin signal to enter an anoestrous state suggests that, unlike the ewe and Pere David's deer hind, this species may require exposure to increasing daylengths to terminate the breeding season (i.e. a direct photoperiodic signal rather than

a photorefractory response). Related to these observations melatonin implants given to pre-pubertal red deer hinds at the winter solstice significantly reduced the amplitude of seasonal changes in prolactin and voluntary food intake (see section 1.2.9) but did not prevent them (Loudon and Brinklow, 1990). Whilst photoperiod is clearly important, the exact role of increasing daylengths during spring in the timing of seasonal changes of red deer remains to be clarified.

It is clear that considerable species variation may occur in seasonal ruminants, with perhaps a common mechanism centered around long-day photorefractoriness (exhibited by all long lived seasonal mammals so far examined) and variable mechanism for switching off breeding seasons on short days.

1.2.5.3. Endocrine control of seasonality

The following section describes the mechanism by which the photoperiod signal is believed to control reproductive status in the female. For a review of the physiological mechanisms controlling seasonal breeding in the red deer stag see Lincoln (1985).

The hypothalamic pulse generator and the oestrous cycle

To understand the mechanism of seasonal control it is first necessary to briefly consider the key endocrine events that take place during the oestrous cycle. The outline described below is based on the model developed for the ewe by Goodman and Karsch (1981) illustrated in Figure 1.2a. (the oestrous cycle length in the ewe is 16 days).

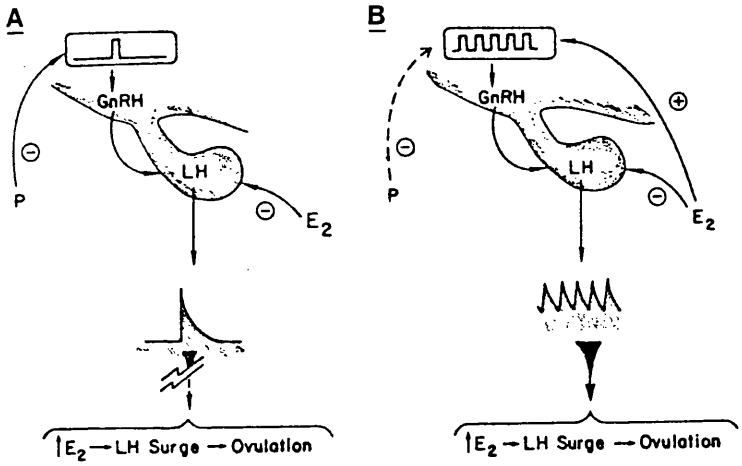
The hypothalamic pulse generator (located in the median basal hypothalamus) produces episodic discharges of gonadotrophin-releasing hormone (GnRH) which stimulate pulsatile release of luteinizing hormone (LH) from the anterior pituitary gland (Clarke and Cummins, 1982). This hormone acts on the ovary to promote follicular maturation and secretion of oestradiol. Increasing levels of this steroid positively feedback onto the pulse generator inducing a surge in LH (Karsch *et al.*, 1979) which results in

ovulation (oestrous behaviour coincides with the LH surge). Following this, granulosa cells of the ruptured Graafian follicle begin to luteinize (increase in numbers and size) forming what is refered to as the *corpus luteum* (CL). Coincident with morphological changes, this tissue begins progesterone synthesis and secretion. The CL regresses on day 15 due to the lytic action of prostaglandins formed in the endometrium under the influence of progesterone (Short, 1972). As a result progesterone levels abruptly decline. The hormonal changes during the oestrous cycle are summarized in Figure 1.2b.

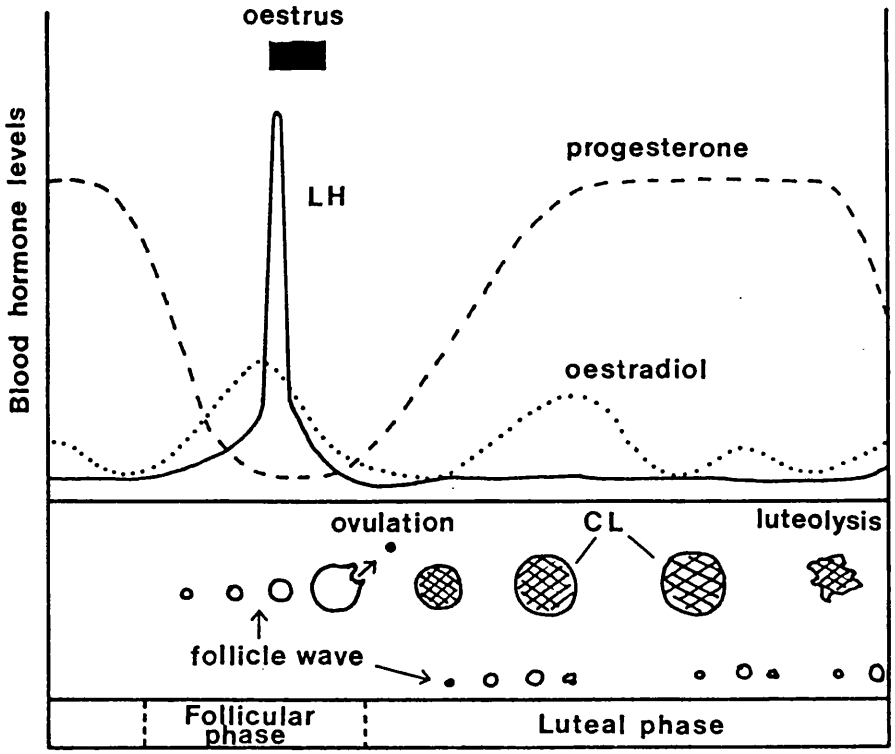
The key element in this model determining ovulation is the pulse frequency of LH (Karsch *et al.*, 1984). High frequency pulses are required to induce the sustained elevation in LH necessary to promote follicular maturation and the preovulatory oestradiol rise. The pattern of LH secretion is modified by the actions of gonadal steroids. Progesterone acting on the pulse generator reduces the pulse frequency of GnRH secretion (Karsch *et al.*, 1987) resulting in low LH release. In contrast, oestradiol reduces LH pulse amplitude (Goodman and Karsch, 1981) acting, at least in part, upon the anterior pituitary to decrease responsiveness to GnRH (Goodman and Karsch, 1981; Karsch *et al.*, 1984). As a result, high progesterone levels produced by the CL reduce LH pulse frequency below the threshold necessary for periodic increases in oestradiol to initiate the pre-ovulatory events (the *'luteal phase'*)(Hauger *et al.*, 1977). When the CL regresses and circulating progesterone concentrations decline, the pulse frequency increases to its endogenous periodicity initiating the sequence of the events leading to ovulation (the *'follicular phase'*). Thus, the bi-weekly pattern of ovulation responsible for the oestrous cycle is primarily determined by the clamping action of progesterone on the pulse generator.

The endocrine control of the oestrous cycle in deer has yet to be described in detail. The length of the cycle in red deer is reported as between 18-21 days (18.3, Guinness *et al.*, 1971; 18.2, Kelly *et al.*, 1985; 21 days, Adam *et al.*, 1985). In both red (Kelly *et al.*, 1985) and white-tailed (Plotka *et al.*, 1980) deer the pattern of changes in progesterone, oestradiol and tonic LH during the oestrous cycle are similar to those found in the ewe (see Figure

FIGURE 1.2: (a) Model for control of ovulation during oestrous cycle of the ewe. (i) Luteal phase: preovulatory sequence (bottom) is blocked by elevated progesterone (P) which reduces the frequency of the GnRH pulse generator. (ii) Follicular phase: frequency of GnRH pulse generator increases due to combined effect of P withdrawal and oestradiol (E_2) stimulation. This permits the preovulatory sequence. Modified from Goodman and Karsch (1981).



(b) Schematic representation of major hormonal changes and pattern of follicular development during oestrous cycle of the ewe. Modified from Hauger *et al.* (1977).



1.2b). The only information about the pulsatile pattern of LH secretion in any species of deer is from the Pere David's deer. In this species, pulse frequency is significantly higher during the follicular than luteal phase, as observed in the ewe. However, in contrast to the latter (Goodman and Karsch, 1981), but broadly consistent with observations in cattle (Page *et al.*, 1987), LH pulse amplitude does not increase during the luteal phase. Despite differences, the model outlined for the control of the oestrous cycle in the ewe is regarded as generally appropriate for female deer.

Photoperiodic regulation of LH pulse generator

The activity of the pulse generator is critical in determining the seasonal reproductive state (Goodman and Karsch, 1981). Evidence suggests that failure to cycle during anoestrus is not due to the inability of the ovary to respond to LH. Ovulation can be brought about by GnRH (after progesterone removal) in anoestrous ewes (McLeod *et al.*, 1982, 1983; Wright *et al.*, 1983) and deer (McLeod *et al.*, 1991). Reproductive failure appears to be a consequence of insufficient LH due to a reduction in GnRH pulse frequency (Goodman and Karsch, 1981; Bittman, 1985).

In general, photoperiod is believed to act via a *steroid dependent* mechanism. Specifically, it appears that seasonal changes in the potency of oestradiol negative feedback on LH secretion, underly changes in reproductive status. This has been demonstrated by treating ovariectomized ewes with an implant providing a fixed basal level of oestradiol. Under natural photoperiods treated ewes exhibited suppressed serum LH levels during the summer anoestrous period but elevated levels during the normal breeding season of intact animals. Manipulating the photoperiod regime by alternating long and short photoperiods every 90 days resulted in intact ewes undergoing 2 breeding and anoestrous seasons in a year. Coincident with reproductive transitions in the latter were striking fluctuations in serum LH levels of oestradiol-treated castrates (Legan and Karsch, 1980).

During the breeding season oestradiol does not significantly reduce LH pulse frequency (see above). However, its suppressive effects during anoestrus appear to result from an inhibitory effect on the hypothalamic pulse generator. This results in a reduction in the frequency of GnRH (and hence LH) pulses (Goodman and Karsch, 1981). This was demonstrated by comparing the pulse frequency of long-term ovariectomized ewes during the breeding and anoestrous seasons before and after oestradiol treatment (Goodman and Karsch, 1981). During the breeding season, oestradiol simply led to a reduction in LH pulse amplitude with no affect on pulse frequency. In contrast, during anoestrus there was a significant decline in pulse frequency. The fact that ewes remained highly responsive to a small dose of exogenous GnRH suggests oestradiol's influence was acting above the level of the pituitary (see Karsch *et al.*, 1984).

There is also evidence for a direct, *steroid independent* drive on the reproductive axis which has so far only been investigated in the ewe. Following castration, LH levels remain elevated at all times of the year (i.e. loss of oestradiol negative feedback)(Karsch *et al.*, 1984). Frequent blood sampling, however, has revealed that LH pulse frequency undergoes distinct seasonal changes (Karsch *et al.*, 1984) which can be driven by photoperiodic manipulation and depend on the presence of the pineal gland for expression (Bittman *et al.*, 1985). Short-days produce high frequency, low amplitude LH pulses whereas long-days produce low frequency, high amplitude pulses similar to the anoestrous period. The photoperiodic signal is believed to act directly on the frequency set by the hypothalamic pulse generator, although the mechanism remains a mystery (Karsch *et al.*, 1984).

Thus, the photoperiodic signal determines reproductive status by determining whether LH pulse frequency is maintained above (= breeding season) or below (= anoestrous period) the rate required to elevate oestradiol to concentrations adequate to trigger a preovulatory LH surge. This is supported by the observation that late follicular phase concentrations (i.e. preovulatory levels) of oestradiol are able to induce unambiguous GnRH and subsequent LH

surges irrespective of the seasonal reproductive state of the ewe (Moenter and Karsch, 1990). In the ovary-intact animal pulse frequency is primarily determined by seasonal changes in the negative feedback effects of oestradiol. The role of the other ovarian steroid (progesterone) is to determine the sequence of events within the oestrous cycle by reducing LH pulse frequency during the luteal phase.

In the ewe available evidence suggests the onset of anoestrus is characterized by a rapid drop in LH pulse frequency which subsequently remains consistently low until approximately a week before the begining of the next breeding season (Legan *et al.*, 1977; l'Anson and Legan, 1988). In contrast, in the Pere David's deer at least, anoestrus is not a uniform state. Both LH pulse frequency and the pituitary response to exogenous GnRH are significantly lower during its early stages gradually increasing as the next breeding season approaches (Curlewis *et al.*, 1991). This suggests that in contrast to the ewe, in the Pere David's hind, oestradiol negative feedback may gradually decline over a period of several months as anoestrus progresses.

Prolactin and seasonal reproduction

Plasma prolactin concentrations in many mammals including deer and sheep show pronounced seasonal changes (red deer: Suttie and Kay, 1985; Loudon *et al.*, 1989; white-tailed deer: Mirachi *et al.*, 1978; roe deer: Schams and Barth, 1982; sheep: Walton *et al.*, 1977). Comparison of the plasma prolactin profiles of 3 different deer species (red, roe, and white-tailed deer: reviewed by Lincoln, 1985) with different breeding seasons has shown that there is no fixed relationship between timing of the plasma prolactin and reproductive cycles. A similar lack of relationship has been reported between sheep of different breeds (Carr and Land, 1982; Webster and Haresign, 1983; Lincoln, 1990). In most cases plasma prolactin concentrations closely follow the seasonal pattern of daylength changes, with the highest levels in summer and lowest in mid-winter (see Figure 1.1 for red deer prolactin cycle). An exception to this

is the Pere David's deer which originated from China. In this species, the seasonal changes in plasma prolactin concentrations (as well as VFI, reproduction, coat growth and T_3) of animals maintained in the U.K., are phase advanced by about 2 months compared to these cycles in red deer under the same ambient photoperiod (Loudon *et al.*, 1989). As a result plasma prolactin concentrations peak earlier in Pere David's deer than in any other indigenous northern temperate zone ungulates so far studied.

High suckling frequencies by offspring are associated with delayed conception in red deer hinds (Loudon *et al.*, 1983), humans (McNeilly, 1979) and lower pregnancy rates in cattle (Bastidas *et al.*, 1984). The onset of oestrous cyclicity has also been reported as commencing later in lactating than early weaned hinds (10 days: Adam *et al.*, 1985). As high suckling frequencies are also associated with elevated plasma prolactin concentrations (deer: Loudon *et al.*, 1983; humans: McNeilly, 1979) it has been suggested that suckling effects on reproduction may be relayed via prolactin, although an independent influence on the LH secretory mechanism is an alternative (McNeilly, 1984). The failure of either suppression (with bromocriptine) or stimulation (using domperidone) of prolactin levels during summer to alter the timing of the breeding season, implies, however, that this hormone is not involved (Milne *et al.*, 1990). The role of prolactin in timing reproductive events is, however, potentially complex since delaying the normal spring rise in plasma prolactin concentrations using the dopamine agonist bromocriptine results in a delay in the onset of anoestrus (as well as spring increases in VFI and the moult of winter coat). This suggests prolactin concentrations may modify reproductive changes at the end of the breeding season (Curlewis *et al.*, 1988b).

The significance of prolactin's role in the control of reproductive activity remains ambiguous, however, its affect appears to be to modify rather than drive seasonal changes. This issue is discussed again in chapter 4 in the light of data gathered in experiment 2 (chapter 4).

1.2.6. LINKAGE OF SEASONAL CYCLES

The temporal pattern of changes in reproduction, pelage and metabolism are inevitably linked as they have evolved in response to the same seasonal fluctuations in temperature and food availability. Evidence suggests that linkage extends beyond common ultimate factors and reflects a dependence on the same environmental stimuli to control seasonality (i.e. photoperiod). Manipulating the photoperiod regime by compressing the annual pattern of daylength changes into 6 months, has failed to significantly disrupt the phase relationship of seasonal changes in appetite, growth, and gonadal activity of red deer stags (Simpson *et al.*, 1983/4; Suttie and Simpson, 1985) and rams (Simpson *et al.*, 1983/4). A similar response has been reported following administration of melatonin during summer to induce a short-day response and thereby advance the onset of the breeding season (for explanation of treatment see section 1.2.3.5.). Associated with the advance in reproductive activity were advances in the autumn moult, liveweight nadir (Fisher *et al.*, 1988, 1990) and seasonal appetite decline (Milne *et al.*, 1990) of hinds, and phase of antler growth of stags (Fisher *et al.*, 1988). Further evidence, indicating linkage in the control of seasonal responses, has been reported in a study in which hinds were treated during the breeding season in late winter for 8.5 months with a slow release preparation of the dopamine agonist, bromocriptine (Curlewis *et al.*, 1988). Treatment resulted in a delay in the spring increase in plasma prolactin concentrations, the onset of anoestrus, the moult of the winter coat and subsequent growth of summer pelage, as well a suppression of food intake.

The relationship between the different seasonal responses is consistent with not only the existence of a common proximate factor controlling seasonality, but with a common mechanism determining the response to particular photoperiod signals. As a result, the following sections reviewing seasonal changes in pelage and metabolism will only briefly refer to the role of photoperiod. It is assumed that the critical photperiodic information is the same for these cycles as that described for reproductive activity in section 1.2.5.

1.2.7. PHOTOPERIODIC CONTROL OF COAT GROWTH

Mature red deer undergo two changes of coat each year. This includes a 'winter' coat comprising guard hairs of about 60mm length and a fine wool undercoat, up to 20mm length. This usually starts growing in August and is completed by about December. Growth of the 'summer' coat is normally first evident in May and reaches a maximum length of approximately 50mm during September. Unlike the winter coat there is very little or no underwool (Ryder and Kay, 1973; Ryder,1977).

The role of photoperiod in the control of coat growth has been demonstrated by subjecting deer to the annual daylength cycle compressed into periods of less than 12 months. For example, when red deer stags were exposed to six month cycles they exhibited a doubled frequency of coat changes (Kay and Ryder, 1978). Pinealectomy of seasonal white-tailed deer leads to loss of synchrony of individuals with enviromental changes, indicating the involvement of the pineal gland in the transmission of photoperiodic information (Plotka *et al.*, 1982). This has been further demonstrated by daily or continuous release melatonin treatment during summer. The response to this is a phase shift in seasonality similar to that observed in reproductive activity, with the autumn moult occuring about one month earlier than normal (implants: Fisher *et al.*, 1988; daily injections: Webster and Barrell, 1985; daily oral dosing: Milne *et al.*, 1990).

The mechanism by which photoperiod controls the activity of hair follicles is not understood. The fact that both pelage and reproductive cycles show similar responses to photoperiodic manipulation suggests the possiblity of a causal link, in particular that reproductive changes may control coat growth. This, however, is not supported by experimental evidence. Duncan and Goldman (1984a) demonstrated gonadectomy of male and female Djungarian hamsters (*Phodopus sungorous*) does not disrupt the response to either long or short day photoperiods.

Martinet *et al.* (1984) reviewing evidence in temperate zone mustelids, which like deer exhibit seasonal pelage cycles, suggested that prolactin may be involved in mediating photoperiodic effects. In mink (*Mustela vison*), red deer and sheep, spring and autumn moults are correlated with increases and decreases in circulating prolactin concentrations respectively (mink: Martinet and Allain, 1985; deer: Loudon *et al.*, 1989; sheep: Lincoln, 1990). In the mink, bromocriptine (a dopamine agonist) administered to animals kept on long days (≃ summer) led to a rapid decline in plasma prolactin concentrations and an advance in the autumn moult. This is a similar response to that obtained by exposure to short days (Martinet *et al.*, 1984) or using melatonin (Allain and Rougeot, 1980). Comparable results have been obtained in the Djungarian hamster following bromocriptine treatment, in addition to a reversal of bromocriptine effects using exogenous prolactin (Duncan and Goldman, 1984b). In red deer, bromocriptine treatment suppressed prolactin and disrupted winter coat growth, although it is unknown whether the time of moult was affected (Milne *et al.*, 1990).

Administration of exogenous prolactin whilst in winter pelage (i.e. when endogenous levels are normally low) resulted in an advance in the spring moult in mink (Martinet *et al.*, 1983) and early growth of the summer pelage in hamsters (Duncan and Goldman, 1984a). Consistent with this, delaying the rise in plasma prolactin concentrations in the spring in red deer (using bromocriptine) was associated with a delay in the moult of the winter coat (Curlewis *et al.*, 1988). It is important to note, however, that maintaining either high or low plasma prolactin levels only modified the timing of seasonal changes in mink and did not prevent moulting. This suggests hair growth can exhibit refractoriness to the prolactin signal (Martinet *et al.*, 1984).

The relationship between plasma prolactin concentrations and coat growth is further supported by the two month advance in the spring rise in both these parameters seen in Pere David's deer compared to red deer (Loudon *et al.*, 1989). In domestic sheep breeds (e.g. Merino) which exhibit continuous growth of pelage throughout the year the plasma prolactin concentrations during winter are

relatively higher than in wild sheep and domesic breeds with pronounced pelage growth cycles (Lincoln,, 1990).

To conclude, evidence suggests that photoperiod may control coat growth via a melatonin signal regulating prolactin release. The mechanism by which prolactin influences the hair follicle activity cycle remains to be determined.

1.2.8. PHOTOPERIODIC CONTROL OF METABOLISM

The most obvious expression of seasonal metabolic changes is the appetite cycle. Enhoused red deer maintained on *ad libitum* diets show a clear annual pattern of voluntary food intake (VFI) reflecting changing appetite (stags: Milne *et al.*, 1978; Kay, 1979; Suttie and Kay, 1985; Fennessy *et al.*, in press; hinds: Loudon *et al.*, 1989). Characteristically, VFI rises rapidly in spring peaking in mid-summer and then declines progressively reaching a nadir in mid-winter (see Figure 1.1). Stags exhibit an additional reduction in appetite during the rut (Fennessy, Thompson and Suttie, in press) Associated with this is an annual cycle in live-weight (Loudon *et al.*, 1989). The persistence of these related cyles in deer fed to appetite demonstrates that they are not simply the consequence of food availability, but represent an important physiological strategy in the deer's repertoire of seasonal adaptations. As the pattern of appetite changes roughly corresponds to the seasonal variation in plant production of temperate zone habitats, it suggests this cycle is an adaptation synchronising energy and nutrient requirements with annual fluctuations in resource availability.

Photoperiod's role in the control of the appetite cycle is best illustrated by compressing the annual pattern of day length changes into a period of six months. Under these conditions red deer exhibit six month cycles of VFI (stags: Pollock, 1975; Brown *et al.*, 1979; Kay, 1979; Simpson *et al.*, 1983/4). As with reproduction and pelage, melatonin treatment during summer advances the phase of seasonality, leading to an early decline in appetite of hinds kept on an *ad libitum* diet (Milne *et al.*, 1990).

The annual appetite cycle is associated with two of the principle determinants of metabolism. Primarily it reflects seasonal variation in metabolic rate (i.e. total energy expenditure) due to the changing energy requirements for growth, reproduction and maintenance (= energy necessary for essential metabolic processes but not growth). However, even when fed to appetite, red deer may lose weight during winter months (hind: Loudon *et al.*, 1989; stag: Fennessy *et al.*, 1981; white-tailed deer: Wood *et al.*, 1962). Losses in stags are in addition to those resulting from rut effects on grazing behaviour (see below). This indicates that appetite is suppressed to a level that maybe insufficient to meet maintenance energy requirements. As a result stored fat reserves are utilized. The use of energy reserves implies that under natural conditions food availability during winter is insufficient to meet energetic requirements. This does not explain, however, why deer are unable to extend feeding activity to meet maintenance requirements if food is plentiful. This could be related to the costs of maximising food consumption when the latter normally is in short supply. Prolonging grazing activity raises energy expenditure and exposes deer to wind chill, particularly if it is necessary to forage away from shelter. It also results in a greater predation risk. Evidence in white-tailed deer indicates that in the wild, animals show a decrease in activity with an increase in predicted heat loss (Moen, 1976). Trials have also demonstrated a substantial difference in maintenance requirements between animals in sheltered and exposed postures (Moen, 1985). This suggests that suppressed appetite accompanying a loss of feeding opportunism, and obligatory use of fat reserves, may have evolved due to the consistency of winter food shortage in seasonal habitats and the costs of foraging under such circumstances (i.e. costs incurred normally exceeding benefits of attempting to increase food consumption).

As stated above the appetite cycle is related to seasonal variation in energy expenditure. By targeting biological processes which have high energy demands (e.g. lactation) with peak food availability, and reducing energy needs during winter, deer could

maximise both survival and reproductive success. The major components exhibiting seasonal variation are as follows:

Maintenance requirements

Energy requirements for maintenance vary due to seasonal changes in basal metabolic rate, climate and animal activity.

There is some evidence to suggest the existance of an underlying seasonal cycle in basal metabolic rate (which is independent of food intake). In both white-tailed deer (Silver *et al.*, 1969) and sheep (Blaxter and Boyne, 1982), significantly higher estimates for fasting metabolic rate (FMR = basal metabolic rate plus energy expended due to activity in the respiration chamber) have been reported during summer than in winter. For example, average FMR of predominantly male deer varied from a peak of 624 kJ/day/$kg^{0.75}$ in July, to 346 kJ/day/$kg^{0.75}$ in December (Silver *et al.*, 1969). A lower basal metabolic rate during winter could be important in reducing energy expenditure when food resources are scarce. Although there may be a lower defended body temperature during winter, the most likely explanation of differences in FMR is seasonal variation in the insulatory quality of the pelage. The winter coat of deer is longer and contains wool fibres which are largely absent in the summer coat (Ryder and Kay, 1973; Ryder, 1977). Measurements of FMR in studies described above were all made at relatively constant environmental temperatures (16-21.5 ^{0}C: Silver *et al.*, 1969; 11-13 ^{0}C: Blaxter and Boyne, 1982) and thus seasonally changing pelage could have accounted for the variation in metabolic rate between summer and winter. Seasonal changes in the insulatory quality of pelage have important consequences for maintenance energy expenditure in thermo-regulation. It is predicted that the effects of low environmental temperatures during winter will in part be compensated for by changes in pelage. The extent to which this reduces absolute energy expenditure during winter and may contribute to the reduction in appetite is unclear. In white-tailed deer fawns, predicted maintenance energy requirements have been shown to be lower during winter than summer for enhoused animals kept at ambient temperatures in some (Thomson *et al.*, 1973) but not all studies (Holter *et al.*, 1977).

It is important to note that not all studies have demonstrated seasonal changes in basal metabolism. Pekins *et al.* (1990) failed to demonstrate any seasonal increase in FMR between winter and summer of non-pregnant white-tailed deer, although there was a difference in pregnant animals. These authors suggested that the difference between this and earlier observations in this species maybe related to animal activity within metabolism chambers. In this study all animals had extensive experience of the chambers before use in trials (200 hours) and measurements were only made after a lengthy period of inactivity. As a result observations should have approximated to basal metabolic rate. Earlier studies (e.g. Silver *et al.*, 1969) assumed there were no seasonal affects of animal activity. Preliminary findings of a recent study of pre-pubertal red deer indicate that level of animal activity maybe important. Seasonal differences in FMR were observed whilst animals were active but not when resting (A.M. Sibbald, *pers. comm.*).

The proportion of time deer spend foraging each day will affect maintenance energy requirements. The reduction in activity observed in white-tailed deer during winter discussed earlier may constitute an important element of the animal's repertoire of seasonal adaptations (Moen, 1976).

Growth and fat deposition

As already mentioned, red deer fed to appetite exhibit pronounced seasonal changes in live weight. This reflects changes in growth and fat deposition. By suppressing growth during winter when food abundance is low, deer limit energy expenditure and increase their chances of survival. Suppression of growth not only occurs in adults but also to a lesser extent in calves (Wood *et al.*, 1962; Milne *et al.*, 1987; Loudon *et al.*, 1989).

Reproduction

Reproduction influences metabolic rate and appetite in two distinct ways. It can modify energy requirements and suppress appetite, irrespective of the energy costs.

In stags, antler growth, muscle development and fat deposition in preparation for the rut are associated with substantial increases

in VFI and body weight compared to castrate males (Kay, 1979). In females energy requirements depend on the stage of the reproductive cycle and whether individuals conceive or not. Pekins *et al.* (1990) observed a 1.6 fold peak difference in the BMR of pregnant compared to non-pregnant white-tailed does. In red deer hinds the energetic cost of lactation is estimated at 2.6 times the maintenance requirements of non-lactating animals (Arman *et al.*, 1974). In both sexes peak energy requirements are synchronised with peak seasonal food availability

Appetite suppression, irrespective of energy expenditure, occurs in both sexes as a result of mating behavior, although its effects are greatest in the male. Time spent grazing by stags drops from a pre-rut peak of 44%, to 5% of the day (Clutton-Brock *et al.*, 1982a). As a result males can lose about 14-17% of their carcase weight (Mitchell *et al.*, 1976). The extent of weight loss depends on length and intensity of rutting activity which is influenced by factors like dominance status and ultimately testosterone levels (Fennessy *et al.*, in press). In hinds, feeding activity is significantly reduced during the duration of oestrus and may be affected by interference from the hareem-holding male (Clutton-Brock *et al.*, 1982a). This, however, is relatively insignificant compared to the impact of rutting behaviour on the male.

To summarise, the seasonal appetite cycle appears to reflect a combination of changing metabolic rate (due to the annual pattern of growth, reproduction, pelage and possibly foraging activity), and the additional effect of appetite suppression during mid-winter (and additionally during the rut in stags).

1.2.8.1. Endocrine control of metabolism

Steroid dependent effects

In females the low VFI associated with behavioural oestrus is attributed to the high levels of oestrogens during this period (sheep: Forbes, 1972). In males, castration (red deer: Kay, 1979) or autoimmunisation against GnRH (sheep: Argo, 1986) results in a reduction in circulating testosterone concentrations and leads to a

significant reduction in the amplitude of the seasonal cycle of VFI. If food intake is corrected for metabolic body weight (Kleiber, 1961) differences between intact and castrate males disappear, indicating that the effect of steroids on appetite is a consequence of their influence on growth (Argo, 1986).

Steroid independent effects

The persistence of a cycle of food intake in castrates demonstrates that the role of steroids is to modify the amplitude rather than generate the appetite cycle. Similar observations have been made in photoperiodic rodents (Syrian hamster: Bartness and Wade, 1984; Djungarian hamster: Wade and Bartness, 1984). For example, ovarectomized Syrian hamsters exhibited changes in body weight and energy metabolism in response to a short-day photoperiod that were 80-90% of those in gonadally intact animals (Bartness and Wade, 1984). Thyroid hormones may be important in the expression of steroid independent changes in metabolism. However, although thyroidectomy reduces liveweight gains in summer, as with gonadal steroids the rhythm persists in their absence (Shi and Barrell, 1990). As exogenous growth hormone (GH) and prolactin are both able to influence growth (see Bauman *et al.*, 1982) these hormones may be involved in the control of appetite.

1.2.9 DEVELOPMENT OF THE SEASONAL RESPONSE

If transferred to summer solstical photoperiods at the first winter solstice of life, female red deer exhibit an advance in the timing of the subsequent rise of plasma prolactin concentrations and food intake during spring (Loudon and Brinklow, 1990). Thus, by at least six months of age red are able to respond to photoperiod changes. Evidence from other mammals, however, suggests photoperiod can influence seasonal changes from much earlier in life. Circadian melatonin rhythms have been demonstrated in foetal lambs (Yellon and Longo, 1988). These respond appropriately to photoperiodic manipulation and are abolished by maternal pinealectomy, implicating a maternal origin (Yellon and Longo, 1988). Evidence from montane voles (*Microtus montanus*) indicates this signal can influence the

post-natal response to photoperiod. This was demonstrated by exposing voles to either long (16L:8D) or short (8L:16D) daylengths during gestation and then exposing them to the same intermediate photoperiod (14L:10D) during lactation. To confirm photoperiodic information was not transferred during this period some voles were raised by foster mothers that had experienced a different photoperiod during pregnancy (Horton, 1985). Although there were no differences at weaning, resulting body and testicular size at 74 days old were lowest for animals gestated under long days (whether raised by own or foster mother). This was consistent with previous observations of the effect a transfer from a 16L:8D to 14L:10D photoperiod on growth and development indicating that gestational photoperiod influences the responses of voles later in life. Gestational photoperiod signals may be important in ensuring that the animal's development proceeds appropriately under the environmental conditions at and immediately after birth. This could be important as evidence in sheep suggests melatonin rhythms in ruminants, do not become established until 3-4 weeks after birth (Nowak *et al.*, 1990). Thus, implying they may be unable to determine daylength changes during this period.

1.2.10. EVIDENCE FOR THE CONTROL OF SEASONALITY BY CIRCANNUAL RHYTHMS

The evidence discussed in previous sections suggests that the timing of seasonal changes in the ewe, and possibly female deer hind, are primarily controlled by the loss of sensitivity to either initially inductive or inhibitory photoperiods, resulting from previous exposure to the same or a similar stimulus. This hypothesis, however, cannot easily explain certain observations. The onset of reproductive activity in ewes held on winter solstice photoperiods from this solstice, was delayed by one month the following autumn but not prevented (O'Callaghan *et al.*, 1990). The ewes had not experienced long photoperiods, and therefore it seems improbable that refractoriness to an inhibitory long-day signal could have been the stimulus initiating reproductive activity. A similar argument applies for the onset of seasonal anoestrus. If

this is initiated by refractoriness to an inductive short-day photoperiod (see section 1.2.5.2.) blocking interpretation of the seasonal decline in daylengths by pinealectomy at the summer solstice should prevent anoestrus occuring. In fact not only does anoestrus still occur but the duration of the breeding season is substantially reduced by such treatment (Wayne *et al.*, 1988; O'Callagher *et al.*, 1989). While inconsistent with the hypothesis that simple photorefractory responses control seasonal changes, these observations are compatible with three alternatives. Persistent cyclicity could have resulted from uncontrolled environmental variables (e.g. temperature) acting as proximate factors in the absence of appropriate photoperiod changes (see section 1.2.1.2.). Secondly, as the studies described above were for less than a year in duration, responses could reflect a sequence of events initiated by a photoperiodic signal which continued for a full annual cycle in the absence of further input. Finally, seasonal changes may have been the consequence of an endogenous annual rhythm (circannual rhythm). In this case the role of the photoperiod could be simply to entrain the rhythm to environmental periodicity, rather than to directly control seasonal events. In the next section evidence for the existance of circannual rhythms in ungulates is reviewed.

To convincingly demonstrate the presence of circannual rhythms it is necessary to show persistence of rhythmicity for at least two cycles under constant environmental conditions. This indicates that annual cycles are not simply the result of a sequence of events initiated by an environmental signal experienced by the animal prior to exposure to constant conditions (Gwinner, 1981b).

The best evidence of circannual rhythmicity in a mammal comes from studies on the Golden-mantled ground squirrel (*Spermophilus lateralis*). Food intake, body weight and hibernation have been demonstrated to persist under constant photoperiod and temperature for 3-4 annual cycles with a free-running period of between 229-445 days (Pengelly and Fisher, 1957, 1963; Pengelley and Amundson, 1974). It is significant that the squirrel's subjective year deviated from an environmental year (i.e. 365 days) and that the period varied between individuals (resulting in loss of sychrony

between animals). This suggests that the observed rhythm was not the consequence of uncontrolled exogenous factors.

Evidence that ungulates exhibit circannual rhythms is limited. This is largely due to the technical problems associated with maintaining large mammals under constant environmental conditions for a sufficient period to demonstrate persistence of annual cycles. Most studies have been of short duration, usually of less than 2 years (exceptions: 2.5 yrs, Ducker *et al.*, 1973; 3 yrs, Jackson *et al.*, 1990; 4 yrs, Lincoln *et al.*, 1989; 5 yrs, Karsch *et al.*, 1989). Another short-coming has been the failure to control exogenous factors that could act as proximate factors. The majority of studies have concentrated solely on providing a constant photoperiod and ample food. Under these conditions persistent rhythms have been demonstrated in the growth and shedding of antlers by sika deer (*Cervus nippon*, Goss, 1969a,b) and in reproductive activity of ewes (Ducker *et al.*, 1973; Karsch *et al.*, 1989). Similar persistence of annual cycles has been observed in studies where the animal's perception of the photoperiodic signal has been blocked by surgically removing or denervating (by superior cervical ganglionectomy) the pineal gland resulting in the loss of the daily plasma melatonin profile. Although this has a disruptive effect on seasonal changes (e.g. ewes pinealectomised in spring commenced oestrous cycling 2.5 months later than intact animals, Wayne *et al.*, 1989) ewes subsequently exhibited circannual rhythmicity with a similar pattern to unoperated controls. Such persistence has been demonstrated in antler (red deer: Lincoln, 1985) and also pelage cycles (white-tailed deer, Brown *et al.*, 1978; Plotka *et al.*, 1982), food intake (deer, Brown *et al.*, 1978; sheep, Suttie *et al.*, 1983/4) and reproductive status (deer, Plotka *et al.*, 1982; sheep, Robinson and Karsch, 1985; Woodfill *et al.*, 1990).

Although the above evidence suggests the existence of circannual rhythmicity, none of the studies listed maintained animals under constant environmental temperatures. Despite the limited evidence that temperature has a role as a proximate factor, in the absence of photoperiodic information it remains possible that deer are able to utilise temperature to control long-term cyclicity. In addition, a number of the studies failed to isolate

pinealectomized individuals from intact animals (e.g. Plotka *et al.*, 1982) or used males receiving natural photoperiods to assess reproductive status (e.g. Ducker *et al.*, 1973). Evidence, discussed later in this review, indicates that social contact can transmit photoperiodic information. Whilst these failings raise doubt over claims for circannual rhythmicity, there is indirect evidence suggesting that the uncontrolled exogenous factors were not important. Karsch *et al.* (1989), for example, demonstrated unambigous annual cycles of LH and PRL over 5 years in ewes kept on a constant short day (8L:16D). LH cycle periodicity not only differed from 365 days (in contrast to controls under normal photoperiods) but also showed desynchronisation between individuals housed together and with respect to the control ewes. Similar cycle desynchronisation between individuals was observed in some pinealectomy studies (deer, Plotka *et al.*, 1982; sheep, Woodfill *et al.*, 1990). These observations imply that cyclicity was not induced by uncontrolled environmental factors, but was the result of an endogenous rhythm.

Further support for the existence of circannual rhythmicity is provided by a sub-tropical deer species. Kept in a temperate zone environment (52°N) axis deer stags (*Axis axis*) exhibited antler cycles of approximately 13 months (Loudon and Curlewis, 1988). These were associated with cycles of testis size, testosterone secretion, neck girth and body weight displaying a consistent phase relationship. Significantly the cycles of individual males were not synchronized to one another. In addition, melatonin implants administered during summer to stags with growing antlers, failed to advance antler cleaning or testosterone secretion, in marked contrast to red deer treated under similar circumstances (Lincoln *et al.*, 1984). Taken together these observations suggest that photoperiod has no significant role in the control of reproduction of this sub-tropical species, and that annual cycles are generated by free running circannual rhythms. It suggests that cycles observed in seasonal deer species may therefore reflect endogenous rhythms that are entrained rather than driven by photoperiod. However, some caution is necessary when using evidence of male reproductive rhythms to support circannual rhythmicity in the female. Males need

to shed their antlers periodically in order to grow a new antler. Since the antler cycle is driven by testosterone, endogenous reproductive cyclicity in male deer may simply reflect the need to maintain control over this cycle.

The strongest evidence against circannual rhythmicity has been provided by Jackson *et al.* (1990). In this study ewes were maintained under a constant equatorial photoperiod (12L:12D), constant temperature, and isolated from other sheep (including rams). As a result only 2/12 ewes exhibited distinct, alternating periods of anoestrus and ovarian cyclicity. In the remainder, oestrous cycles occurred throughout the year at variable intervals, rather than in distinct breeding seasons. In addition there was no evidence of a consistent photorefractory response between individuals. These results imply there is no endogenous circannual organisation (at least in reproductive activity) and that seasonal changes are driven directly by environmental changes. It also suggests rhythmicity reported in previous studies may have been due to temperature changes. These conclusions are supported by evidence from rams of a variety of cross-breeds kept under constant short-day lengths and temperatures (Langford *et al.*, 1987). Over two years these animals maintained a high level of testicular activity with no indication of the spontaneous regression demonstrated by Almeida and Lincoln (1984), for rams kept on short-day lengths but under environmental temperatures.

In red deer, the principal evidence against the existence of circannual rhythmicity remains the failure of hinds to exhibit seasonal anoestrus when treated with an exogenous short-day melatonin signal from mid-winter for 16 months (Adam *et al.*, 1989). This contrasts with the response of ewes (Nett and Niswender, 1982) and Pere David's deer hinds (C.M. Argo, unpublished observations) in which the breeding season was only extended by a few weeks by melatonin treatment (see section 1.2.5.2.). Whilst the response of ewes and Pere David's deer are consistent with the involvement of an endogenous mechanism, the evidence in the red deer hind suggests direct photoperiodic control. Similarly, pre-pubertal red deer hinds treated with melatonin implants from the winter solstice showed disrupted seasonal changes in prolactin and food intake (Loudon and

Brinklow, 1990). However, in the same study, calves maintained on constant long days from birth appeared to exhibit normal cyclicity of food intake, coat growth and prolactin (Loudon and Brinklow, 1990). This difference between the response to constant long-days or melatonin implants (≃ short-day signal?) suggests expression of annual cyclicity in this species can be profoundly influenced by exposure to different constant 'photoperiod' signals. It raises the possibility that circannual oscillations exist, but unlike circadian rhythmicity, can be relatively easily disrupted or masked by particular environmental conditions. If true, variation between studies may be the result of differences in the experimental regimes under which rhythmicity was investigated. It is apparent from a number of studies that the response to different constant photoperiods varies. In the studies of sika deer by Goss *et al.* (1974), for example, stags exposed to photoperiods deviating by 1 or more hours from 12 hours light/day showed annual patterns of antler growth and shedding, but those maintained on 12L:12D failed to shed their antlers. This response is consistent with the difference observed between the reproductive activity of ewes kept at 8L:16L (Karsch *et al., 1987,* 1989) and 12L:12D (Jackson *et al.,* 1990) discussed above. Jackson *et al.* (1990) proposed that there could be a selective advantage for the disruptive affect of equatorial photoperiods on endogenous rhythmicity. This would enable ungulates in such an environment to breed at any time of year in response to proximate factors like food availability. How exactly such a system might have evolved is not clear.

If circannual rhythms exist, it is logical to propose that the linkage between seasonal cycles reviewed previously (see section 1.2.6.) may reflect a single neural oscillator controlling all endogenous annual rhythms. Karsch *et al.* (1989) reported that in ewes maintained under short photoperiods for 5 years plasma LH, but not plasma prolactin, cycles became desynchronised between individuals. This implies that, whilst LH may have required photoperiod to entrain seasonality, prolactin was synchronised by an alternative environmental variable (possibly temperature) suggesting the existence of separate circannual oscillators. In general, however, the temporal relationships of plasma prolactin and LH

cycles remained 180 degress out of phase with each other despite the fact the cycles are believed to be functionally independent. Thus, using the endocrine system as a marker of internal physiological state it is clear that circanual rhythms may be driven by two or more linked oscillators.

The selective advantage of endogenous circannual rhythmicity in mammals is unclear (Gwinner,1981a,b). Gwinner (1981b) suggests rhythms may improve precision of timing of seasonal activities by providing inertia that buffers against atypical fluctuations in the physical environment. It is possible circannual rhythmicity was inherited from tropical mammals in which had it evolved as a means of maintaining the correct phase relationship between dependent biological processes in an environment providing relatively unreliable external cues.

1.2.11. THE ROLE OF OTHER EXOGENOUS FACTORS IN THE CONTROL OF SEASONALITY

There is considerable evidence that photoperiod provides the principal zeitgeber (entraining signal) responsible for synchronising seasonality with environmental periodicity in deer. However, there is evidence that other exogenous factors are involved in modifying the expression of seasonal changes. The possible role of temperature, social factors and nutrition are discussed below.

1.2.11.1. TEMPERATURE

Fluctuations in environmental temperatures do not in themselves drive seasonal changes in reproductive activity (Wodzicka-Tomaszewska, *et al.*, 1967 - discussed in section 1.2.1.1.). However, there is evidence in the ewe that temperature may modify the rate of response to a changing photoperiod. The response intervals of ewes to a shift from an inductive (8L:16D) to an inhibitory (16L:8D) photoregime (i.e. during transition to anoestrus) exhibited a strong negative correlation with external temperatures. There was, however,

no evidence that temperature influenced the onset of reproductive activity (Legan and Karsch, 1980; Jackson *et al.*, 1989).

In domestic cattle maintained under natural or 16L:8D photoperiods high temperatures stimulated and low temperatures inhibited the circulating levels of prolactin (Peters and Tucker, 1978). Evidence that this hormone may be important in the control of seasonal pelage changes (see section 1.2.7.) suggests that environmental temperature may affect coat growth via prolactin.

The most obvious effect of environmental temperature on seasonality stems from its impact on maintenance energy requirements. Despite a relatively thick winter coat (Ryder and Kay, 1973; Ryder, 1977) the temperature at which it becomes necessary for red deer to raise their metabolic rate to maintain core body temperature (the lower critical temperature) has been reported to be as high as 10°C (Brockway and Maloiy, 1968). This is a relatively high value compared to environmental temperatures experienced in the British Isles, indicating that energy requirements for maintenance will be closely related to external temperatures. This is confirmed by evidence that maintenance requirements of stags kept out-doors in winter were 50% greater than those enhoused (Fennessy *et al.*, 1981). It seems likely that under natural conditions seasonal variation in temperature will profoundly modify expression of the seasonal metabolic changes demonstrated in enhoused deer and sheep (see section 1.2.8).

1.2.11.2. SOCIAL STIMULI

Red deer form loose associations with other individuals throughout the year. Except for the rut, mature males and females normally remain segregated. Group sizes vary but female herd (including calves) can exceed 100, especially in open habitats during winter (Clutton-Brock *et al.*, 1982a; personal observation). This provides extensive opportunity for the action of social cues.

Experimental studies with sheep have revealed that photoperiodic information can be transmitted through social contact. Legan and Karsch (1980) observed that changes in the reproductive activity of surgically blinded ewes remained synchronous with 90 day

alternations of photoperiod (16L:8D to 8L:16D) only if maintained in contact with a sighted ram. Ewes pinealectomised at the spring equinox and maintained in isolation of pineal-intact sheep exhibited a 2.5 month delay in the onset of reproductive activity. In contrast, those kept in contact with intact animals (females and males) did not (Wayne *et al.*, 1989). There is also evidence of intra-sex transmission in males (Lincoln *et al.*, 1989) and females (Sunderland *et al.*, 1990). Ewes maintained on winter solstice photoperiods, from the solstice, but in contact with ewes which received 35 long days during April exhibited a smaller delay in the onset of reproductive activity and greater within group synchrony than a group maintained in isolation throughout (Sunderland *et al.*, 1990). In spite of this evidence, under normal conditions it seems unlikely that social cues would have a significant role in relaying photoperiodic information. Instead the clear effects demonstrated, suggest social factors may have an important role in synchronising the phase of seasonality amongst associating individuals.

Intra-sex synchrony

Free ranging red deer hinds associating during the rut tend to show greater synchrony of oestrus (and therefore calving) than non-associates (Iason and Guinness, 1985). Similar observations have also been reported in sheep (Wayne *et al.*, 1989; Jackson *et al.*, 1990; Sunderland *et al.*, 1990). The evolutionary function of this is probably to synchronise calving to reduce individual predation risks.

Inter-sex synchrony

The reproductive success is clearly dependent on synchrony of reproductive status between males and females. In seasonal mammals photoperiod entrains the reproductive rhythm of both sexes ensuring this coincides with the appropriate season. This implies synchrony will occur in the absence of social stimuli, as all animals in the population receive the same photoperiodic information. Social cues, however, may be important in the 'fine tuning' of synchrony, particularly in overcoming the effects of varying nutrition and/or lactational status between individuals.

Male vocalisation as well as presence of males can advance ovulation (McComb, 1987; Wilson, 1990) and winter coat growth in hinds (M.W. Fisher, *unpublished data*). In sheep the presence of a male has also been shown to extend the duration of the breeding season (Sunderland *et al.*, 1990). Females are equally capable of influencing males. Red deer hinds given exogenous melatonin to advance time of first oestrus significantly advanced roaring activity of non-treated males (M.W. Fisher, *unpublished data*). In sheep the introduction of ewes can cause an acute increase in secretion of LH and testosterone in rams (Illius *et al.*, 1976).

Social effects on puberty

There is also circumstantial evidence that social stimuli are important in influencing the timing of puberty in red deer. Pre-pubertal hinds maintained on an *ad libitum* diet, but isolated from stags or mature females, showed delayed first oestrus (compared to the latter). They also exhibited a relatively low incidence of puberty (3/7) despite high live-weights (Loudon and Brinklow, 1990 and *unpublished observations*).

In stags a different social effect has been reported. Juvenile animals maintained on an *ad libitum* diet in isolation from mature stags displayed rutting behaviour during their second autumn. When kept in contact with adults, however, rutting behaviour was suppressed (Fennessey, Thompson and Suttie, *in press*). This indicates mature males may delay expression of physiological maturity in younger and presumably subordinate males.

1.2.11.3. NUTRITIONAL FACTORS

Food availability is the major ultimate factor responsible for the evolution of seasonality in temperate habitats. Thus in theory, if the seasonal responses are well adapted, nutritional requirements and availability should coincide. Plant production, although exhibiting a marked seasonality, does however show considerable annual and local variation. This is due both to climatic fluctuations and biological factors like competition. As a result food availability may exert a direct effect on expression of

seasonality and reproductive success, especially in over-populated or marginal habitats (e.g. typical of Scottish red deer populations in the 1980's). The most obvious impact of limited food resources will be on expression of the appetite cycle and on growth. Stag calves held on a restricted plane of nutrition during winter showed compensatory growth during the following summer associated with a signficant elevation of food intake (Suttie *et al.*, 1983). Even with access to *ad libitum* food, however, the winter- restricted stags failed to achieve the mature live weights of unrestricted animals. It suggests that under natural conditions fluctuations in food abundance can have major long term effects on body size. This may explain the relatively small size of wild Scottish red deer compared to English and European counterparts (Kay and Staines, 1981; Suttie *et al.*, 1983). Details of the effects of food abundance on intake and grazing strategies are reviewed in section (1.2.12).

There is also evidence that reproductive activity in red deer is influenced by the effects of food abundance. Hind fertility is strongly correlated to live weight (Hamilton and Blaxter, 1980; Albon *et al.*, 1983, 1986). For example, Hamilton and Blaxter (1980) reported that farmed red deer in eastern Scotland fail to calve if their live weight is less than 52 kg, but at 60 and 80 kg the probability of calving increased to 0.49 and 0.91 respectively. In ewes, restricted food intake and low body condition were associated with a delay in the onset of the breeding season (Gunn and Doney, 1975) and reduced ovulation rate (Gunn and Doney, 1975; Doney *et al.*, 1981; Rhind and McNeilly, 1986). Delays in conception can have a profound affect on hind reproductive success. For every day after the median birth date a calf is born there is a 1% increase in mortality (Clutton-Brock *et al.*, 1987) and a 1% reduction in the female's probability of being fertile the next year (Clutton-Brock *et al.*, 1983).

The mechanism by which nutrition influences fertility is still unclear. Associated with poor nutritional status is evidence of a reduced LH pulse frequency (Rhind *et al.*, 1989; Thomas *et al.*, 1989) and lower mean plasma levels of this and FSH (Thomas *et al.*, 1989; Tatman *et al.*, 1990). Tatman *et al.* (1990) also observed higher

concentrations of GnRH in the median stalk eminence of poorly nourished ewes. From this they concluded that GnRH release may have been reduced in these animals, which could explain the lower LH pulse frequency and mean plasma levels. A sufficient reduction in LH pulse frequency could result in delayed or suppressed reproductive activity. The relation between live weight and fertility, and the fact that body fat comprises the major component of weight loss, suggests nutrition could influence the LH secretory process via its effect on fat reserves. In both ewes (Tatman *et al.*, 1990) and humans (Frisch, 1988) there appears to be a threshold amount or concentration of fat required for reproduction to occur.

The nutritional requirements of lactating hinds fed to appetite have been estimated to be as much as 2.6 times that of non-lactating animals (Arman *et al.*, 1974). Considering the relation between body condition and fertility discussed above, it is not suprising that in marginal habitats the fertility of lactating hinds is lower than that of non-lactating females (Guinness *et al.*, 1978). There is also evidence that the duration of seasonal anoestrus is extended in lactating hinds (10 days, Adam *et al.*, 1985). Increased energetic requirements might affect reproduction by reducing fat reserves. There is, however, evidence to suggest in lactating animals nutritional effects may in part be relayed via the offspring. Loudon *et al.* (1983) observed that suckling frequency was highest in red deer calves whose mothers grazed an impoverished pasture and produced relatively little milk. Increased suckling activity appears to be the result of an attempt by these calves to obtain an adequate milk intake to maintain growth at its maximum potential (Loudon *et al.*, 1983). Associated with this was a 6.2 day delay in the timing of conception. The mechanisms by which suckling activity might influence reproductive activity are discussed in section 1.2.5.3 'prolactin and reproduction').

Nutrition also has a role in determining the timing of puberty. The relatively limited food abundance of many Scottish habitats is believed to explain why wild red deer hinds often conceive for the first time 1 or 2 years later than in captive and eastern European populations (Kay and Staines, 1981; Clutton-Brock *et al.*, 1982a;

Loudon *et al.*, 1989). Nutrition is believed to exert its influence primarily by affecting growth rates and body size. Evidence in the ewe suggests growth related cues are used to regulate the activity of the GnRH pulse generator. Only when these are appropriate does its sensitivity to the inhibitory feedback action of oestradiol decrease. This results in an increase in LH pulse frequency, ovarian development and subsequent ovulation (Foster and Ryan, 1981; Foster *et al.*, 1985). Although nutrition influences timing of puberty, the latter can only occur during the appropriate season. This is determined by the endogenous reproductive rhythm and the entraining effects of photoperiod (Foster *et al.*, 1985). Evidence indicates that poor nutrition cannot prevent ewe lambs accumulating photoperiodic information. For example, when undernourished ewes previously exposed to either long- or short-day photoperiods were placed on inductive short days (with *ad libitum* food) only those experiencing a decline in daylength exhibited subsequent reproductive activity (Foster and Yellon, 1985). This indicates that growth-retarded lambs were able to differentiate between photoperiods. The major effect of nutrition on puberty is, therefore, to determine whether or not animals are in a sufficiently developed state to attain sexual maturity in a particular breeding season.

1.2.12. CONTROL OF FOOD INTAKE AND GRAZING BEHAVIOUR

The dietary niche of red deer has been described as intermediate between grazers and browsers (Hofmann, 1973) with considerable flexibility in diet choice (for review see: Mitchell *et al.*, 1977; Kay and Staines, 1981; Clutton-Brock *et al.*, 1982a). In common with other herbivores living in temperate habitats, red deer experience pronounced seasonal changes in the quality and availability of food as well as in their own nutritional requirements. This raises the question as to the extent to which the grazing ecology of these animals is determined by prevailing food availability (extrinsic factor) or endogenous seasonal appetite state (intrinsic factor). This part of the review will outline current evidence for the role of each and conclude with a discussion

of their relative importance in determining the grazing ecology of red deer. There have been few detailed investigations of the effect of herbage availability on grazing behaviour in deer, and thus where necessary evidence from domestic sheep will be reviewed. Most of these studies have used agricultural pastures which are considerably more uniform than the plant communities grazed by wild ungulates. The advantage of these simple grazing systems is that it is easier to quantify the effects of herbage charactersitics on behaviour. The information obtained can then be used to assist interpretation of grazing strategies in wild animals.

1.2.12.1. ROLE OF PREVAILING FOOD AVAILABILITY

The main components of food availability: herbage mass; nutrient content; digestibility; water content; presence of metabolites that depress intake; botanical composition; palatability; plant spacing, and plant height and accessibility (Allden and Whittaker, 1970) vary not only between communities but also with season.

If prevailing food abundance determines food intake and grazing behaviour, it is predicted that level of intake should in general terms, reflect the annual pattern of production. In addition, grazing strategies should be closely associated with the relative abundance, nutritional value and physical characteristics of the food species. The evidence for this is discussed in relation to different aspects of grazing behaviour.

Rate of intake (mass of herbage consumed/min).

This can be regarded as a function of *bite size* and *bite rate*. Little detailed work has been undertaken on the effect of food availability on intake rates in red deer or any other cervids regarded as intermediate feeders. Most workers have relied on indirect estimation from the effect of pasture characteristics on grazing times (Clutton-Brock *et al.*, 1982a) or based conclusions on the extensive literature for domestic sheep (Clutton-Brock *et al.*, 1982a; Loudon *et al.*, 1984).

The work of Trudell and White (1981) on reindeer (*Rangifer tarandus*) is an exception. These authors demonstrated that the rate of intake (mass ingested per min.) increased with plant biomass for all types of herbage (except forbs which had a high intake rate even when scarce). This observation is in general agreement with other studies of reindeer (Skogland, 1984), wapiti (*Cervus elaphus nelsonii*, Collins *et al.*, 1978) and sheep (Allden and Whittaker, 1970; Black and Kenney, 1984; Forbes and Hodgson, 1985). No correlation was observed between bite mass and either total or selected plant biomass. This contrasts with evidence in sheep, where bite mass appears to be the primary determinant of intake rate (Hodgson and Milne, 1978; Jamieson and Hodgson, 1979). The difference may reflect the dietary preferences of reindeer and differences in the plant diversity of pastures studied. If a reindeer grazes selectively on a diverse pasture, then as biomass of prefered forage increases, it is possible that search time between bites will decline and bite rate increase. Thus, rate of intake may more closely reflect bite rate rather than bite mass. This is supported by evidence of positive correlation between bite rate and both increases in biomass of selected plant parts and their intake. In contrast, in sheep grazing a relatively low diversity and structurally uniform sown pasture the influence of plant selection on bite rate will be comparatively small. In fact evidence indicates that on these pastures bite rate declines as biomass increases (Allden and Whittaker, 1970; Jamieson and Hodgson, 1979; Black and Kenny, 1984) due to increasing bite mass (see below). Thus, the positive correlation between intake rate and plant biomass primarily reflects changes in bite mass. On highly diverse hill pastures sheep also graze selectively (Milne *et al.*, 1979) further suggesting that the differences observed in grazing behaviour between sheep and reindeer at least partly reflect pasture differences.

Trudell and White (1981) also demonstrated that the botanical composition of selected plant communities can influence prehension patterns and consequently intake rate. A significant difference was observed in bite rate and bite mass between vascular plants and lichens (177 *vs* 205 bites/min. and 21 *vs* 32 mg/bite respectively). It is therefore likely that the relative proportion of time spent grazing

different communities has a significant effect on total intake rate. This was also suggested as a possible explanation of bite rate differences observed between red deer grazing indigenous hill and sown pastures (33 *vs* 56 bites/min: Loudon *et al.*, 1984), and for the longer grazing times of red deer selecting short grass rather than higher biomass communities (Clutton-Brock *et al.*, 1982a).

As already mentioned, grazing studies on sheep have demonstrated a positive correlation between rate of intake and herbage availability (Allden and Whittaker, 1970; Hodgson and Milne, 1978; Jamieson and Hodgson, 1979; Black and Kenney, 1984; Forbes and Hodgson, 1985). This relationship is summarised in Figure 1.2a. Initially unaffected by a decline in availability, a stage is reached when the rate of intake becomes progressively restricted. Allden and Whittaker's (1970) study concluded that the most important component of availability was sward height, there being little relationship between intake and herbage mass of dry matter per unit area. This they demonstrated by manipulating the spatial relationships of the sward with the purpose of disrupting the strong correlation normally observed between herbage mass per unit area and plant height. The association between the latter, and bite rate and bite mass is illustrated in Figure 1.3b. Briefly, the mass of a bite increased almost linearly with changing sward height, where as following a small increase the bite rate decreased. At lower sward heights the sheep were unable to compensate for reduced bite mass leading to a reduction in intake rate.

Since this study, more recent work has demonstrated a significant relationship between intake rate and herbage mass per unit area (Hodgson and Milne, 1978; Black and Kenney, 1984). With the exception of Black and Kenney (1984), however, few have attempted the difficult task of separating the influences of plant height and density. These authors concluded that the best prediction of intake rate was herbage mass per unit area covered by one bite. Since the rate of intake was related to sward height only when density was constant and to herbage mass only at similar sward heights (see Figure 1.3c). Bite mass, which declined with a reduction in both sward height and density, was also best described in this fashion.

Grazing time

Considerably more is known about the role of food availability in determining daily grazing budgets in deer. Generally, as food resources diminish and rate of intake declines, herbivores attempt to compensate by grazing longer (red deer: Clutton-Brock *et al.*, 1982a; Loudon *et al.*, 1984; reindeer: Trudell and White, 1981; Skogland, 1984; sheep: Allden and Whittaker, 1970; Hodgson and Milne, 1978 and Jamieson and Hodgson, 1979; Forbes and Hodgson, 1985).

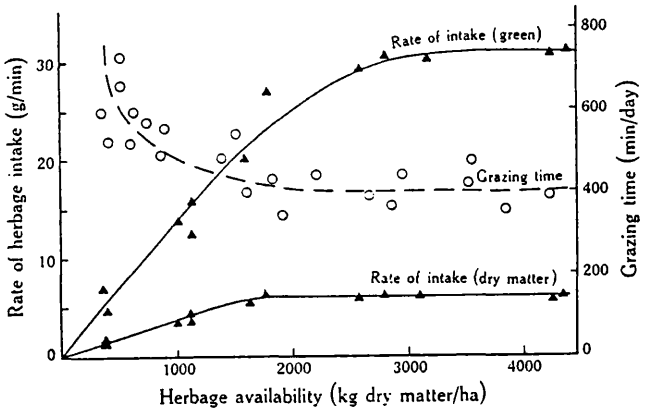
The long-term study of red deer on the Scottish Isle of Rhum (Clutton-Brock *et al.*, 1982a) revealed grazing times for mature stags of 10.40 h/day in summer compared to 12.88 h/day in winter. This was attributed to variation in selected plant biomass. This suggestion is inconclusive as differences could have resulted from appetite changes. There is some support for such an explanation from evidence that grazing times were also significantly longer on short grass pastures, with a low biomass, than other plant communities. More controlled studies with sheep have confirmed that available plant biomass strongly influences grazing times. Allden and Whittaker (1970) showed that grazing times ranged between 6 - 13 h/day depending on food available (see Figure 1.2a).

Species composition of swards has also been shown to influence grazing time. Lactating red deer grazing an indigenous pasture with high plant diversity grazed an average of 11.7 h/day whereas similar hinds on a sown pasture spent only 6.0 h/day grazing (Loudon *et al.*, 1984). This difference is probably the consequence of selection for preferred plant parts increasing search time per bite, and/or more lengthy manipulative jaw movements when tackling the non-uniform sward structure of indigenous pastures. Prehension patterns have also been found to differ greatly between plant types in reindeer (Trudell and White, 1981).

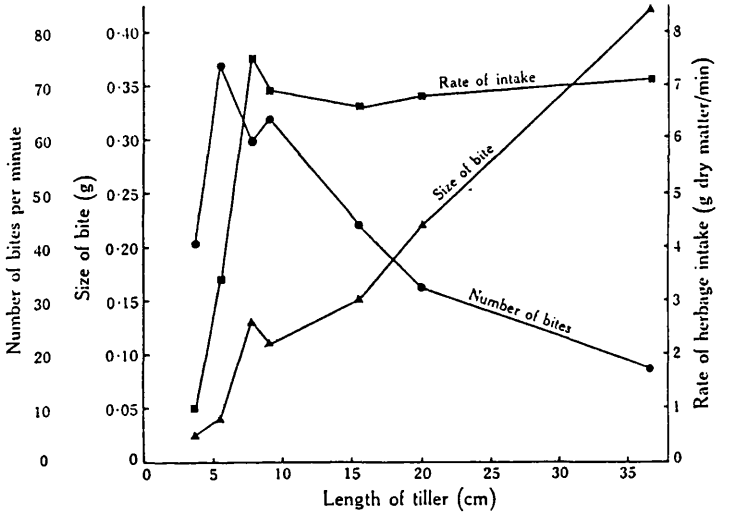
Another aspect of the relationship between availability and grazing time has been reported in sheep studies (where accurate estimates of food intake were possible). It appears that increases in grazing time can only compensate for a reduced intake rate (as availability declines) up to a certain point, and that beyond this

FIGURE 1.3:

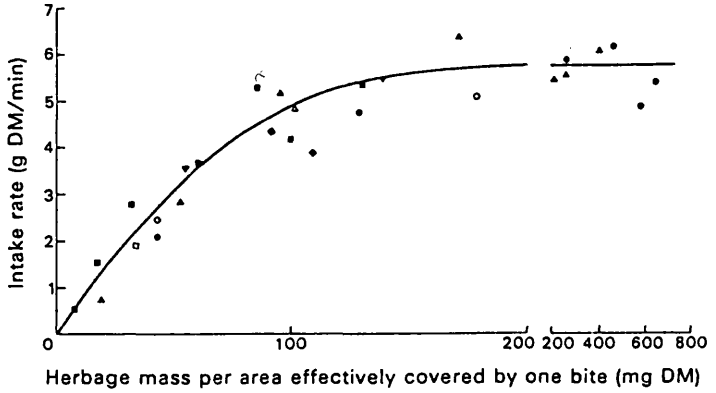
(a) Relation of rate of intake of both dry and green herbage (▲) and of grazing time (O) to herbage mass. - from Allden and Whittaker, 1970.



(b) Relation of rate of herbage intake, rate of biting, and size of bite to length of tiller. - from Allden and Whittaker, 1970.



(c) Relation of rate of intake and herbage mass per area effectively covered by one bite. - from Black and Kenney, 1984.



daily intake falls (Allden and Whittaker, 1970; Arnold, 1975; Hodgson and Milne, 1978; Jamieson and Hodgson, 1979). Whether this is also the case in deer has yet to be established as no accurate estimates of intake in grazing deer exist.

A critical factor preventing full compensation is an apparent ceiling limit to daily grazing time imposed by the need to ruminate. Evidence suggests this limit is approximately 12 h/day. Maximum observed grazing times include, for red deer: stags 12.88 h/day, hinds 11.76 h/day (Clutton-Brock *et al.*, 1982a), 11.70 h/day (Loudon *et al.*, 1984); for sheep: 11.2 h/day (Arnold and Dudzinski, 1967), 13 h/day (Allden and Whittaker, 1970); for wild reindeer: 12 h/day (Skogland, 1984) and tame reindeer: 12.72 h/day (Trudell and White, 1981).

Overall intake

Herbage intake is the product of intake rate and daily grazing time. These are both influenced by herbage availability and as a result there are significant differences in intake for herbivores (sheep) grazing either similar pastures with different mass/sward height (Arnold, 1975), or different species composition (Doney *et al.*, 1981). There are, however, no accurate estimates of intake in free ranging deer to demonstrate whether intake follows the seasonal pattern of plant production, predicted if food resources determine grazing activity. Indirect evidence (e.g. body weight: Blaxter *et al.*, 1974; Mitchell *et al.*, 1976) suggest this is the case but do not rule out the possibility that an appetite cycle is important.

Diet selection

Diets are selected as the result of the interaction between:
(a) Energy and nutritional requirements of the animal.
(b) Animal limitations (due to the prehensive and digestive capabilities: Gordon and Illius, 1988; Gordon, 1989).
(c) Availability of food plants.
(modified from Kossak, 1976 and Grant *et al.*, 1985).

In herbivores the preferred diet is usually that with the

highest nitrogen and carbohydrate, and least fibre, consistent with mineral requirements (red deer: Kay and Staines, 1981; Clutton-Brock *et al.* 1982a; reindeer: Skogland, 1984; moose: Belovsky, 1978). Diet choice should therefore be strongly influenced by seasonal variation in herbage availability. This prediction has been confirmed by various studies and, as a general rule, the range of food types eaten (both species and plant parts) is inversely related to the availability of preferred forage.

Belovsky (1981) found it was possible to predict with reasonable accuracy the diet composition of moose (*Alces alces*). This was based on nutrient content of plants, the size of food items, and their relative abundance. Density and quality were also found the best criteria to predict diets in wild reindeer (Skogland, 1984). Similar relationships between plant availability and selection have been observed in red deer (van de Veen, 1979). Kay and Staines (1981) stated that the "seasonal sequence of grazing on hill pastures (by red deer) generally relates to plant digestibility which in turn depends on the times a particular species starts to grow, mature, etc." For example, *Molinia caerulea*, normally rejected may be eaten during its rapid growth phase in spring and early summer by deer on Rhum (Clutton-Brock *et al.*, 1982a). Similarly utilisation of *Agrostis/Festuca* swards (highest proportion of digestible dry matter and available protein in all months) depended on their biomass. When this fell an increasing proportion of time was spent grazing other communities (e.g. *Calluna-dominated*) which although of lower nutritional quality were present in greater abundance.

Experimental studies investigating sheep forage selection support these observations. Milne and colleagues (1979) demonstrated that sheep grazing heather selected a progressively poorer quality diet as the severity of defoliation increased. Initially grazing shoot tips (highest nitrogen and least cell wall content) and then regrazing, ingesting a higher proportion of less nutritious woody shoots. Black and Kenney (1984) investigated the effect of availability, in terms of spatial distribution and sward height, on selection. They found that diet preference was strongly influenced by the rate at which forage can be eaten (i.e. when offered a choice

of two pastures with different sward characteristics they generally preferred the one they could eat faster). They also noted that sheep, at least, discriminated less when offered choices of forages with high intake rates than those with low rates.

1.2.12.2. ROLE OF SEASONAL APPETITE STATE

As discussed in earlier sections red deer exhibit pronounced seasonality in reproduction, pelage changes and numerous metabolic parameters. These seasonal strategies are adaptations to the variation in climate and food availability experienced in temperate habitats and one of their functions is to synchronise energy and nutrient requirements with availability (Kay, 1979; Kay and Staines, 1981; Clutton-Brock *et al.*, 1982a; Suttie *et al.*, 1983; Loudon and Kay, 1984). Clearly with such seasonal variation in requirements it would be suprising if there was not a significant influence of appetite state on grazing behaviour and intake (Clutton-Brock *et al.*, 1982a).

Rate of intake (mass of herbage consumed/min)

Virtually nothing is known about the effect of appetite state on rate of intake. Arnold (1975) studying grazing behaviour of four breeds of sheep in different reproductive states observed higher rates of intake in pregnant and lactating ewes than non-lactating animals on the same pasture. Similar differences between lactating and non-lactating ewes were obtained in an earlier study (Arnold and Dudzinski, 1967). Skogland (1984) noted similar but non-significant differences in reindeer.

None of these studies however investigated whether differences were due to changes in the bite mass and/or bite rate.

Grazing time

More convincing evidence of the influence of appetite comes from observations of red deer hinds on the Isle of Rhum (Clutton-Brock *et al.*, 1982a,b). During summer, lactating hinds grazed

significantly longer than non-lactating females, extending both their day and night-time activity budgets. Overall daily grazing times were 11.8 and 9.8 h/day respectively. This difference was attributed to the greater nutritional requirements of lactating hinds. This has been demonstrated in enhoused studies as being 2.6 times the maintenance requirements of non-lactating animals (Arman *et al.*,1974). By late winter differences in grazing times had disappeared (average for both, 11 h/day).

A similar relationship has been observed in sheep (Arnold and Dudzinski, 1967; Arnold, 1974). Grazing times of 11.2 and 9.9 h/day were observed for lactating and non-lactating ewes respectively (Arnold and Dudzinski, 1967).

In stags reproductive status has a profound impact on grazing time during the rut (Clutton-Brock *et al.*,1982a). Hareem holding males reduce the proportion of day time grazing activity from the summer average of 44% to just 5%. This is in sharp contrast to juveniles and non-hareem holders who show only a marginal reduction. This influence persists as long as a stag actively pursues hinds. Within 24 hours of relinquishing his hareem, grazing activity has returned to pre-rut levels.

Overall intake

Studies of enhoused deer fed *ad libitum* have demonstrated a clear seasonal pattern of appetite (see Figure 1.1.). The magnitude of changes, at least, under these conditions is profound. Fed a diet of *Agrostis/Festuca* and heather castrate males showed a 70% increase in VFI between January and April (Milne *et al.*, 1976). Evidence that appetite changes influence food intake in grazing animals, however, is sparse. Partly because of the difficulties of maintaining a constant quality and quantity of herbage over sufficient periods to show appetite changes. Adam *et al.* (1986) attributed a reduction in live weight of melatonin-treated hinds, compared to non-treated animals, during September to an early seasonal decline in appetite following a phase shift in the cycle. More convincing evidence comes from comparing animals in different reproductive states. In sheep, lactating ewes have been shown to have up to 30% higher intakes than

non-lactating individuals (Arnold and Dudzinski, 1967; Arnold, 1975; Doney *et al.*,1985). The effect of pregnancy is less clear, and in only one study was intake higher.

In stags the rut has a profound influence on food intake. Even in the absence of females and abundant food there is a significant reduction in intake of enhoused animals (Kay, 1979; Suttie *et al*,. 1983). Grazing behaviour (Clutton-Brock *et al.*, 1982a), body weight and condition losses (Mitchell *et al.*, 1976) indirectly suggest free ranging stags also experience a reduction in intake at this time, regardless of food availability.

Diet selection

The best evidence of appetite state influencing diet selection is provided by observations of grazing behaviour of red deer on the Isle of Rhum (Clutton-Brock *et al.*, 1982a,b). During both summer and winter lactating hinds spent a greater proportion of their daily grazing budget, compared to non-lactating animals, grazing the most strongly selected plant communities (i.e. short and herb rich greens). The herbage masses of these are low, but nutritional quality, in terms of nitrogen, carbohydrate and fibre content, is high. Two reasons are proposed to explain this behaviour. Firstly, the behaviour of lactating females may be associated with the requirements of calves for a high nitrogen/energy diet from herbage, and/or related to the demands of lactation on the hind. Non-lactating hinds in contrast, may benefit from greater utilisation of heather because of its larger standing crop (and associated higher intake rate) reducing grazing time, even if diet quality is less. Hinds were also observed making more use of higher quality mesotrophic communities than stags during winter (Gordon, 1989).

1.2.12.3. DISCUSSION

The grazing ecology of red deer is determined by the interaction of a variety of factors, not just food availability and appetite status. Also implicated are: the animal's physical limitations (e.g. digestive and prehensive: Freeland and Janzen,

1974; Gordon and Illius, 1988); climate (Mitchell *et al.*, 1976; Clutton-Brock *et al.*, 1982a); disturbance (Mitchell *et al.*, 1976; Gordon, 1989); social behaviour (Mitchell *et al.*, 1976) and age and sex (Clutton-Brock *et al.*, 1982a). Current evidence relating to the relative importance of two of the factors reviewed here is inconclusive. Herbage availability clearly modifies diet selection and grazing time, with indirect evidence from sheep suggesting it also influences rate and overall intake. Equally there is evidence indicating a significant role for appetite status, at least during certain periods of the year. In the stag, for example, the extent of rut inappetance precludes any signficant effect of herbage abundance on grazing behaviour. There have, however, been few comparative studies investigating the interaction of these factors in grazing ruminants.

The limited data available suggests the influence of appetite on grazing behaviour is, at least in certain circumstances, dependent on food availability.

The daily grazing activity of lactating red deer grazing a high quality rye grass/white clover sward declined as lactation progressed (8.5 h/day at day 44 and 5.6 h/day at day 100): Loudon *et al.*, 1984). Herbage availability was maintained at approximately the same level throughout the study and therefore this change probably reflects changing energy requirements. In contrast, hinds on an indigenous hill pasture not only grazed substantially longer (11.7 h/day) but did so at approximately the same level throughout the summer. The difference in grazing behaviour between hinds maintained on the the two pastures reflected differences in herbage availability. The long grazing times of hinds on the indigenous pasture are similar to the maximum lengths recorded for red deer and other ungulates (see earlier section). This suggests that these hinds grazed at or near their maximum potential. Taking account also of the lower bite rates, diet digestibilities and poorer lactational performance of hinds on the indigenous pasture it seems likely they were unable to satisfy their nutritional requirements at any time during the study. On the rye grass/white clover sward, food availability was apparently sufficient to enable hinds to achieve

intake requirements. Consequently as these fell a corresponding reduction in grazing time was observed.

A similar observation has been made in sheep (Doney *et al.*, 1981). Following deliberate experimental depression of body condition in early spring, the time taken for a difference in food intake to be expressed between lactating and non-lactating ewes was closely related to the level of herbage availability. After 3 weeks grazing improved reseeded pastures non-lactating animals exhibited significantly lower intakes than lactating ewes. When grazing an indigenous (poor) pasture, however, it took 8 weeks for a difference to appear.

These studies suggest the influence of appetite on grazing behaviour is modified by food availability. Perhaps, where this is sufficiently limiting, animals graze to their maximum abilities inspite of their potential appetite. In such situations herbage availability primarily determines grazing behaviour. Where food resources are more abundant then a greater, but as yet undetermined, role for appetite is predicted.

1.2.13. SUMMARY

This review has considered the control of seasonality in a the red deer hind. Evidence suggests that a hierarchy exists within the organization of its seasonal responses. Directing the sequence of changes in reproductive activity, metabolism, *etc.* are endogenous circannual rhythms. Quite possibly these are generated by a single internal 'clock'. Rhythms are entrained to environmental periodicity primarily by seasonal photoperiod changes. These ensure that biological processes are synchronised with the appropriate external conditions to maximise reproductive success and survival. In addition, there appears to be a subordinate role for other exogenous factors (e.g. temperature, food resources and social factors) which modify expression of seasonal changes.

1.3. EXPERIMENTAL AIMS

Although wild red deer are exposed to substantial variation in herbage availability, the influence that this exerts on the expression of seasonal changes is poorly understood. It was the purpose of this study to examine the interaction between herbage abundance and the endogenous processes controlling seasonality. In addition, the study also investigated how reproduction influences seasonal changes by modifying expression of an underlying seasonal rhythm(s). These were investigated in three experiments; the specific objectives of which are outlined below.

Experiment 1 (chapter 3)

The objective of this experiment was to examine how herbage availability and seasonal appetite changes interact to determine herbage intake and foraging behaviour during autumn. This was investigated by comparing hinds in different seasonal appetite states grazing pastures providing either abundant or limited herbage resources. Appetite state was manipulated by administering exogenous melatonin between July and October to advance the seasonal decline in appetite.

Experiment 2 (chapter 4)

The first experiment examined the interaction between herbage availability and endogenous seasonal state over short periods, and in animals with relatively low nutritional requirements. In this experiment, the interaction was investigated during the period between mid-summer and autumn, to determine the consequences of low herbage availability for hinds with elevated nutritional requirements. This was tackled in two ways. Firstly, by manipulating the food supply of grazing hinds and, secondly, by examining how differences in nutritional requirements due to lactation and manipulation of the phase of the seasonal cycle by melatonin, influence the hind's response to limited food resources.

Experiment 3 (chapter 5)

It was the objective of this study to determine if ovarian steroids secreted during the breeding season influence the timing of seasonal changes in VFI, live weight, coat growth and plasma prolactin concentrations during spring.

This was investigated in two ways, (i) by suppressing the breeding season in a group of mature females, to simulate the steroid hormone environment of pre-pubertal females, and (ii) by simulating the steroid profiles of cycling hinds in pre-pubertal animals.

CHAPTER 2

MATERIALS and METHODS

2.1. NUTRITIONAL PARAMETERS

2.1.1. ESTIMATION OF VOLUNTARY FOOD INTAKE, DIGESTIBILITY AND DIET COMPOSITION.

Two techniques were used to estimate voluntary food intake, the *food refusal* method for enhoused animals, and the *n-alkane* method for grazing animals.

2.1.1.1. THE FOOD REFUSAL TECHNIQUE

Dry matter intakes of individual animals were measured by offering each animal a known weight of feed each day and at the same time on the following day weighing residues. Animals were offered approximately 15% more than they consumed the previous day. The feed offered and residues were measured to an accuracy of 5 g. The intakes derived were corrected for dry matter content of food offered and residue.

The diet used was a ration of pelleted lucerne (Dengie Ltd, U.K.) with a daily mineral supplement. Using pellets manufactured in the same batch from the same source it was possible to ensure consistency of the diet throughout an experiment.

2.1.1.2. THE n-ALKANE TECHNIQUE

This technique was developed by Dr.R.W. Mayes and colleagues at Macaulay Land Use Research Instøitute (Pentlandfield, Roslin, Midlothian, U.K.). Its principle is to utilize a combination of different dosed and naturally occurring long-chain n-alkanes in herbage as markers to estimate intake, digestibility and diet composition of grazing ungulates. The technique requires the measurement of the concentrations of n-alkanes in food and faeces. The suitability of n-alkanes as markers was first described by Mayes and Lamb (1984) and in more detail by Mayes, Lamb and Colgrove (1986). Validation of the technique was carried out in sheep (Mayes

et al., 1986) and goats (Duncan, 1986). Since then it has also been applied successfully in cattle. This was the first major use of the technique in red deer.

Alkanes have a number of useful properties relevant to their use as markers. Firstly, they are simple saturated and relatively inert hydrocarbons (C_nH_{2n+2}) that can be relatively easily analysed and manufactured. They are also relatively indigestible, with faecal recoveries increasing with chain length (C35 = 96-98% recovered). Alkanes are also present universally in the cuticular waxes of plants. They are present as discrete compounds making it possible to use the same techniques to analyse concentrations in herbage and faeces. Finally, n-alkanes found in plants have predominantly odd carbon chain-lengths in the range C25-C35 (approximately 96% of the total). This makes it possible by using n-alkanes with an even carbon chain length to distinguish dosed and naturally occuring alkanes in the faeces, yet makes it likely that both markers will be processed similarily by the animal's digestive system.

HERBAGE INTAKE ESTIMATION

Food intake = Faecal output / proportion of food apparently undigested

The *proportion of food undigested* is determined by measuring the difference in concentration (mg/kg DM) of a specific odd-chain herbage alkane between samples of ingested herbage and of faeces. The concentration of this n-alkane in herbage and faecal dry matter (DM) are represented by [Herbage alkane] and [Faecal alkane] respectively.

$$\text{Proportion of apparently undigested DM} = \frac{\text{[Herbage alkane]}}{\text{[Faecal alkane]}}$$

The use of such a plant component if undigested as a marker has the advantage that digestibility is measured directly *in vivo*.

Choosing the appropriate herbage n-alkane is critical for the successful application of this technique. *Ideally* it should be completely indigestible and present as a significant proportion of the alkane component of the diet. The faecal recovery of pentatriacontane (C35) is almost quantitive (0.96: R.W.Mayes, *pers. comm.*). However, its relatively low concentration in common plant species (e.g. 12-15 mg/kg DM in perennial ryegrass: Mayes *et al.*, 1986 and this thesis) limits its potential for the accurate estimation of herbage intake and digestibility. In contrast, nonacosane (C29), hentriacontane (C31) and triatriacontane (C33), are present in concentrations 5-9 times greater than that of C35. However, these have lower faecal recoveries resulting in underestimates of digestibility and intake. Thus, to be used as accurate markers, faecal recoveries of the alkanes must be known and universally consistent. This permits correction for gut loss as follows:

$$\text{Proportion apparently undigested DM} = \frac{[\text{Herbage alkane}] \times \text{Recovery of n-alkane in faeces}}{[\text{Faecal alkane}]}$$

for the specific odd-chain alkane.
Therefore, following substitution and rearrangement:

$$\text{Food intake (FI)} = \frac{([\text{Faecal alkane}] / [\text{Herbage alkane}]) \times \text{Faecal output}}{\text{Recovery of n-alkane in faeces}}$$

Faecal output of DM is determined from the dilution of a known amount of dosed even-chain alkane (mg/day) to its concentration (mg/kg DM) in the faeces. The trace amounts of this n-alkane present in herbage and its apparent partial disappearance in the gut are taken into account:

$$\text{Faecal output (FO)} = \frac{\text{(Dosed alkane + [Herbage alkane] x FI)}}{\text{[Faecal alkane] / Recovery of alkane in faeces}}$$

for the specific even-chain alkane.

Partial disappearance of dosed n-alkane results in an over-estimation of intake. Clearly, if faecal recoveries are the same for both alkanes then the errors will cancel each other out. Mayes *et al.* (1986) demonstrated this to be true if pairs of alkanes with a difference in a single carbon atom are selected. Validation trials in sheep revealed the alkane pair C32/C33 gave intake estimates identical to actual intake measured directly. Total faecal recoveries were 0.889 and 0.891 for C32 and C33 respectively. On the basis of this study and subsequent experiments in sheep, cattle and goats (Duncan, 1986; R.Mayes, unpublished data) this pair was chosen for the estimation of intake in this study.

The faecal recovery of C32 is described as follows, where [F32] and [F33] are the concentrations of faecal C32 and C33 respectively, and [H32] and [H33] are the concentrations of herbage C32 and C33:

$$\text{Faecal recovery of C32} = \frac{\text{FO x [F32]}}{\text{Dosed C32 + [H32] x FI}}$$

Assuming the recovery of C32 = the faecal recovery of C33, by substituting the above:

$$\text{Food intake} = \frac{\text{([F33] / [H33]) x FO}}{\text{(FO x [F32]) / Dosed C32 + ([H32] x FI)}}$$

This can be rearranged as follows:

$$\text{(a)} \quad \text{FI} = \frac{\text{[F33] x (Dosed C32 + [H32] x FI)}}{\text{[F32] x [H33]}}$$

(b) $FI \times [F32] \times [H33] = [F33] \times (\text{Dosed } C32 + [H32] \times FI)$

(c) $$\frac{FI \times [F32] \times [H33]}{[F33]} = \text{Dosed } C32 + [H32] \times FI$$

(d) $$\frac{FI \times [F32] \times [H33]}{[F33]} - [H32] \times FI = \text{Dosed } C32$$

(e) Dividing through by FI:

$$\frac{[F32] \times [H33]}{[F33]} - H32 = \frac{\text{Dosed } C32}{FI}$$

(f) $$\text{Food intake} = \frac{\text{Dosed } C32 / [F32]}{([H33] / [F33] - [H32] / [F32])}$$

DIGESTIBILITY OF THE DIET

The proportion of food apparently digested by the animal (the digestibility of the diet) can be calculated using either naturally occurring herbage or dosed alkanes with almost complete faecal recoveries. The most suitable are alkanes, C35 and C36, which have recoveries of about 0.96 (R.Mayes *pers. comm.*). These are fairly consistent permitting correction for partial disappearance. A limitation is that it is not possible to estimate digestibilities for individual diet components.

Estimation of diet digestibility using dosed C36

Method used in experiment 1.

Digestibility of the total diet was calculated from the ratio of faecal output to food intake as follows:

$$\text{Digestibility of DM} = 1 - \frac{\text{Faecal output of DM}}{\text{Food intake of DM}}$$

Food intake (g DM/day) was calculated from the ratio of C32:C33 (as described above) and faecal output (g DM/day) from the dilution of a known amount (mg/day) of dosed C36 as follows:

$$\text{Faecal output} = \frac{0.96 \times \text{Amount of dosed C36}}{[F36]}$$

The concentration of C36 in the faeces ([F36]) was corrected to account for the 4% apparent disappearance in the gut.

Estimation of diet digestibility using C35

Method used in Experiment 2.

Digestibility was estimated from the ratio of the concentration of C35 in herbage DM ([H35]) to C35 in faecal DM ([F35]), as follows:

$$\text{Digestibility} = 1 - \frac{0.96 \times [H35]}{[F35]}$$

As before the faecal alkane estimate is corrected to account for apparent disappearance in the gut.

A problem with the use of C35 is its relatively low concentration in most herbage. This is typically between 12-15 mg/kgDM in perennial ryegrass (the major grass species of swards used). To test the accuracy of using the C35 method, a comparison was made between estimates of the digestibility of the diet using the latter method with that of the C36 method. Analysis of variance confirmed that there was no statistical difference between the two methods. The use of C35 was therefore deemed satisfactory for the estimation of digestibility in experiment 2.

COMPOSITION OF THE DIET

Plant species differ widely in the relative proportions of individual n-alkanes present in their waxes. This has been exploited to develop a technique using these n-alkanes to establish the proportion of different plant species ingested by grazing herbivores.

The technique is based on the following principles:
(i) Proportions of diet components are estimated from the patterns of concentrations of n-alkanes found in the faeces relative to the patterns of concentrations of n-alkanes in the plant species in the diet.
(ii) The variable recovery of herbage n-alkanes of different chain length is overcome by dosing animals with a range of even-chain alkanes (of similar length to act as internal standards) to enable prediction of herbage alkane recovery and correction of faecal concentrations.

To utilize this technique different plant species in the diet require contrasting n-alkane profiles. In practice most plants differ little in the range of long-chain n-alkanes they possess. In addition, alkanes present in low concentrations and those with shorter chain lengths are difficult to analyse accurately. Thus, the number of suitable alkanes is relatively low, generally between 3-5 in the range C25 to C35.

Although the technique is being further refined, it is currently capable of determining the ratio of 2 or 3 major plant species in the diet. In this study it was used to measure the proportions of heather and acid-grass species in the diet of hinds by comparing the concentrations of n-alkanes C29, C31, C33 and C35 in the herbage and faeces.

The proportions of heather and grass in the diet are defined as those values which most accurately predict the proportion of each alkane found in the faeces from the levels present in the herbage. A minimising computer routine (NAG routine E04JAF: Gill and Murray, 1976) was used to calculate this proportion by minimizing the

following expression, and selecting those giving the least difference between predicted and actual concentrations in the faeces.

$$\text{Minimise} \left\{ \left[\left(\begin{array}{l} \textit{predicted} \\ \text{proportion of} \\ \text{alkane in faeces} \end{array} - \begin{array}{l} \textit{actual} \\ \text{proportion of} \\ \text{alkane in faeces} \end{array} \right)^2 \right]_{x \ldots x+n}^{\text{alkane}} \right\}$$

The *predicted* proportion of alkane of chain length 'x' is estimated for each selected proportion of heather (α) and grass (β) as follows:

$$\textit{Predicted}\ \text{proportion} = \frac{\alpha.H_x + \beta.G_x}{\alpha.H_t + \beta.G_t}$$

where H_x and G_x are the concentrations of alkane 'x' in the heather and grass respectively, and H_t and G_t are the total concentrations of all alkanes under consideration (in this case: C29, C31, C33 and C35) in heather and grass.

The *actual* proportion of alkane 'x' in the faeces is calculated as follows:

$$\textit{Actual}\ \text{proportion} = \frac{\text{Concentration of 'x' in faeces}}{\text{Total alkane concentration in faeces}}$$

The concentration of each alkane requires correction due to apparent disappearance in the gut, which increases with reduction in carbon chain-length. Faecal recovery values calculated during previous trials (Duncan, 1986) were used. The values used were: C29 = 0.745; C31 = 0.85; C33 = 0.895; C35 = 0.94.

The procedures for using the n-alkane technique to estimate food intake, digestibility and composition of the diet are similar.

The general principle is to dose animals with even-chain n-alkane(s) for a sufficient period to allow their concentration in the faeces to become constant and, while continuing to administer the alkane(s), to collect samples of faeces and also herbage representative of that ingested. Faecal and herbage samples are analysed to determine the concentration of naturally occuring- and dosed- n-alkanes. With a knowledge of the amount dosed these can be used to estimate food intake, digestability and composition of the diet.

Method of administering even-chain n-alkane(s)

Even-chain n-alkane(s) was administered orally in pellet form. Each pellet contained 170 mg (for adults) or 100 mg (for calves) of each n-alkane used. This choice was based on experience with sheep and cattle grazing comparable pastures. It was designed to achieve similar concentrations of dosed even-chain and naturally occurring odd-chain alkanes in the faeces (for ease of analysis).

To prepare 330-340 170 mg C32 n-alkane pellets the following procedure was used (the same quantities will produce 560 100 mg C32 pellets):

(i) 20 sheets of Whatman no.1 filter paper were placed in an aluminium tray of the same dimensions as the filter and heated in an oven at 80°C for ten minutes.

(ii) 57.5 g of C32 was dissolved in n-heptane (total solution volume = 900 ml). The heated solution was then poured evenly over the hot tray ensuring all sheets soaked up the liquid. The sheets were then hung up to dry in a well-ventilated room.

(iii) Drying was completed by drying each sheet for 3-4 minutes in an oven at 80°C. This allowed the paper to absorb the amount of n-alkane.

(iv) When cool the paper was cut into 15 x 2.5 cm stripes with a guillotine before shredding to produce 2.5 x 2.5 cm strips.

(v) Each pellet for dosing hinds and calves was made from 1.70 g and 1.0 g of this paper respectively as follows: The paper was weighed out and compacted into a pellet by squeezing through a tube of 1.2

cm internal diameter. This was then wrapped in a 7 x 5 cm sheet of tissue paper and glued together using starch paste.

Only pellets made from a single batch of paper were used during a measurement period.

Length of dosing period prior to faecal sampling

In sheep and goats it has been established that at least 6 days are required to elapse for the dosed n-alkane to achieve a steady state concentration in the faeces (Duncan, 1986; Mayes *et al.*, 1986). In red deer however, the retention time of digesta in the gut is significantly shorter than for sheep for a variety of diets (see Kay and Goodall, 1976; Milne *et al.*, 1976). This suggests that the length of this pre-sampling period could be shortened. To investigate this possiblity a trial was carried out during which faecal samples were collected on each day C32 alkane was administered. Concentrations of C32 and C33 in the faeces were measured to determine the time taken for ratio of these n-alkanes to achieve a constant state.

The results of this trial are illustrated in Figure 2.1. Hinds achieved a constant ratio of n-alkanes C33 to C32 within 2-3 days of the start of dosing. This suggests a shorter pre-sampling period is possible. However, the length was kept to a minimum of 6 days for all trials to allow animals to adjust to the experimental regime.

Collection of herbage

As already mentioned samples of plant material were collected to enable characterization of the n-alkane concentrations of ingested herbage. These needed to be representative for reliable intake and diet composition estimates to be made. In domesticated animals, samples are commonly obtained using oesophageal-fistulated individuals grazing along-side the experimental animals. An alternative approach, utilized in these studies, was to clip samples of herbage from where animals were seen taking bites. This procedure was repeated with a variety of individuals until approximately 200 g wet matter had been collected. For measurements of diet composition

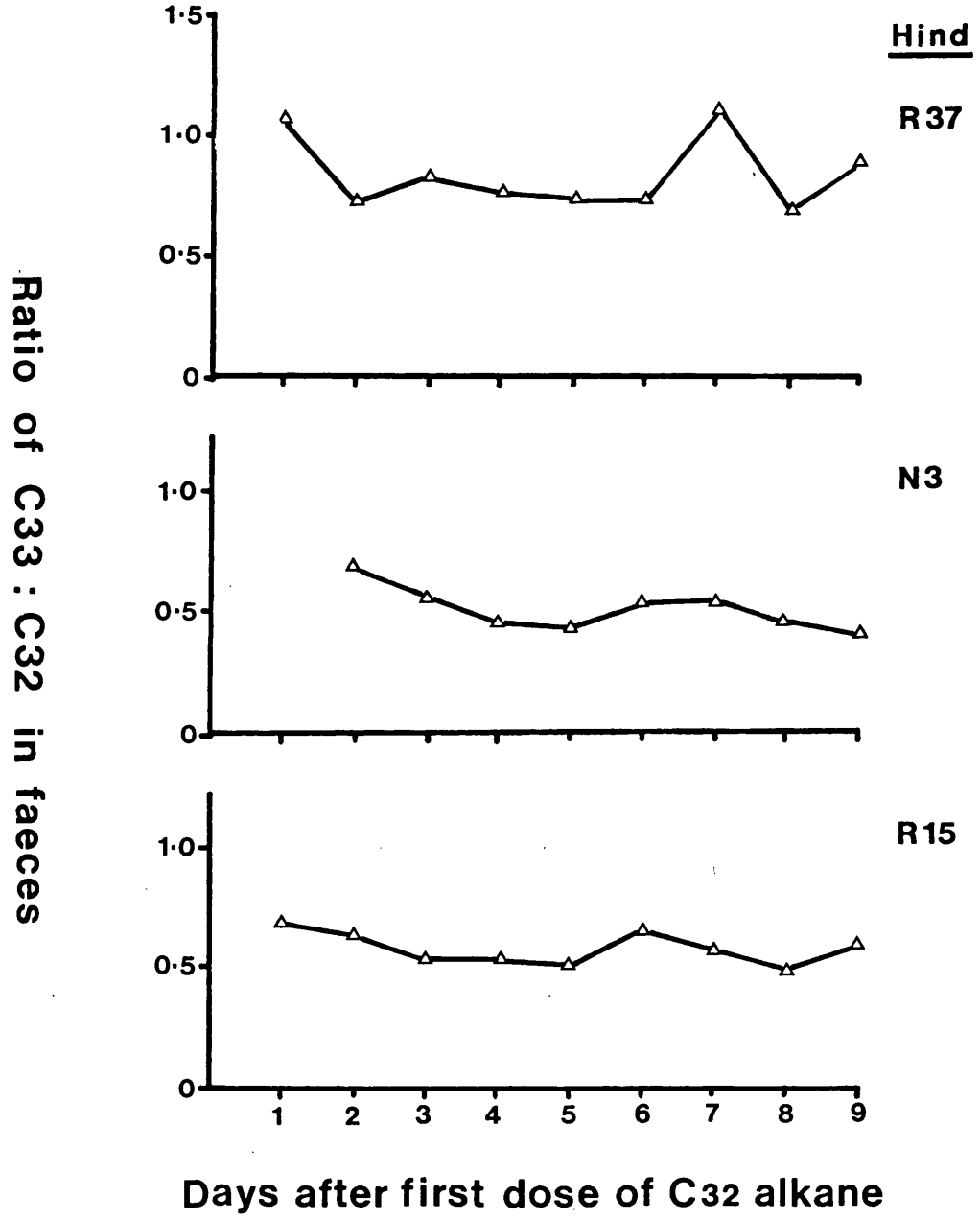
trials this was carried out for each plant community grazed. Samples were then frozen and stored at -20°C until freeze dried and milled through a 1mm screen prior to analysis.

This technique is not considered significantly inferior to the use of oesophageal fistulated animals on uniform grass swards similar to those used in all but one comparison of experiment 1 (R. Mayes, *pers. comm.*).

Experimental procedure

(i) Hinds were allocated to the appropriate pastures 1-2 weeks prior to the onset of each measurement period to permit acclimatization to pastures and the experimental regime.
(ii) For 14 consequtive days in experiment 1, and 12 days in experiment 2, the animals were gathered into handling yards adjacent to the experimental paddock at approximately 08.30 hr.
(iii) On all but the last day each animal was orally dosed with a pellet containing even-chain n-alkane(s) using a plunger-type cattle dosing 'gun'. From day 8 to 14 in experiment 1 and day 7 to 12 in experiment 2 a faecal sample (approx. 20g wet matter) was collected. This was obtained by either grab-sampling (see section 2.7., Methods of restraint) or if a hind was seen to defecate, by collecting the faeces in the field, (approximately 50 % of samples were obtained in this fashion). Evidence from previous studies (Mayes *et al.*, 1986) suggests that there is no daily change in the pattern in alkane excretion, so that collection of faecal samples at different times in the day should not lead to bias in intake estimation.
(iv) Herbage samples were collected during the period of days faeces were collected.
(v) All samples were frozen and stored at -20°C until freeze dried, bulked for each animal over the trial period and ground into a fine powder using a domestic Moulinex coffee grinder (faeces) or a heavy duty industrial grinder (herbage).

FIGURE 2.1: Changes in ratio of herbage C33 n-alkane concentration to dosed C32 n-alkane concentration with time (days) after onset of dosing.



Analysis of n-alkanes by direct saponification

Faecal samples

Analysis of samples was carried out in duplicate as follows:

(i) 0.5g of the bulked faeces was weighed out (to 4 decimal places) into a 20 x 100 mm thickwalled screw-topped Pyrex test tube (Sovirel tube) with a PTFE lined cap (to prevent solvents escaping). These had been washed out with n-heptane followed by acetone to ensure that they were contamination free.

(ii) Added to this was a weighed amount of a solution tetratriacontane (C34) as an internal standard (approxiamtely 0.4 g of solution providing about 0.2 g of internal standard).

(iii) In the saponification stage, 7 ml of a 1M potassium hydroxide solution (in ethanol) was added. The stoppered tube was left for 30 minutes to allow the solution to soak the sample. The tube was then heated in a dry block heater at 90°C for at least 3 hours, or overnight.

(iv) After partial cooling 7 ml n-hexane and 2 ml distilled water were added and tubes shaken vigourously (for 30 sec.) to extract the alkanes. Gentle agitation with an orbital mixer ('Whirlimixer') assisted the formation of a non-aqueous hexane layer containing the alkanes above the alcoholic potassium hydroxide solution. The top layer was removed using a Pasteur pipette and retained. The above procedure was repeated with a further 7 ml hexane. The collected solvent (containing the alkanes) was evaporated to dryness to remove any remaining alcohol.

(v) The residue was then redissolved in 2 ml hexane and warmed at 60°C for 10 minutes while remaining capped. The solution was then applied to the top of a small disposable plastic column (Supelco Inc.,Bellafonte, P.A., U.S.A.) containing silica gel (Kiesel gel 60, 70-230 mesh, Merck, Darmstadt, F.G.R.). with a bed volume of 5 ml. The hydrocarbons were eluted with 10 ml hexane.

(vi) The hexane was evaporated off and the hydrocarbons redissolved in 300 µl of heptane (assisted by heating very briefly on a hot plate) and the solution transfered to a GLC vial and capped.

(vii) The concentrations of n-alkanes were measured as follows. 1µl of sample was injected onto a 1.6m x 4mm glass column containing 3%

SE-30 on 100-120 mesh Supelcoport (Supelcoport Inc., P.A., U.S.A.) in a Model 104 gas chromatograph fitted with a flame ionisation detector (Pye Unican Ltd., Cambridge, U.K.). The chromatograph oven was maintained at 275°C and the carrier gas N_2 had a flow rate of 30ml/min. The peak areas of n-alkanes were determined using a Pye Unican CDP1 computing integrator. The carbon chain lengths of the n-alkanes present in samples were deduced by their retention times relative to known alkanes, (present in standards analysed at the same time). Concentrations of n-alkane were recorded as percentages of the internal standard C34 (which had been added to each of the samples).

Herbage samples

Analysis of samples was carried out in quadruplicate by the same procedure as described above for faeces except:

(i) 1.5g sample was weighed into 22 x 200 mm Pyrex screw-topped test tubes.

(ii) Saponification was carried out using 14 ml of alcoholic potassium hydroxide and extracted with 2 ml of distilled water and 2 x 14 ml n-hexane.

The concentration of n-alkane in herbage and faeces (mg/kgDM) was calculated from the measurements made by the gas chromatograph as follows:

$$\text{Concentration of alkane 'x'} = 10 \times \frac{A_x \times IW \times IC}{SW \times DM \times DRF}$$

where A_x represents the amount of alkane 'x' in the herbage or faeces as a percentage of the Internal Standard (C34); IW represents the weight of Internal Standard solution used (g); IC represents the concentration of the Internal Standard in the solution (mg/ml); SW represents the sample weight (g); DM the proportion of dry matter in the freeze dried sample (see 'Other analyses'), and DRF the detector response factor.

The detector response factor is a correction to take account of the differences between the known amount of n-alkane in the chromatograph standards and that estimated by the gas chromatograph. It is calculated as follows:

$$\text{Detector response factor} = \frac{\text{observed alkane } \alpha}{\text{expected alkane } \alpha}$$

Dosed alkane pellets

The n-alkane content of 5 pellets (randomly selected) from each batch was analysed as follows:

(i) Each pellet was chopped into fragments and put into an extraction flask. To this were added approximately 150 mg of C34 internal standard (roughly equivalent to the amount of even-chained n-alkane in each pellet), 70 g of petroleum spirit (B.P. 60-80°) and some anti-bump granules. This was refluxed for 2 hours.

(ii) The solution was allowed to cool to approximately 30°C (any lower and the alkane crystallised) and 200 µl of the extract was added to a GLC vial. The solution was evaporated to remove the petroleum spirit and the residue redissolved in 300 µl of n-heptane.

(iii) The extracted n-alkanes were then analysed using the gas chromatograph as described for the faecal samples above.

The amount of each dosed alkane in a pellet (mg) was calculated from the measurements made by the gas chromatograph as follows:

$$\text{Weight of alkane in pellet} = 1000 \times \frac{(A_x \,/\, 100) \times IS}{\text{Detector response factor}}$$

where A_x represents the amount of alkane 'x' in the pellet as a percentage of the Internal Stardard (C34); IS represents the weight of Internal Standard added (g), and the detector response factor is calculated as described above.

(iv) Results for the 5 pellets analysed were averaged to provide a mean alkane content for the pellets of that batch.

Other analyses

(i) Dry matter content of the freeze dried herbage and faecal samples were determined by oven drying 1 g of sample at 105°C overnight. This was necessary as the process of freeze drying only removed about 92% of the water content.
(ii) To enable the calculation of organic matter intakes from dry matter intakes, the proportion of organic matter in the herbage samples was determined. To estimate this 1 g (in triplicate) of oven dried herbage sample from each pasture was ashed overnight in a muffle furnace at 550°C. From the weight of ash (≃ inorganic material) remaining the proportion of organic material in the dried herbage was calculated.

Calculations

Herbage intake, digestibility and composition of diet were calculated using computer programmes based on the equations described above written by Mrs Angela Sibbald (Macaulay Land Use Research Institute, Pentlandfield, Roslin, Midlothian, U.K.).

2.1.2 MEASUREMENT OF LIVE WEIGHT

Live weight of hinds was measured to an accuracy of ± 0.5 kg. Live weight of calves was measured to an accuracy of ± 0.25 kg in experiment 2, and in experiment 3 to ± 0.1 kg until groups reached mean weight of c. 50 kg when it was measured to an accuracy of ± 0.5 kg.

2.1.3 BODY CONDITION SCORING

Introduction

The objective of condition scoring is to obtain a simple and reliable index of the level of body fat reserves in a live animal.

The technique used in this study was a modification of that developed at the East Scotland College of Agriculture for the condition of scoring cattle (Lowman, Scott and Sommerville, 1976).

The technique was used in experiment 2 to supplement information from live-weight measurements as the latter can can be substantially affected by gut-fill. In addition, animals with similar live weights may have markedly different relative body composition. Drawbacks of the technique include its subjectivity (the same person carried out all measurements to minimize subjective differences) and the difficulties of identifying small changes or differences in body condition. It is, therefore, most usefully applied where a wide range of condition exists, in which case it provides valuable additional information to live-weight and aids the interpretation of treatment effects.

Procedure

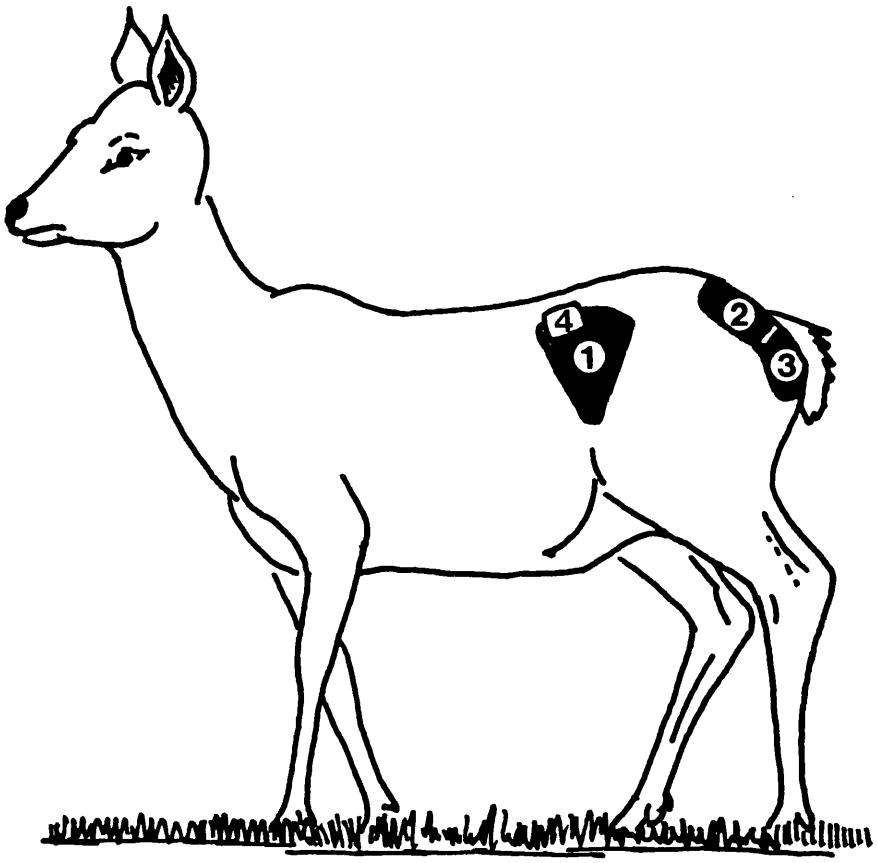
This involved assessing the level of fat cover on 2 areas of the animal's body. These are:

(i) The spinous processes of the lumbar vertebrae (area between the hip bone and the last rib; see Fig. 2.2). This was the major assessment region for animals in poor to average condition. The degree of prominence of the process was judged by placing the fingers firmly on the neural spines of the 4-5th lumbar vertebrae and the thumb on the transverse processes.

(ii) The sacral vertebrae, between the hips and tail head (crup region). The degree of prominence of the spinous processes of these vertebrae are judged by applying pressure with the fingers. The general appearance of the tail head in relation to the fat cover around it was also noted.

The only significant modification of the technique from that described by Lowman *et al.* (1976) for use in cattle, was the emphasis on assessment of fat cover over the sacral vertebrae rather than tail head. This was necessary because applying pressure to the tail head resulted in an aggressive response from most hinds.

FIGURE 2.2: Regions used for body condition scoring and winter coat measurement in red deer.



KEY

1 - spinous processes of lumbar vertebrae
2 - crup region
3 - tail head
4 - approximate region of coat measurement

Condition scores

Animals were given a score based on the following criteria between 1 and 5 with increments 0.5 if they fell between categories:

Score 1: Individual spinous processes are sharp to touch and easily distinguished in both regions.

Score 2: Spinous processes can be identified when touched but feel rounded rather than sharp.

Score 3: The spinous processes can be felt with very firm pressure in the lumber region but can still be felt with moderate pressure in the crup region.

Score 4: Lumbar spinous processes cannot be felt. Fat cover on the crup region is substantial and noticable fat deposited about the tail region.

Score 5: The bone structure of the animal is indistinguishable and the tail head almost completely buried. Spinous processes in either region cannot be felt. The deer has a rounded appearance.

2.2 HERBAGE MEASUREMENTS

Sward height

This was estimated weekly from the mean of approximately 50 height measures per hectare (minimum of 50/paddock) using the Hill Farming Research Organization sward stick (Barthram, 1986) measuring to an accuracy of 0.5 cm. To ensure representative sampling the field was paced in a zig-zag pattern with measurements taken approximately every ten paces.

Herbage mass

This was estimated at two-weekly intervals by clipping to ground level approximately 4-5 quadrats (145.5 x 13.5 cm) per hectare in each paddock. This was carried out using commercial sheep shearers powered by a portable electric generator. Samples collected were dried in an oven at 80°C for 24 hours and then weighed to provide estimates of mean herbage mass of DM per hectare.

2.3 BEHAVIOURAL TECHNIQUES

2.3.1 OESTROUS BEHAVIOUR

In conjunction with endocrine monitoring of reproductive status incidence of oestrous behaviour was recorded. Oestrus was detected by observing hinds for specific behavioural patterns. The more common of these are cited below, with illustrations of some in Plate 1.

(i) Mounting of hind in oestrus by another female in a posture similar to that adopted during copulation by the male. Note was also taken of the mounting hind, or any animal that showed partcular interest in the oestrous hind, as these were often approaching oestrus themselves.

(ii) a significant reduction in grazing and an increase in activity often associated with pacing along field boundaries, particularly if adjacent field contained males.

(iii) distinct temperament changes sometimes resulting in strong attraction to humans and generally a much reduced tendency to flee at their approach. Hinds often become difficult to move and would stand rigid if pressure was applied to either their back or rump. In such situations hinds would usually lift their tails which is characteristic of mating posture and a signal of willingness to mate (the Lordosis response) - see Plate 1, and

(iv) rubbing head and neck against posts, other deer, humans and even their own back.

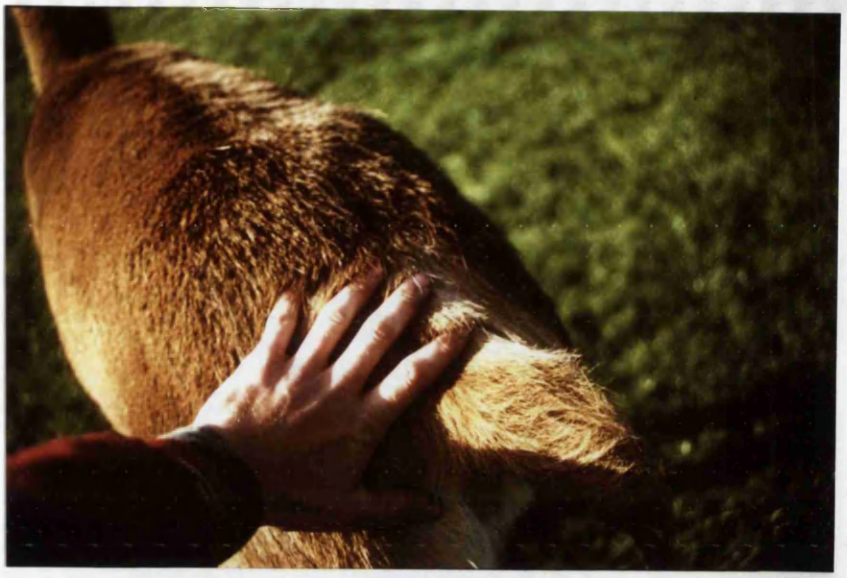
2.3.2 GRAZING BEHAVIOUR

Observations of grazing behaviour were carried out in experiments 1 and 2 at the end of each intake comparison. The purpose of these was to observe the effects of treatment on grazing behaviour, particularily to relate differences in herbage intake to variation in behaviour.

Bite rate

Bite rate wa estimated by recording the length of time required by a hind to take 20 bites (Loudon *et al.*, 1984). This was repeated a minimum of 10 times for each animal throughout the observation

PLATE 1: Examples of behavioural activity characteristic of red deer hinds in oestrus.



period in order to derive an estimate for the mean number of bites taken per minute while grazing.

Activity budget

Day long observations examining the activity budget of individual hinds were carried out. All animals were observed simultaneously to overcome the potential problems created by daily differences in weather and disturbance.

The observation period consisted of a single unbroken period the length of which depended on daylength (i.e. available light) and on the availability of night viewing equipment. In experiment 1 observations were limited to the period between dawn and dusk. In experiment 2 an image intensifier was used to extend observations to an average of 19.7 hr per day.

Throughout each period of observation measurements of behaviour were made at 5-15 minute intervals. The interval length differed between comparisons due to the location and topography of paddocks and within comparisons due to changes in day-light. To assist individual identification hinds wore colour and bar-coded collars.

Hind behaviour was categorised as follows:
(i) GRAZING: defined as head down and taking bites.
(ii) ACTIVE NON-GRAZING: either walking or standing.
(iii) NON-ACTIVE: defined as the hind sitting down, not grazing.
(iv) SUCKLING: suckling bouts occurring at the time of measurement were recorded.

It was impossible to distinguish rumination consistently enough to categorize it separately. Most occured during the period classified as 'non-active'.

To estimate the length of time individuals spent pursuing each activity it was assumed that hind behaviour remained the same throughout the interval between measurements. The total daily duration in each activity was determined from the sum of measurement interval lengths when hinds were observed pursuing that activity.

Note was also taken of any hinds in behavioural oestrus, disturbance to grazing and prevailing weather conditions.

2.4 PELAGE MEASUREMENTS

Coat growth was monitored in experiments 2 and 3. This was carried out by direct measurement of the hair *in situ*. Although subjective. This method is considerably quicker than other methods and permits regular measurements from the same site. To reduce error the same person carried out all measurements in each study. The following parameters were measured:

(i) Primary fibre (summer and/or winter) length was estimated from a sample of at least a dozen hairs measured *in situ* using a steel rule from the same site in the centre of the back, approximately 10cm from the spine (see Fig. 2.2).

(ii) The proportion of winter and summer fibres in the coat was estimated on a scale of 1-5 as follows:

Score 1: full summer coat
Score 2: trace of winter coat
Score 3: intermediate
Score 4: trace of summer coat
Score 5: full winter coat

2.5 METHODS OF RESTRAINT

A number of the experimental techniques necessitated restraining deer. The methods adopted varied with facilities available. The following criteria were used such that the technique:

(i) Permitted measurements and samples to taken safely and consistently.

(ii) avoided distress or injury to the deer, and

(iii) aimed to reduce disruption to a consistent minimum time.

Experiment 1

Two or three hinds were gathered into a high-sided holding pen, approximately 1.5 x 2 m in floor area. Handling several animals at once reduced their ability to move suddenly and had a calming effect. One hind would be singled out and held by two members of

staff. The first would hold the head using his/her body to brace the fore-end of the animal against the side of the holding pen, while the second, pressing one leg between the last rib and hip to restrain the hinds hindquaters. Once held firmly a third person could take the necssary measurements. For the dosing of n-alkanes and collection of faecal grab samples it was usually possible for this to be achieved using two staff. Critical to the success of this technique was familiarity of hinds with the handling procedure.

Experiment 2

Two means of restraint were available. The first followed the same method as described above (carried out by two people) and was used for all experimental procedures except collection of blood samples and coat measurements. For these, hinds were restrained in a 'drop floor' type deer crush (see Plate 2).

The crush was operated as follows. A hind was driven up a ramp and into a 'Y' shaped passage. A hinged floor was then released wedging the hind in the groove. The head was held by one person completing its immobilization. Access was limited to the head, neck, back and rear of the animal. Despite this drawback the crush required fewer staff and was considerably safer. To release the hind one side of the crush swung outwards allowing the animal to slip to the ground and exit through a gated front.

Experiment 3

Handling of hinds was carried out using a deer crush which differed in design from that used in experiment 2. This utilized a sliding side with a yoke at the shoulder and hip to hold the hind firmly while standing. Once held the front of the pivoting side could be opened and the head held. Access to the deer was possible from openings in the side between the shoulder and head, and the hips and shoulder.

During the first two months of the study the calves were too small to hold in the crush. To restrain them they were held horizontally across the lap of a person sitting on a low seat (calf legs on the ground). A second person held the head. This technique was only possible due to the hand-rearing of the calves and proved

PLATE 2: The handling facility used during experiment 2

sufficiently successful to restrain calves up to approximately 50 kg live weight.

2.6 ENDOCRINE TECHNIQUES

2.6.1 BLOOD SAMPLING

Blood samples were collected by jugular venepuncture using the methods of restraint described in section 2.5. The samples were collected using either:

(i) 21 gauge needle with 10 ml vacutainer containing heparin (experiments 1 and 2).

(ii) 19 gauge needle with 10ml syringe, heparinizing the blood in a screw topped test tube containing lithium heparin (ML brand LH 10, Teklab, U.K.; experiment 3).

Blood was then centrifuged and the plasma separated and stored at -20°C until assayed.

2.6.2 HORMONE MANIPULATIONS

2.6.2.1. MELATONIN MANIPULATION

Objective: To advance seasonal changes associated with declining photoperiod in late summer and autumn (eg. onset of breeding, appetite decline and growth of winter coat) by manipulating the transduced photoperiod signal using the pineal hormone melatonin. The purpose was to permit comparison of animals in different seasonal states under the same environmental and nutritional conditions in the field.

As discussed in the section 1.2.3., the secretory profile of melatonin is determined by photoperiod modifying a circadian rhythm. This results in high night-time and low day-time plasma concentrations of the hormone. The seasonally changing profile relays information about daylength which is important in synchronising the animal's seasonal responses with the environment. If the profile is manipulated over a sufficient period using either artifical photoperiods (Kay, 1979; Webster and Barrell, 1985; Poulton *et al*,.1987a; Malpaux *et al.*, 1988b) or appropriate

melatonin administration (see below) then it is possible to alter the phase of seasonal changes. The mechanism by which exogenous melatonin is believed to influence the phase of seasonal changes is discussed in section 1.2.3.5. To advance the changes normally associated with declining photoperiod, exogenous melatonin was administered from mid-summer by one of the following methods:

Daily dosing

(method adopted in experiment 1)

Daily dosing takes a variety of forms including: addition of melatonin to feed (Adam *et al*, 1986; Asher *et al*, 1987; Poulton *et al.*, 1987a); oral dosing in pellet form (Bubenik, 1983; Milne *et al.*, 1990) and injection (Kennaway *et al.*, 1983; Webster and Barrell, 1985). These methods aim to lengthen the period of high night-time melatonin levels by administering prior to dusk (usually in mid-afternoon). Though ideally the time of dosing should advance as day-length shortens animals are usually dosed at a set time (15.30 hrs.: Asher *et al*, 1987; Poulton *et al.*, 1987a; 1600 hrs.: Adam *et al*, 1986; Webster and Barrell, 1985). The advantage of this technique over others is that it creates a plasma melatonin profile similar to that of a short day photoperiod typical of autumn (Poulton *et al*, 1987a). The result appears to be a phase advance of seasonal physiological changes (see section 1.2.3.5.).

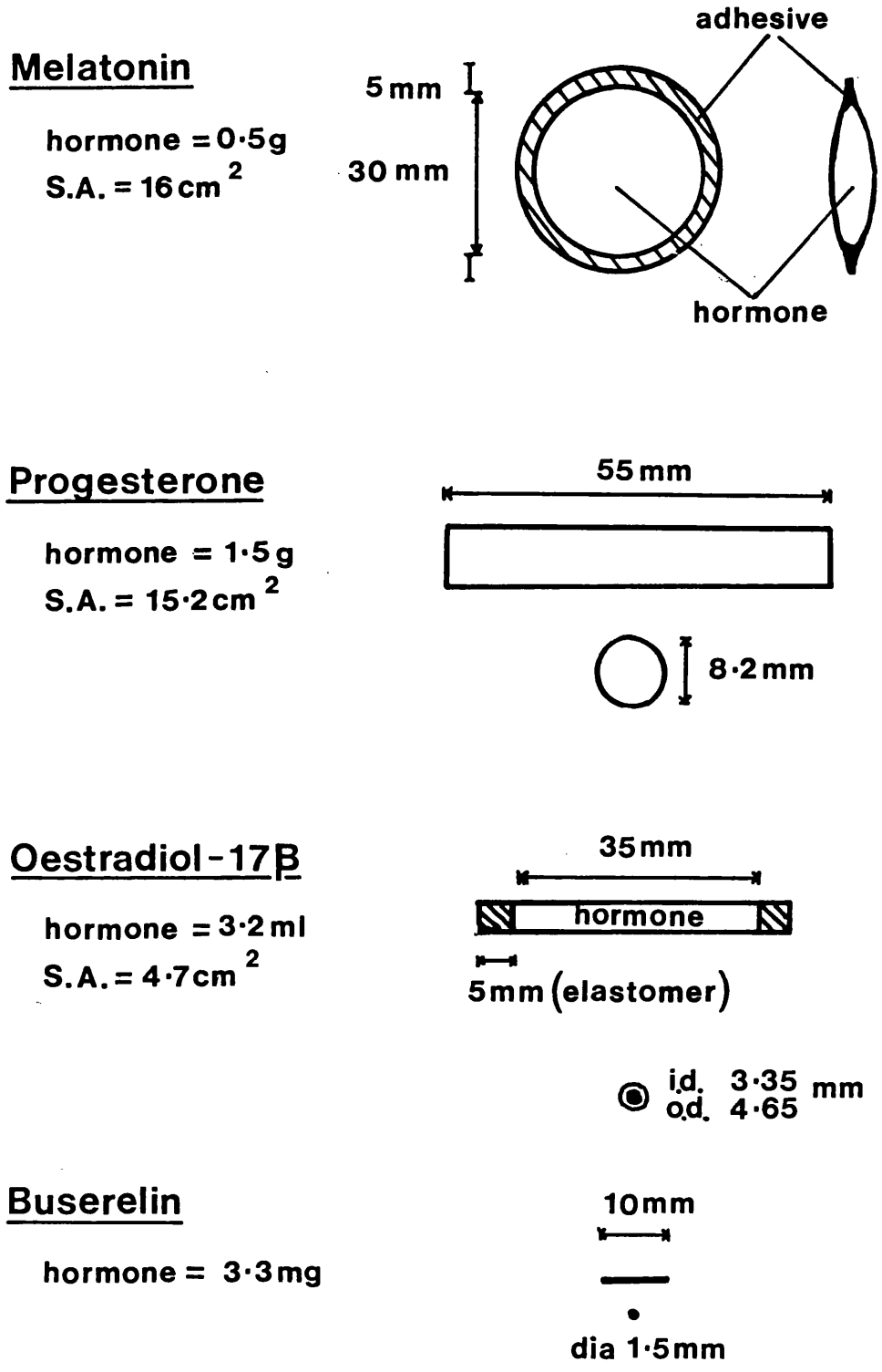
The procedure utilized in experiment 1 was as follows: hinds were given 10mg of powdered melatonin (Sigma Chemical Co., Poole, Dorst, U.K.) in a gelatin capsule orally each day at 1600 hours from the 24 July to the 8 October 1987. The dose given was based on previous experience to give a physiological effect (Milne *et al.*, 1990).

Continuous release

(method adopted in experiment 2)

Continuous release methods also take a number of forms including: degradable subcutaneous implants (Fisher *et al.*, 1988); non-degradable long active implants (Lincoln and Ebling, 1985; Poulton *et al.*, 1987a); intra-vaginal sponges (Nowak and Rodway, 1985) and intraruminal soluble glass boluses (Poulton *et al.*,

FIGURE 2.3: Implants used to manipulate hormone levels (see section 2.8.3 for details).



1987b). These methods differ fundamentally from daily administration by obliterating the normal pattern of melatonin secretion. In deer and sheep responses to the continuous release implants resemble the effects of daily dosing or short-day photoperiod regimes (English *et al.*, 1986; Webster and Barrell, 1985). The mechanism by which continuous release methods may influence seasonal changes is reviewed in section 1.2.3.5.

An advantage of this method of administration is that it removes the necessity of disturbing animals daily or feeding them supplements (impregnated with melatonin). This was important in experiment 2 as this trial involved measurement of intake as well as all day behavioural observations.

The procedure adopted in experiment 2 was as follows: Two implants containing 0.5 g of powdered melatonin in a Silastic packet (see description below) were inserted sub-cutaneously on the 21st June and left *in situ* until the 8 November.

The implant used is illustrated in Figure 2.3 and was based on that described by Loudon *et al.*, 1985. Each implant contained 0.5g powdered melatonin sandwiched between two sheets of Silastic Medical Grade sheeting (ref. no. 500-1 8" x 6" x 0.005"; Dow Corning Corp., Michigan, U.S.A). The approximate surface area was 16 cm^2. Implants were made as follows:

(i) Silastic sheets were first prepared by washing thoroughly with a soft sponge in distilled water to remove sodium bicarbonate (dusted on at manufacture to facilitate handling). They were then dried and cut into 4 x 4 cm squares.

(ii) A single 4 x 4cm piece of sheeting was placed on top of a circular paper template (32 mm diameter).

(iii) 0.5 g of powdered melatonin was spread evenly over the area within the template boundary.

(iv) Silastic Medical Grade Adhesive (Type A) was liberally applied around the hormone boundary using a 2 ml syringe.

(v) A second sheet was then placed on top expelling as much air as possible.

(vi) The implant was then left overnight for the adhesive to set (out of direct sunlight) before trimming off edges to give a round,

smooth-edged implant (see Figure 2.3). This was then immersed in distilled water for several hours to check that the seal was water tight.

(vii) Until use, implants were stored at -20°C.

(viii) To sterilize implants, they were immersed in pevidine solution for 1-2 hours and then washed in sterile saline immediately prior to insertion.

Implants were inserted sub-cutaneously under general anaesthetic on either side of the sternum in the loose skin behind the foreleg.

(i) Hinds were given 65 mg xylazine (Rompun: Bayer Pharmaceuticals, Leverkusen, Germany) in saline (0.9 % NaCl) intravenously.

(ii) The unconscious animal was laid on its side. The implanting area was shaved, scrubbed with pevidine solution and rinsed with surgical spirit.

(iii) Approximately 2ml of lignocaine hypocloride (Lignavet: C-Vet Ltd., Bury St. Edmonds, Suffolk) local anaesthetic was injected sub-cutaneously in the implanting area.

(iv) A scalpel blade was used to cut though the skin and using forceps and scissors connective tissue above the incision was cleared to make a pocket for the implant.

(v) After insertion the wound was closed with at least two sutures using a 40 mm reverse cutting needle with vicryl suture (Ethicon Ltd., P.O. Box 408, Barkhead Av., Edinburgh, U.K.).

(vi) The incision area was then washed and sprayed with an aerosol antibiotic (Oxytetracycline violet - Duphacycline, Duphar Veterinary Ltd., Hedge End, Southampton, U.K.).

(vii) An intra-muscular dose of long acting antibiotic (Terramycin LA: Pfizer Ltd., Sandwich, Kent, U.K.) was given. Dose depended on live weight (1 ml/10kg).

(viii) 0.7 mg Colex (Reckitt and Coleman, Ltd., U.K.) in 2 ml saline was injected intravenously to revive the animals. This occured within 30-60 seconds of administration.

The same general procedure was used to remove implants.

2.6.2.2. PROGESTERONE AND OESTRADIOL IMPLANTS

Objective: Implants of the ovarian steroids progesterone and oestradiol 17β were given to hind calves in experiment 3 to simulate levels normally present in cycling adult hinds. Administration coincided with the period of adult breeding season.

Progesterone

Implants were designed to achieve levels of progesterone in peripheral plasma similar to those present during the luteal phase (3-10 nmol/l). To calculate the required implant size and progesterone content, trials were carried out using adult hinds during anoestrus. Estimates were then corrected for the predicted calf body size range (40-60 kg). The implant design was modelled on the commercial implants used by Curlewis, Loudon and Coleman (1988). These contained 275 mg progesterone in a silicone-elastomer matrix (Sil-estrus. Ceva, Paris, France). Appropriate progesterone concentrations were based on data from experiments 1 and 2. The implant dimensions are illustrated in Figure 2.3.

Implant manufacture: (amounts are for a single implant):
(i) 2.2 g Silastic 382 Medical Grade Elastomer and 1/3 drop of vulcanising agent Catalyst M (stannous octoate; Dow Corning, U.S.A.) were mixed thoroughly. This ratio of elastomer and catalyst provides approximately 15 mins. working time.
(ii) 1.5 g powdered progesterone (Sigma P 0130) were added and mixed to produce a paste.
(iii) The hormone/elastomer mixture was extruded into a mould made from a 2 ml plastic syringe (Plastipak, Becton Dickinson, Spain). Using the plunger the volume of elastomer was adjusted to 2.5 ml and a cap placed on the open tip.
(iv) Implants were left in the moulds for 24 hours at room temperature until set. After removal implants were stored in plastic universal screw topped tubes.

(v) Prior to use implants were pre-incubated in 2 changes of sterile saline over 48 hours at room temperature as advised by Karsch *et al* (1973) to avoid a transient post-insertion peak of hormone.
(vi) Implants were sterilised by exposure to U.V. light for 1 hour in clear plastic universal screw topped tubes, and then immediately prior to insertion by submersion in a pevidine solution for 30 mins. Pevidine was washed off using sterile saline.

Implants were used twice (re-sterilising after first use).

Two implanting procedures were utilised. The first involved general anaesthetic (similar to that described above for melatonin) and was used during implant trials and for the first insertion of implants at the beginning of experiment 3. Subsequently a local anaesthetic was used proving sufficient for the procedures involved and causing less disturbance to the animals. This also removed the necessity to give controls (non-implanted animals) anaesthetic (due to the observed affects of xylazine on food intake: Simpson, Suttie, Sharman and Corrigal, 1983). This procedure was possible due the docile nature of the hand-reared calves. Methods of restraint are described in section 2.5.

The insertion and removal procedures were similar to those described for melatonin imlants, with the following exception. To prevent resistance to antibiotics 3 types were used on an alternate basis. These were Clamoxyl LA, Duphapen LA (Duphar Veterinary Ltd., Hedge End, Southampton, U.K.) and Terramycin LA (Pfzier Ltd., Sandwich, Kent, U.K.).

Oestradiol-17β

Concentrations of oestradiol-17β present in mature and pre-pubertal deer have not been determined. For experimental purposes it was assumed that concentrations are similar to those reported in the ewe. In pre-pubertal ewes, plasma oestradiol-17β concentrations are between 3-10 pmol/l (Foster and Ryan, 1981; Foster *et al.*, 1986). The implant used to deliver oestradiol in this study was designed to elevate these levels by 10 - 15 pmol/l in peripheral plasma. This

should result in a concentration comparable to the average over the ewe's oestrous cycle (c.20 pmol/l, Hauger *et al.*, 1977).

The implant used was a cylinderical Silastic capsule containing crystalline oestradiol-17β which was modelled on the type used by Martin *et al.* (1988; see Fig. 2.3). This in turn was based on the extensively used design first described by Karsch *et al.* (1973). The size of implant was chosen after consideration of hormone elevations achieved in sheep of different body weights, using implants of various sizes (Goodman *et al.*, 1980; Goodman *et al.*, 1981; Martin *et al*, 1988; R. Webb, *pers. comm.*).

Implant manufacture:

(i) One end of a 4.5 cm length of Silastic Medical Grade Tubing (3.35 mm i.d. and 4.65 mm o.d., cat. no. 601-335; Dow Corning, U.S.A.) was blocked with 0.5 cm of Silastic 382 elastomer (with vulcaniser) and allowed to set.

(ii) This was packed with 3.5 cm depth crystalline oestradiol-17β (Sigma E8875) and the open end sealed with elastomer.

(iii) Rough edges at each end were trimmed to avoid irritation to tissue.

(iv) Prior to usage implants were pre-incubated in 2 changes of sterile saline at room temperature over 48 hours, as advised by Karsch *et al* (1973) to avoid a transient post insertion peak of hormone concentration.

(v) Sterilization, insertion and removal of the implants were carried out by the same procedures described for progesterone implants.

2.6.2.3. BUSERELIN IMPLANTS

Objective: To suppress the breeding season in adult hinds. The technique involved administration of the GnRH agonist buserelin (HOE 766: Hoechst, Frankfurt, Germany) generously provided by Dr. med. Jurgen Sandow (Hoechst Atkiengensellschaft, Frankfurt). Administered as a continous release implant buserelin suppresses pituitary responsiveness to GnRH resulting in curtailment of pulsatile LH

activity in a variety of female mammals including cats, dogs, monkeys (J. Sandow, *pers. comm.*) and sheep (Robinson *et al.*, 1989).

The implant used is illustrated in Figure 2.3. Each was approximately 10 mm in length and 1.5 mm in diameter, containing 3.3 mg [D-Ser(But)6]GnRH(1-9) - nonapeptide - ethylamide (buserelin: manufactured by Hoechst). Following advice (confirmed in the trial described below) each animal was given 2 implants every 4 weeks from 14th September 1989 to the 1st March 1990 (8 doses in total). Treatment started approximately 5 weeks prior to the normal breeding season of the study herd and finished after the hinds in the control group finished cycling.

The implanting procedure was as follows:
(i) The implant site was in the side of the neck away from the jugular. This was prepared by shaving the coat, scrubbing with pevidine solution and finally washing with alcohol.
(ii) 1.5 ml Lignavet (local anaesthetic) were injected subcutaneously around the insertion site.
(iii) Two implants were removed from their sterile pouch loaded into the bevel end of a 14 gauge needle (Monoject, Sherwood Medical, Crawley, U.K.) capped to prevent accidental loss of implants.
(iv) Holding the needle horizontally (to prevent implants slipping out) the cover was removed from the tip and inserted sub-cutaneously to a depth of 2.5 cm. This was retracted 1cm to allow room for the implants in the sub-cutaneous tunnel. A sterile stainless steel pushrod was then inserted into the bevel end of the needle expelling the implants into the sub-cutaneous pocket. Care was taken not to remove implants when withdrawing the needle.
(v) The insertion site was sprayed with antibiotic and the hind given an intra-muscular dose of long acting antibiotic.

Implants were not removed.

Pre-experiment trial

Buserelin has been used in female cats, dogs, monkeys and sheep but not previously in deer to suppress the oestrus cyclicity. To

investigate its suitability a trial was carried out. In this the LH response of buserelin treated (n=2) and control (n=2) hinds during anoestrus to a challenge of 250 µg GnRH (in saline) was determined. This dose was designed to produce a maximal LH response. Challenges were carried out prior to implantation, and subsequently 2, 3, 4, 5 and 6 weeks later. The response was measured by assay of plasma samples collected before (t0) and 20 minutes (t20) post-injection, for LH. The results of the trial are summarised in Table 2.1.

Buserelin treatment clearly abolished the response to exogenous GnRH by 3 weeks after implantation, confirming the suitability of the hormone agonist in deer. Although pituitary suppression lasted for at least 6 weeks after dosing with buserelin in this pilot study it was decided to re-implant every 4 weeks to ensure suppression was retained.

TABLE 2.1

The effect of buserelin on the response of hinds to GnRH. Delta values of LH (t20 - t0 µg/l ± s.e.m.) in response to 250 µg GnRH.

Treatment (n)	Weeks after buserelin implanted					
	0	2	3	4	5	6
Control (2)	1.24 ±0.19	3.41 ±1.8	1.20 ±0.66	2.20 ±0.62	1.95 ±0.78	2.56 ±1.33
Buserelin (2)	0.5 ±0.1	0.28 ±0.03	0.06 ±0.05	0 0	0.04 ±0.04	-0.02 ±0.09

2.6.2.4. GnRH CHALLENGES (experiments 2 and 3)

GnRH is secreted by the hypothalamus in a manner causing the pulsatile release of LH (and FSH) from the anterior pituitary gland. The levels and secretory pattern of these hormones determine the reproductive status of the female and are influenced by factors such as photoperiod and nutrition (Kennaway *et al.*, 1986). To assess treatment affects on pituitary responsiveness to GnRH, exogenous releasing hormone was administered. The response was measured by determining the resulting elevation of plasma LH concentration at 20 minutes. This interval was based on the time course of LH response

observed in Pere David's deer (Curlewis, McLeod and Loudon, 1991). The GnRH dose used depended on experimental conditions, and is described for each study in the relevant 'Experimental design' section.

Challenge design was as follows:
(i) A 10ml blood sample was collected (t0) and then the GnRH (in 2 ml saline) was injected into the jugular vein.
(ii) At 20 minutes post-injection a 5ml blood sample was collected (t20).
(iii) In experiment 2 the whole procedure was repeated 2 hours after the first injection. In this case the response of hinds to GnRH was defined as the average of the 2 challenges.
(iv) Plasma samples were stored at -20^{0}C until assayed for LH.
(v) The LH response (delta LH value) was defined as the difference in plasma LH concentration between t0 and t20.

2.6.3 HORMONE ASSAYS

Concentrations of steroid and peptide hormones in plasma samples were measured by radioimmunoassay. In brief, this involves a competative interaction between hormone in the plasma sample and a known amount of radioactively (I^{125}) labelled hormone for specific antibody binding sites. As this is a competative reaction, the number of counts of labelled hormone bound to the antibody is inversely proportional to the concentration of unlabelled hormone in the plasma sample. The actual concentrations are interpolated from these counts and a standard curve produced by assaying samples containing known concentrations of unlabelled hormone.

Abbreviations

c.p.m.	counts per minute (of I^{125} labelled hormone).
T	Total c.p.m. of labelled hormone added to each tube.
B	c.p.m. of labelled hormone bound to antiserum in tubes containing plasma sample or standard hormone.
B_0	c.p.m. of labelled hormone bound to antiserum for maximum binding tubes (i.e. no unlabelled hormone present)

NSB non specific binding (c.p.m. of labelled hormone 'bound' in tubes containing no antiserum). This is used to correct c.p.m. values for binding to assay tube surfaces.

Unless otherwise stated all reagents were obtained from B.D.H. Chemicals Ltd., Poole, Dorset, U.K. and assay tubes from Luckham Ltd., Burgess Hill, Sussex, U.K.

2.6.3.1. PROLACTIN

A double antibody equilibrium radioimmunoassay was used to measure concentrations of prolactin in deer plasma. This was a modification of an assay used to measure prolactin in ovine plasma. The original validation and protocol is described in McNeilly and Andrews (1974).

Materials and Reagents

(i) **Assay buffer (1% BSA/PBS)**

The buffer used for all dilutions (unless stated otherwise) was a 1% solution of bovine serum albumen (BSA: factor V powder, 96-99% albumen, Sigma Chemical Co. Ltd., Poole, U.K.) in 0.05M sodium dihydrogen orthophosphate pH 7.4 containing 0.15M sodium chloride (phosphate buffered saline: PBS) and 0.1% sodium azide (used as a preservative). Buffer was made up in deionised-distilled water and stored at 4°C.

This buffer was selected following investigation of 3 phosphate buffers. These were:

(a) 1% BSA/PBS (see above).

(b) 0.04M NaH_2PO_4; 0.06M Na_2HPO_4; 1.5×10^{-3}M NaN_3 and 0.1% gelatine, pH 7.0.

(c) 0.08M Na_2HPO_4; 0.02M NaH_2PO_4; 0.15M NaCl; 1.5×10^{-3}M NaN_3 and 0.1% gelatine, pH 7.2.

Standard curves were set up using each of the buffers following the original ovine assay procedure (McNeilly and Andrews, 1974).

One of the buffers, (c) failed to produce a recognisable curve. Binding of the zero standard (%$(B_o$-NSB)/T) was 17.6 % and 11.7 % for buffers (a) and (b) respectively. Although the binding was low in

both, suggesting other aspects of the protocol required modification, buffer (a) containing BSA was selected as for use in assay.

(ii) **Prolactin standard**

Ovine prolactin (NIH-OPrl-S13) was serially diluted in assay buffer from a stock of 200µg/l (kept at -20°C) to give 10 dilutions from 200 µg/l to 0.39 µg/l (prolactin was initially solublised in pH 9 buffer).

(iii) **Quality control plasma samples**

Pooled red deer plasma with low (c. 8 µg/l) or high (c. 22 µg/l) levels of prolactin were stored in 250 µl aliquots at -20°C until required.

(iv) **Plasma samples**

These were stored at -20°C until assayed.

(v) **First antibody**

Rabbit anti-ovine prolactin (McN 2532 - *bleed no.51*: M.R.C. Unit of Reproductive Biology, Edinburgh, U.K.) was stored at 1:100 dilution in 100 µl aliquots at -20°C. For assay, this was diluted to 1:100,000 in assay buffer.

The optimum dilution of the antibody was ascertained as follows: The first step was to measure the maximum binding ($(B_o$-NSB)/T) over a wide range of antiserum dilutions to establish an optimum range. Total counts, NSB's and maximum binding tubes at dilutions between 1:50,000 and 1:800,000 were subject to the assay procedures described below (results are summarized in table below).

First antibody dilution	% (B_o-NSB)/T
1:50,000	28.2
1:100,000	20.6
1:200,000	14.5
1:400,000	8.1
1:800,000	4.0

Highest bindings was observed at 1:50,000 dilution. To choose the dilution for use in the assay standard curves were subject to assay procedure over a narrow range of antiserum dilutions between 1:100,000 and 1:200,000 (Figure 2.4). From these results a dilution of 1:100,000 (giving a final dilution of 1:175,000) was selected as the most appropriate for the range of values to be encountered (while remaining relatively conservative of antiserum stock).

(vi) **Tracer**

I^{125}-ovine prolactin (made by Dr Alan McNeilly at the M.R.C., Unit of Reproductive Biology, Edinburgh, U.K.) was diluted with assay buffer to give a solution of 10,000 to 15,000 c.p.m./100 µl. Stock aliquots of tracer were stored at -20°C.

(vii) **Normal Rabbit Serum (NRS)**

Normal rabbit serum at 1:400 dilution in buffer was used to assist separation of bound and free fractions. Stock was stored at -20°C. Choice of optimum NRS dilution is decribed in section (viii).

(viii) **Second antibody**

Separation of bound and free tracer was achieved using a 1:40 dilution of donkey anti-rabbit serum (DARS: Guildhay Antibody Ltd., Guildford, Surrey, U.K.) in 1:400 NRS/buffer to precipitate the bound fraction.

The optimum dilutions of DARS (and NRS) for assay were determined as follows. A series of maximum binding tubes were set up containing DARS at the following initial dilutions: 1:5, 1:10, 1:20, 1:40 or 1:80. For each of these a dilution of either 1:200, 1:400 or 1:800 NRS was used. The results are shown in Figure 2.5. An initial dilution of 1:40 DARS and 1:400 NRS were chosen.

FIGURE 2.4: Standard curves for the prolactin assay at 1:100,000 (●), 1:150,000 (▲) and 1:200,000 (■) initial dilutions of bleed 51 antibody. Concentration of standard is expressed as a logarithmic scale and plotted against % binding (100 x (B-NSB)/T).

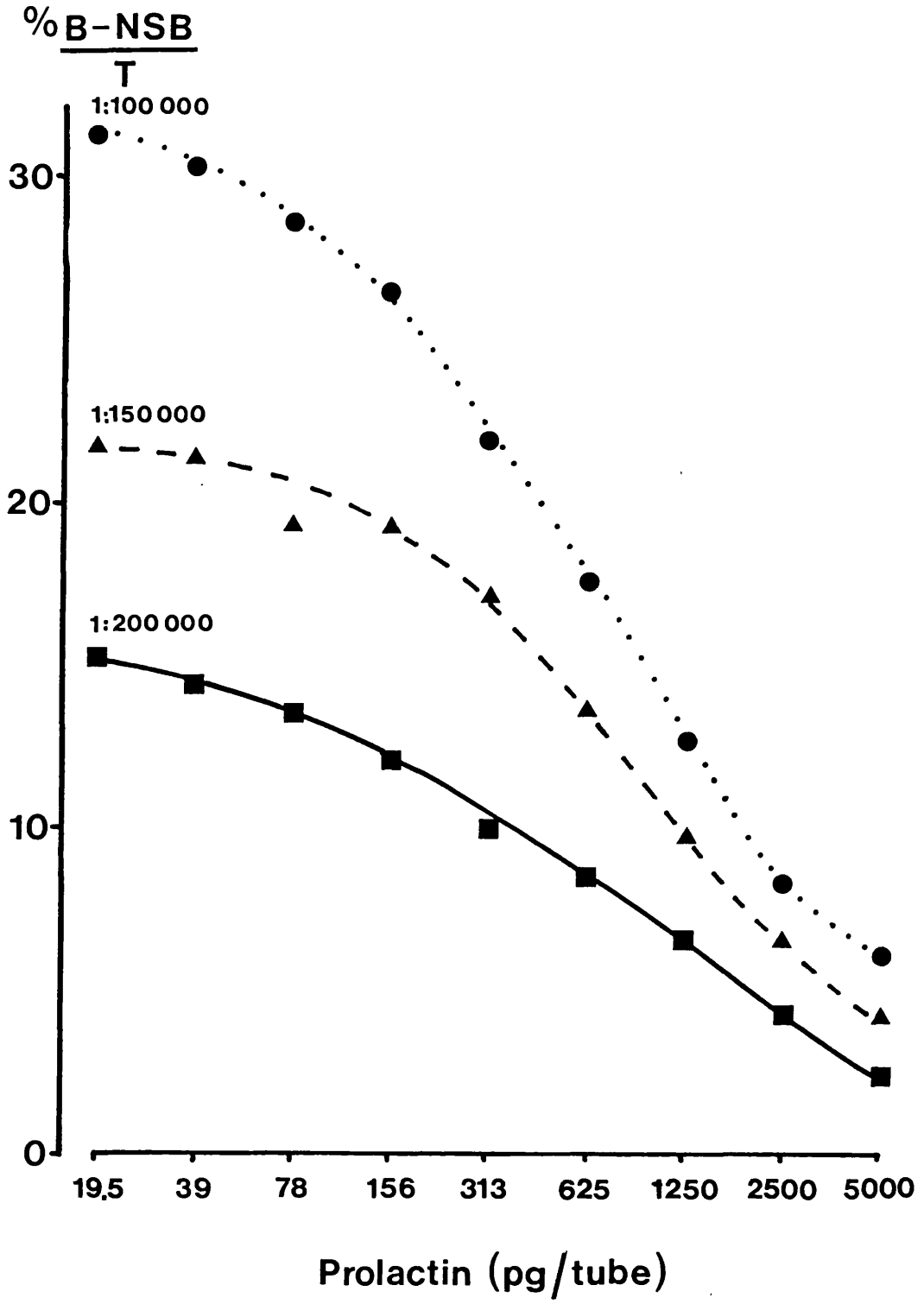
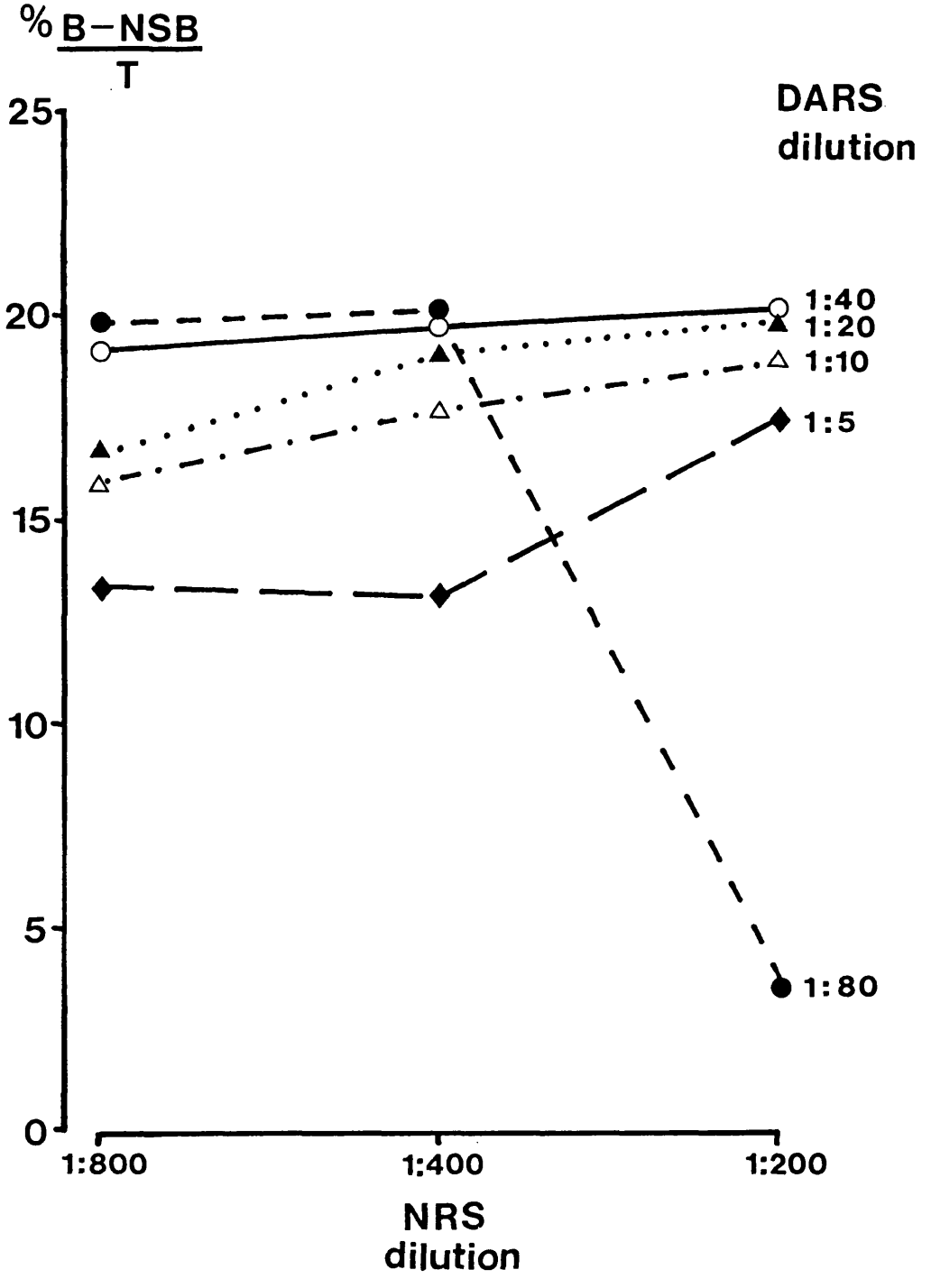


FIGURE 2.5: Maximum binding (100 x (B_o-NSB)/T) for the prolactin assay using various combinations of DARS and NRS dilution.



Assay protocol

Each assay consisted of up to 117 plasma samples with one standard curve.

DAY 1 : Polystyrene LP4 tubes were set up as follows:

Order of tubes and contents (n)		Sample/ QC	Standard	Buffer	First Antibody	Tracer
Total counts	(3)	-	-	-	-	100
NSB	(4)	-	-	250	-	100
Max. binding	(5)	-	-	50	200	100
Standards	(3)	-	50	-	200	100
QC *	(2)	50	-	-	200	100
Samples	(2)	50	-	-	200	100
QC *	(2)	50	-	-	200	100
Total counts	(2)	-	-	-	-	100

All volumes are in µl.

'*' = at low and high levels of prolactin

The samples, QC's and standards were added to the tubes, followed in order by, buffer, antibody and tracer. These were vortex mixed for 10 seconds and incubated at 4°C overnight.

DAY 2

Keeping the tubes at 4°C, 100µl of second antibody was added to all tubes (except total counts). Tubes were vortex mixed for 10 seconds and incubated overnight at 4°C.

DAY 3

1ml saline (4°C) was added to all tubes (except total counts). These were vortex mixed for 10 seconds and then centrifuged at 2400 r.p.m. for 30 minutes at 4°C (causing the first antibody/tracer complex to settle to the bottom of the tube).

Keeping the tubes a 4°C the supernatants in each tube were aspirated off using a vacuum pump, leaving a pellet containing the

bound fraction. The radioactivity of this was recorded over 3 minutes using a Cobra Auto-Gamma counter model 5005 (Packard, Canberra, Australia). The c.p.m. of tubes containing known concentrations of prolactin standard were used to produce a standard curve by a spline interpolation method (smoothed cubic spline curve). Unknown sample concentrations were interpolated from their cpm's and the standard curve.

Results were expressed as µg/l prolactin in plasma.

A modified version of this assay was used to determine plasma prolactin in experiments 2 and 3. Differences are outlined below:

(i) Prolactin standard

Same as above except the top standard was 100 µg/l (serially diluted to 0.39 µg/l in 9 dilutions).

(ii) Quality control plasma samples

Pooled red deer plasma with low (c. 5 µg/l), medium (c. 12 µg/l) or high (c. 45 µg/l) levels of prolactin were stored in 250 µl aliquots at -20°C until required.

(iii) First antibody

Rabbit anti-ovine prolactin (McN 2532 - *bleed no.50*: M.R.C. Unit of Reproductive Biology, Edinburgh, U.K.) was stored at 1:100 dilution in 50 µl aliquots at -20°C. For assay, this was diluted to 1:200,000 in assay buffer.

To determine the optimum dilution of antibody the maximum binding ($(B_0-NSB)/T$) of a range of dilutions similar to that used for bleed no. 51 antiserum was investigated, including short standard curves (6.25, 12.5 and 25 µg/l). The results are illustrated in Figure 2.6. Final choice was based on full standard curves over a narrower range of antiserum dilutions between 1:150,000 and 1:250,000 (Figure 2.7). From this a dilution of 1:200,000 (giving a final dilution of 1:350,000) was selected as the most appropriate for the range of values to be encountered.

(iv) Second antibody

Separation of bound and free tracer was achieved using a donkey anti-rabbit serum bound to iron oxide particles (supplied by Prof. S. Lynch, Dept. of Clinical Biochem. Birmingham Women's Hosptial). The solid phase antibody particules bind to the first

FIGURE 2.6: Short standard curves for the 2 day prolactin assay at 1:50,000 (▲), 1:75,000 (△), 1:100,000 (●), 1:125,000 (○), 1:150,000 (◆) and 1:200,000 (◇) initia dilutions of antiserum. Concentration of standard is expressed on a logarithmic scale and plotted against % binding (100 x (B-NSB)/T).

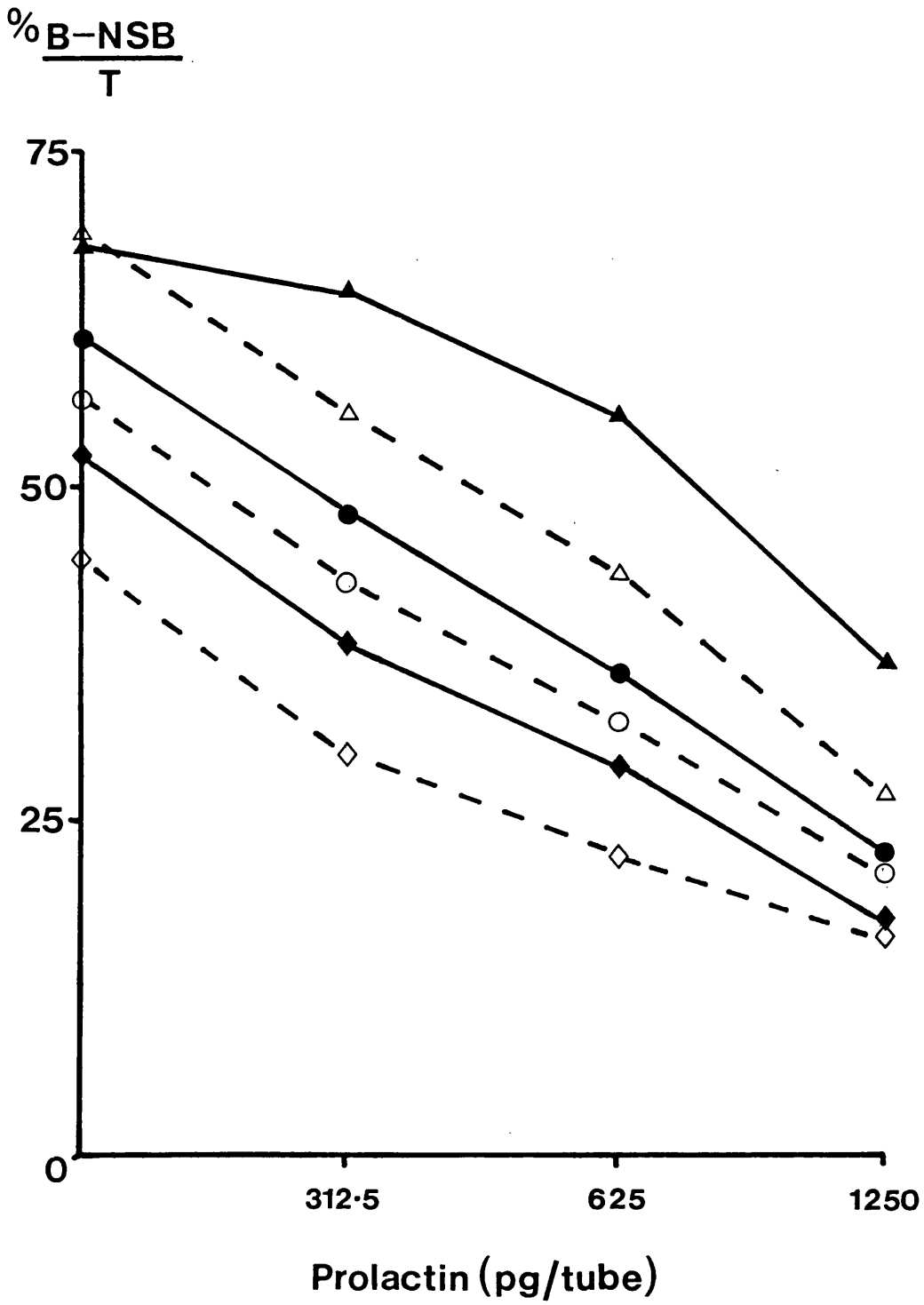
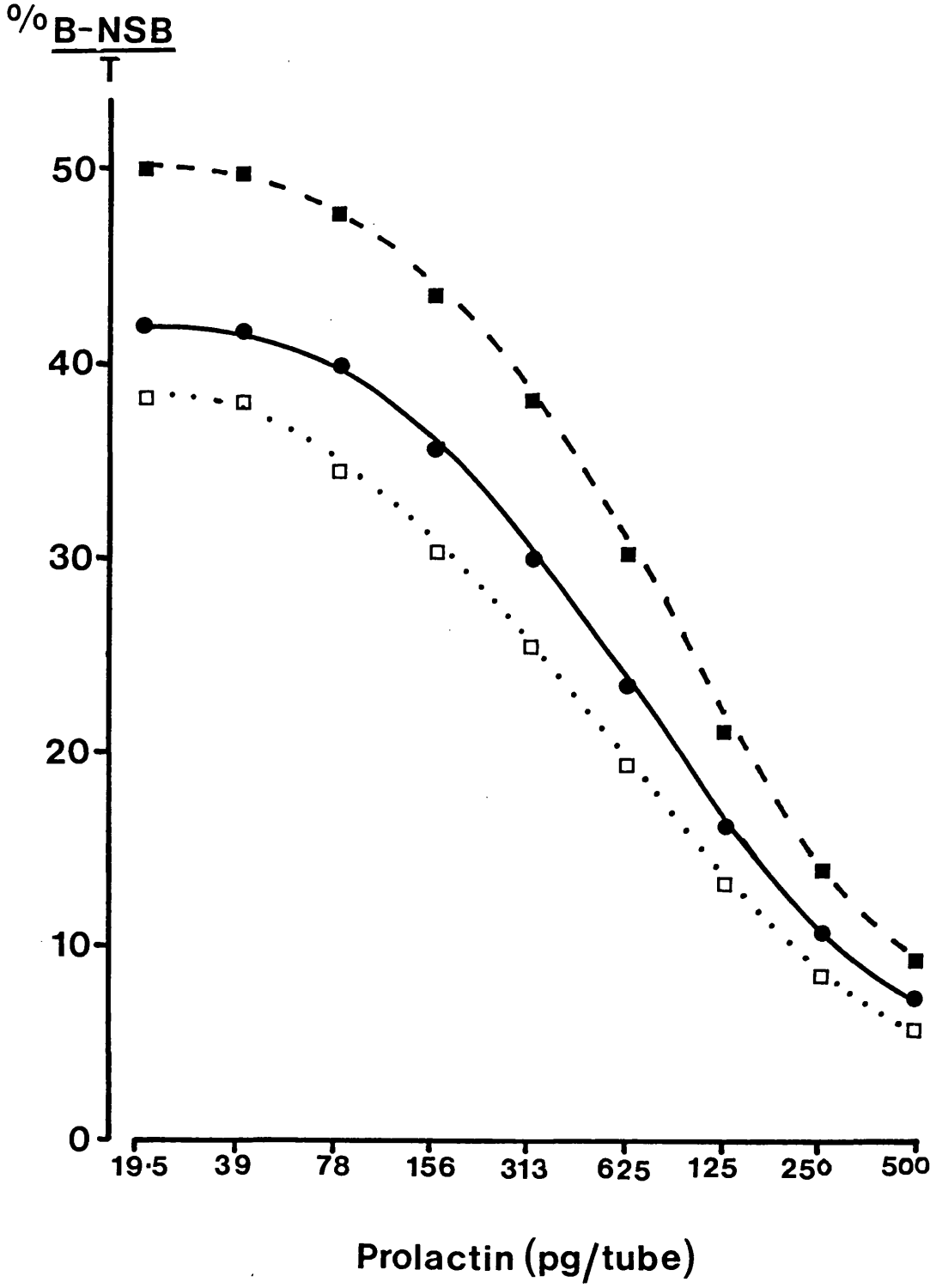


FIGURE 2.7: Standard curves for the prolactin assay at 1:150,000 (■), 1:200,000 (●) and 1:250,000 (□) initial dilutions of bleed no. 50 antibody. Concentration of standard is expressed as a logarithmic scale and plotted against % binding (100 x (B-NSB)/T).



antibody/tracer complex settling to the bottom of the assay tube under the force of gravity (assisted by centrifugation at 2400 rpm for 5 minutes, at 4°C). Antibody was diluted in phosphate buffer (c) (described above) which contained a 0.3% (w/v) solution of hydroxypropylmethyl cellulose (HPMC: Sigma Chemical Co., Poole, U.K.) The purpose of the HPMC was to delay settling-out during incubation by increasing viscosity. 0.5 ml of antibody was added to each tube (except total counts) and incubated at room temperature for 45 minutes. The advantage of this procedure was the short incubation period which reduced the assay from 3 to 2 days duration.

Assay range, sensitivity and precision

A typical standard curve is illustrated in Figure 2.8.

Experiment 1

The mean estimated concentration of standard at 20% binding was 80.8 (± 4.4) µg/l; at 50% was 13.5 (± 0.4) µg/l and at 80% was 3.7 (± 0.3) µg/l (n=13).

Assay sensitivity was defined as 2 standard deviations from the mean of the maximum binding tubes (B_0). Mean sensitivity was 94.8% (% B/B_0), equivalent to 1.79 (± 0.2) µg/l prolactin in plasma (n=13). All samples with a % binding greater than this were recorded as the mean limit of sensitivity. Any sample with fewer radio-active counts than the highest prolactin standard (200 µg/l) was reassayed at an appropriate dilution.

Inter-assay precision, expressed as the coefficient of variation for repeated determinations of the quality controls was 16.6% at 8.4 µg/l and 10.5% at 21.8 µg/l, (n=13). Intra-assay precision, expressed as the coefficient of variation for repeated determinations of prolactin in plasma samples within an assay, was 8.2% at 5.6 µg/l; 7.0% at 12.5 µg/l and 7.4% at 46.7 µg/l (n=12).

The mean (± s.e.m.) for non specific bindings (NSB/T) was 3.0 (± 0.4)%, (n=13). The mean (± s.e.m.) for maximum binding tubes ((B_0 - NSB)/T) was 42.4 (± 1.6)%, (n=13).

Experiment 2

The mean estimated concentration of standard at 20% binding was

40.8 (± 1.6) µg/l; at 50% was 10.5 (± 0.5) µg/l and at 80% was 3.2 (± 0.2) µg/l (n=10).

The mean assay sensitivity was 96.9%, equivalent to 0.62 (± 0.1) µg/l prolactin in plasma (n=10). Inter-assay precision was 10.8% at 4.7 µg/l; 9.8% at 11.2 µg/l and 12.5% at 36.4 µg/l, (n=10). The mean (± s.e.m.) for non specific bindings (NSB/T) was 6.92 (± 0.4)% (n=10) and for maximum binding tubes ((B_0-NSB)/T) was 50.0 (± 2.1)%, (n=10).

Experiment 3

The mean estimated concentration of standard at 20% binding was 35.8 (± 2.0) µg/l; at 50% was 8.75 (± 0.3) µg/l and at 80% was 2.2 (± 0.1) µg/l (n=15).

The mean assay sensitivity was 95.7%, equivalent to 1.0 (± 0.1) µg/l prolactin in plasma (n=15). The mean (± s.e.m.) for non specific bindings (NSB/T) was 3.25 (± 0.2)% (n=15) and for maximum binding tubes ((B_0-NSB)/T) was 40.1 (± 2.5)%, (n=15).

Specificity of antiserum

To test the specificity of the antiserum against cross-reactivity with other polypeptide hormones the following concentrations were subjected to normal assay procedure.

Ovine growth hormone:

250,000 - 61.04 µg/l (13 doubling dilutions).

Ovine follicle stimulating hormone:

20,000 - 39.1 µg/l (10 doubling dilutions).

Ovine luteinizing hormone:

176,000 - 85.9 µg/l (12 doubling dilutions).

For each concentration of hormone the fraction bound was calculated and expressed as a proportion of the bound fraction in the maximum binding tubes (%B/B_0).

The relative potencies of these hormones are shown in Table 2.2 below.

TABLE 2.2

The relative potencies of peptide hormones in the RIA for prolactin.

Hormone	µg hormone ≡ 1µg PRL	relative potency
Prolactin	1	1
Growth hormone	475,000	2.1×10^{-6}
FSH	> 20,000	< 5×10^{-5}
LH	> 176,000	< 5.7×10^{-6}

There was no cross reactivity with LH or FSH over the range of concentrations used. Growth hormone only cross-reacted at 250,000 µg/l. All these concentrations are far in excess of those which could be found in deer plasma under normal circumstances.

Assessment of recovery of prolactin.

To assess the recovery of prolactin, ovine prolactin in doubling dilutions from 50 - 0.39 µg/l was added to aliquots of a plasma sample with a known concentration of hormone. Recovery was calculated from the difference between the observed and expected concentrations of prolactin in the plasma.

The mean % recovery between 0.39-50 µg/l prolactin was 96.9 ± 1.6 %.

Parallelism

To ensure cervine plasma samples behave in the same way as ovine standards in the assay system doubling dilutions of plasma samples assayed as containing high levels of prolactin were compared to the curve obtained from ovine standard preparations, and parallelism assessed. The results are illustrated in Figure 2.8. The plasma samples clearly diluted in parallel.

Comparison with previous prolactin assay.

Prolactin concentrations in plasma samples assayed using the previous heterologous assay used at the Institute of Zoology

FIGURE 2.8: Investigation of parallelism in prolactin assay. Comparison of % binding (B/B_0 x100) of serial doubling dilutions of deer plasma samples (● ○ ■) and of standard preparations (▲).

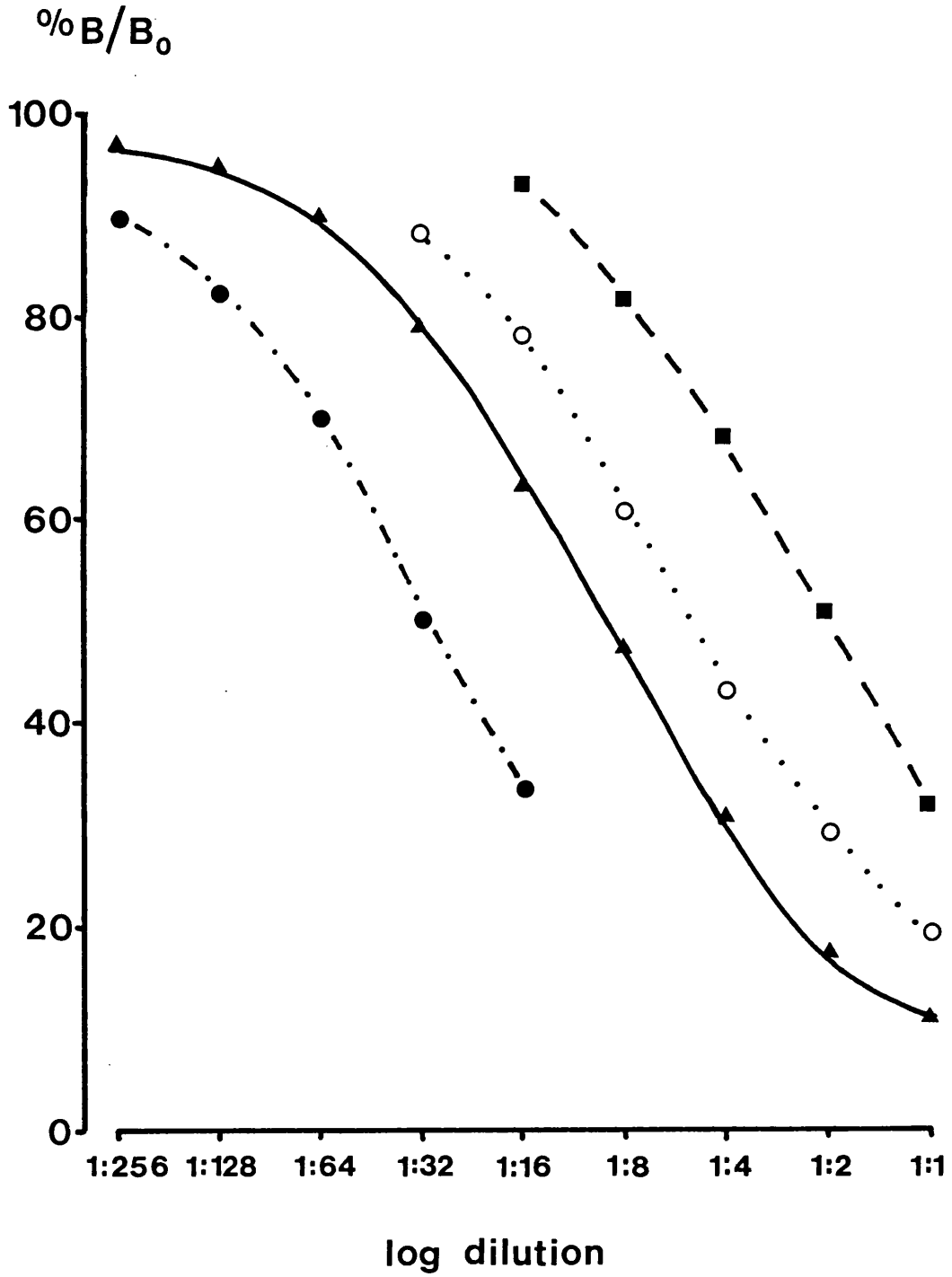
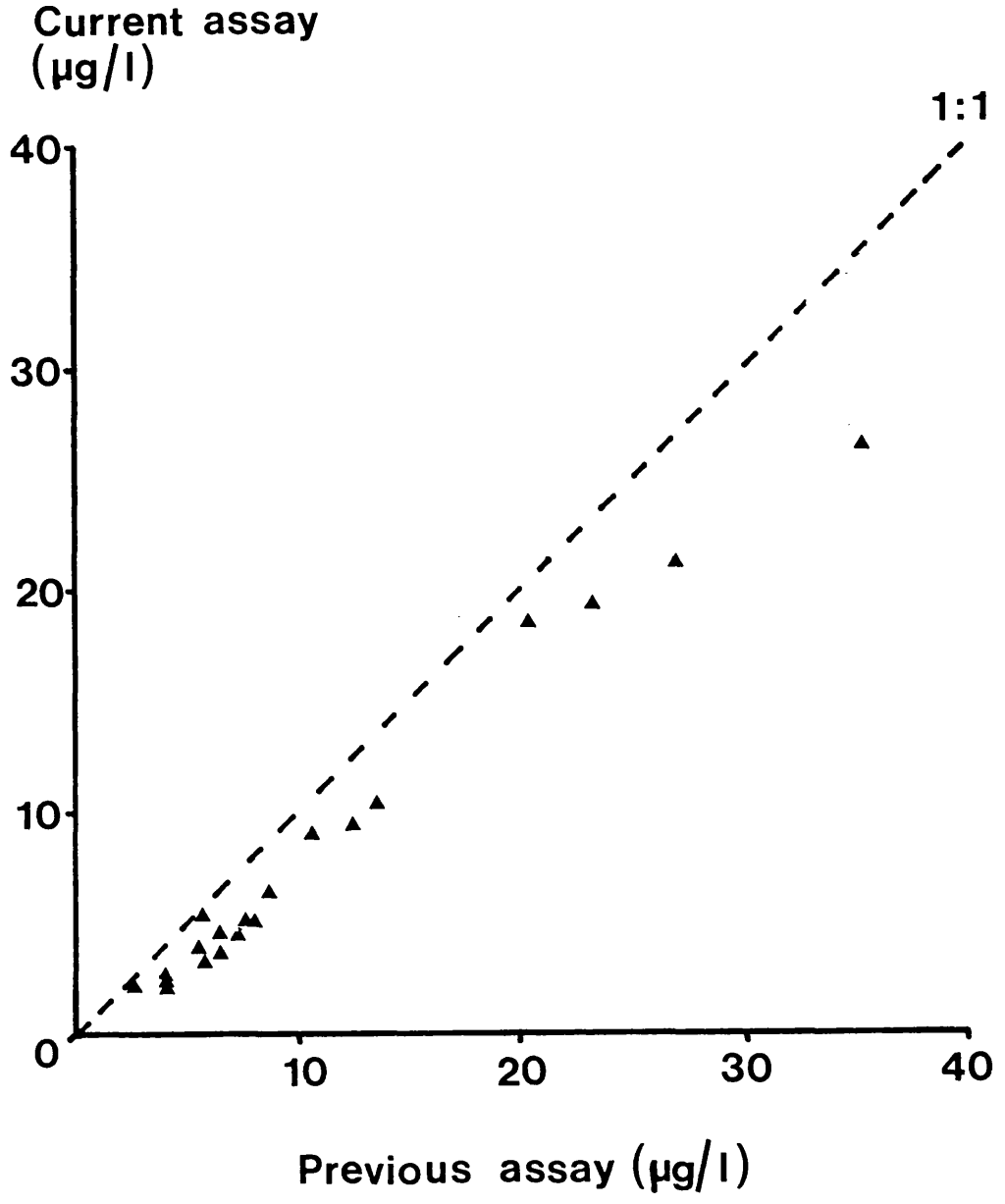


FIGURE 2.9: Comparison of prolactin levels in red deer plasma samples estimated using the current and previous assay used to measure prolactin. $y = 0.76x - 0.15$, $r^2 = 0.96$, $P<0.0001$.



(McNeilly and Freisen, 1978; validated for use in red deer by Curlewis *et al.*, 1988b) were determined using the current assay. The results of this comparison are shown in Figure 2.9. The correlation coefficient was very high (r^2 = 0.96) and analysis of variance upheld the Null hypothesis that differences in estimates were due to chance (P = 0.29; equation of line, y = 0.76x - 0.15, P<0.0001).

2.6.3.2. LUTEINIZING HORMONE

Plasma LH concentrations were measured using the heterologous ovine double antibody radioimmunoassay of Foster and Crighton (1974), with the modifications described by McLeod *et al.* (1982b). Validation of this assay for measuring LH in deer has been reported by Loudon *et al.* (1990).

Materials and Reagents

(i) **Assay buffer**

The buffer used for all dilutions in the assay was a 0.5% solution of egg albumen (A5253: Sigma Chemical Co., Poole, U.K.) in 0.013M di-sodium hydrogen orthophosphate with 0.014M sodium dihydrogen orthophosphate, pH 7.0, 0.15M sodium chloride, 0.015M sodium azide (used as a preservative) and 0.001M ethylenediaminetetra-acetic acid (disodium salt). Buffer was made up in deionised distilled water and stored at 4°C. Egg albumen was added before use.

(ii) **LH standard**

LH standard (NIH-LH-S16) was stored as a stock solution of 50µg/l at -20°C until required. For assay, this was serially diluted in buffer from 25µg/l to 0.195µg/l in 8 dilutions.

(iii) **Quality control plasma samples**

Pooled red deer plasma with low (c. 2 µg/l), medium (c. 6 µg/l) or high (c. 15 µg/l) levels of luteinizing hormone were stored in 500µl aliquots at -20°C until required.

(iv) **Plasma samples**

These were stored at -20°C until assayed.

(v) **First antibody**

Rabbit anti-ovine LH (R07/7: Dr Philip Knight, Sch. Animal and

Microbial Sci., Reading University, Berks., U.K.). Stored as 1:100 dilution (in 100µl aliquots) at -20°C until required. For assay, a dilution of 1:15,000 in 1:200 NRS was used.

(vi) **Tracer**

I^{125}-ovine LH (Reading University, Reading, Berkshire, U.K.). For assay, it was diluted with buffer to give a solution of 10,000-12,000 c.p.m./50µl. Stock was stored at 4°C.

(vii) **Precipitation medium**

Separation of bound and free tracer was achieved by a polyethylene glycol (PEG) attenuated second antibody method. This used a 1:20 dilution of donkey anti-rabbit serum in a 4% (w/v) solution of PEG 6000 in assay buffer. The purpose of the latter was to enhance precipitation and reduce incubation time.

(viii) **Normal rabbit serum (NRS)**

Rabbit serum at 1:200 dilution in buffer was used to assist separation of bound and free fractions. Stock was stored at -20°C.

Assay protocol

Each assay consisted of up to 125 plasma samples with one standard curve.

DAY 1

Polystyrene LP4 tubes were set up as follows:

Order of tubes and contents (n)		Sample/ QC	Standard	Buffer	First Antibody
Total counts	(3)	-	-	-	-
NSB	(4)	-	-	250	-
Max. binding	(5)	-	-	200	50
Standards	(3)	-	100	100	50
QC *	(2)	100	-	100	50
Samples	(2)	100	-	100	50
Qc *	(2)	100	-	100	50
Total counts	(2)	-	-	-	-

All volumes are in µl.

'*' = at low, medium and high levels of LH.

Tubes were then vortex mixed for 10 seconds and incubated overnight at 4°C.

DAY 2

50µl of tracer was added to each assay tube. These were then vortex mixed for 10 seconds and incubated overnight at 4°C.

DAY 3

200µl of the second antibody/PEG solution was added to each tube (except total counts). These were vortex mixed for 15 seconds and incubated for 45 minutes at room temperature (c. 25°C).

1 ml of buffer was added to each tube (except total counts). Tubes were vortex mixed for 10 seconds and immediately centrifuged at 2400 r.p.m. for 30 minutes at 4°C.

Keeping the tubes at 4°C supernatants were aspirated off (using a vacuum pump) leaving a pellet containing the bound tracer. The radioactivity of this was recorded over 3 minutes using a Cobra Auto-Gamma counter model 5005. The c.p.m. of tubes containing known concentrations of LH standard, were used to produce a standard curve by a spline interpolation method (smoothed cubic spline curve). Unknown sample concentrations were interpolated from their cpm's and the standard curve.

Results were expressed as µg/l LH in plasma.

Assay range, sensitivity and precision

The mean estimated concentration of standard at 20% binding was 12.7 (± 0.2) µg/l; at 50% was 3.2 (± 0.09) µg/l, and at 80% was 0.96 (± 0.04) µg/l (n=15).

The mean assay limit of sensitivity (B_0 - 2SD) was 93.1 %, equivalent to 0.39 (± 0.04) µg/l LH in plasma (n=15). All samples with a % binding greater than this were recorded as the mean limit of sensitivity.

Inter-assay precision was 12.7 % at 1.3 µg/l, and 11.1 % at 7.0 µg/l (n=15). Intra-assay precision was 11.8 % at 1.6 µg/l, and 7.9 % at 6.9 µg/l, (n=10).

The mean (± s.e.m.) for non specific bindings (NSB/T) was 5.2 (± 0.3) %. The mean (± s.e.m.) for maximum binding tubes ((B_0 - NSB)/T) was 39.5 (± 0.9) % (n=15).

2.6.3.3. PROGESTERONE

Levels of progesterone in deer plasma were measured by direct radioimmunoassay using one of two different ovine antibodies.

361 ANTIBODY

Ovine progesterone antibody 361 (A.S. McNeilly, M.R.C., Unit of Reproductive Biology, Edinburgh, U.K.). RIA method described for sheep by McNeilly and Fraser (1987) and previously validated for measuring progesterone in deer by McLeod *et al.* (1991). This method was used to determine plasma progesterone concentrations in samples from experiments 1 and 2.

Materials and Reagents

(i) **Assay buffer**

The buffer used for all dilutions (unless stated otherwise) in the assay was a phosphate-citrate buffer (PCB), pH 6.0, consisting of 0.126M di-sodium hydrogen orthophosphate (Na_2HPO_4); 0.036M citric acid and 0.001 % (w/v) Thiomersal (used as a preservative: Sigma Chemical Co. Ltd., Poole, U.K.). Buffer was made up in deionised distilled water and stored at 4°C.

(ii) **Assay buffer with gelatine (PCBG)**

This consisted of a 0.1% (w/v) solution of gelatine in PCB. The gelatin was dissolved in the PCB by heating to 80°C.

(iii) **8-anilino-1-napthalene sulfonic acid (ANS)**

ANS was used to separate the progesterone molecules from binding proteins in the plasma. It was used as a solution of 1mg ANS/ml PCB initial dilution.

(iv) **Progesterone standard**

Progesterone (4-pregnen-3,20-dione: Sigma Chemical Co. Ltd., Poole, U.K.) was made up as a stock solution of 10µg/l (31.8nmol/l) in PCB and stored at -20°C until required. For assay, this was serially diluted in PCBG from 31.8 to 0.124 nmol/l in 9 dilutions (plasma equivalent = 63.58 to 0.248 nmol/l).

(v) **Non-detectable plasma (NDP)**

To provide a source of progesterone free plasma to add to tubes not already containing plasma, blood was collected from prepubertal

hinds. This was assayed to confirm concentrations were non-detectable in this assay.

(vi) **Quality control plasma samples**

Pooled red deer plasma with low (c. 1 nmol/l), medium (c. 7 nmol/l) or high (c. 15 nmol/l) levels of progesterone were stored in 250 µl aliquots at -20°C until required.

(vii) **Plasma samples**

These were stored at -20°C until assayed.

(vii) **Antibody**

Used at an initial dilution of 1:10,000 in PCBG. Stock was stored at -20°C.

The optimum dilution of the antibody was determined from a series of antiserum dilution curves. Standard curves for a range of dilutions between 1:5000 and 1:40,000 were subject to the assay procedure described below. For results see Figure 2.10. The range of progesterone levels most relevant to the separation of the oestrus and anoestrus states are those between 1 - 10 nmol/l ($\simeq$ 5 - 50 $x10^{-5}$ nmol/assay standard tube). A dilution of 1:10,000 (giving a final dilution of 1:35,000) was selected as the most appropriate for this range of values.

(viii) **Tracer**

11 Progesterone gluceronide-Tyramine-^{125}I was diluted with PBS-ANS to give a solution of 12,000-15,000 c.p.m./100µl. Stock label was stored at -20°C. The tracer used to analyse samples in Experiment 1 was generously provided by Dr Alan S. McNeilly (M.R.C. Unit of Reproductive Physiology, Edinburgh, U.K.) and for Experiment 2 by NETRIA Ltd. (St. Bartholomew's Hospital, London, U.K.).

(ix) **Precipitation medium**

Separation of bound and free tracer was achieved by precipitation of proteins, including the bound fraction, using a 25% (w/v) solution of polyethylene glycol 6000 (PEG) in a 0.9% saline solution containing 0.2% Tween - 20 (detergent to reduce surface tension).

Assay protocol

Each assay consisted of up to 117 plasma samples with one standard curve.

DAY 1

LP4 tubes were set up as follows:

Order of tubes and contents (n)		Sample/ QC plasma	NDP	Standard	Buffer (PCBG)	Antibody	Tracer
Total counts	(3)	-	-	-	-	-	100
NSB	(4)	-	50	-	200	-	100
Max. binding	(5)	-	50	-	100	100	100
Standards	(3)	-	50	100	-	100	100
QC *	(2)	50	-	-	100	100	100
Samples	(2)	50	-	-	100	100	100
QC *	(2)	50	-	-	100	100	100
Total counts	(2)	-	-	-	-	-	100

All volumes are in µl.

* = at low, medium and high levels of progesterone (see text)

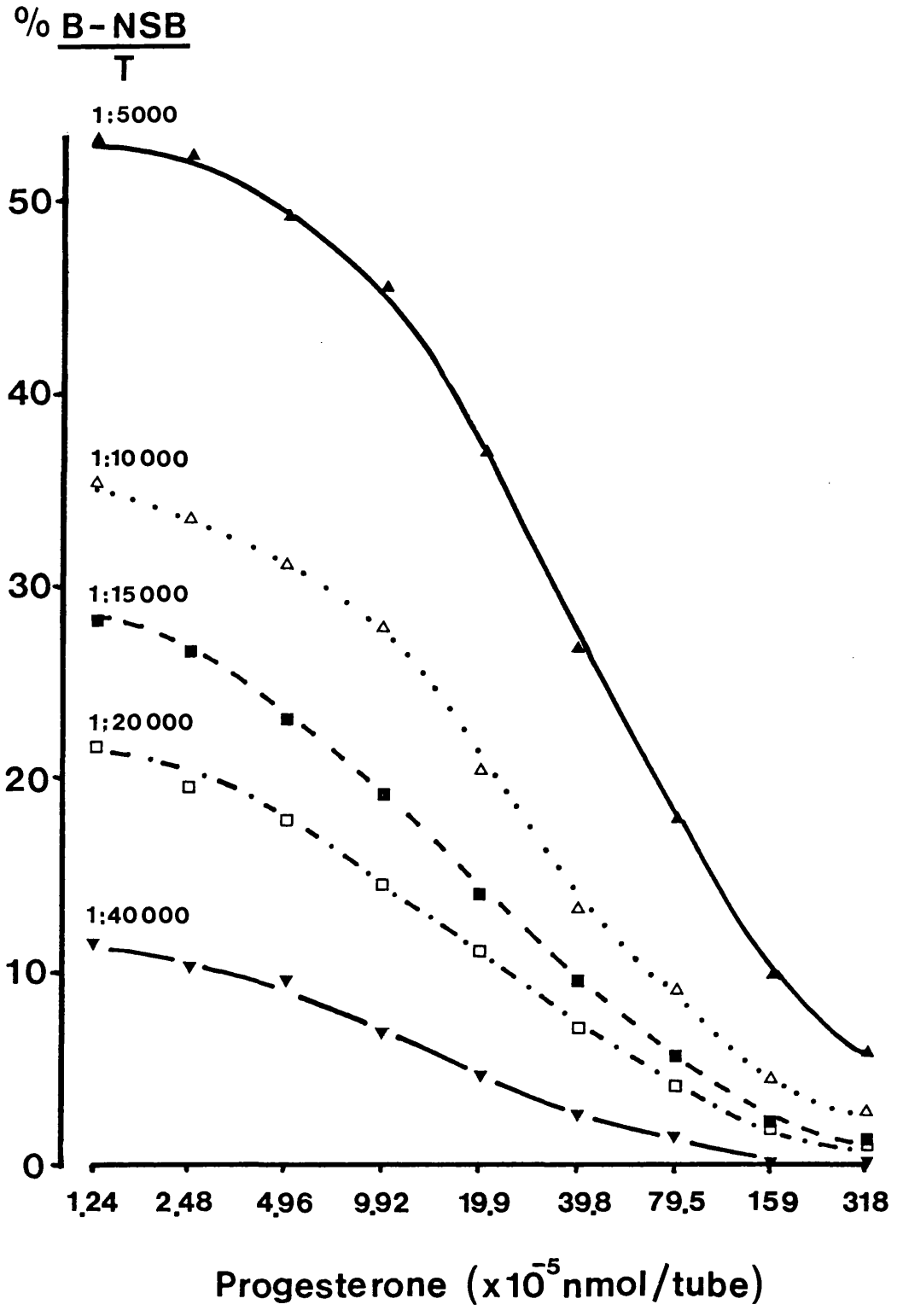
The samples, QC's, non-detectable plasma and standards were added to the tubes, followed in order by, buffer, antiserum and tracer. These were vortex mixed for 10 seconds and incubated overnight at 4°C overnight

DAY 2

Keeping the tubes at 4°C, 1ml of the precipitation medium was added to all tubes except the total counts. These were vortexed thoroughly and immediately centrifuged at 2400 r.p.m. for 30 min. at 4°C.

Keeping the tubes on ice the supernatants were aspirated off (using a vacuum pump) leaving a pellet containing the antibody bound faction. The radioactivity contained in this fraction was recorded over 3 min. using a Cobra Auto-Gamma counter model 5005. The c.p.m. of tubes containing known concentrations of progesterone standard, were used to produce a standard curve by a spline interpolation method (smoothed cubic spline curve). Unknown sample concentrations were interpolated from their cpm's and the standard curve.

FIGURE 2.10: Standard curves for progesterone assay at 1:5000 (▲); 1:10,000 (△); 1:15,000 (■); 1:20,000 (□) and 1:40,000 (▼) initial dilutions of antibody. Concentration of standard is expressed on a logarithmic scale and plotted against % binding (100 x (B - NSB)/T).



Results were expressed as nmol/l progesterone in plasma.

Assay range, sensitivity and precision

A typical standard curve is illustrated in Figure 2.11

The mean estimated concentration of standard at 20% binding was 19.1 (± 0.6) nmol/l; at 50% was 4.8 (± 0.2) nmol/l, and at 80% was 1.2 (± 0.05) nmol/l, (n=26).

The mean limit of sensitivity (B_0 - 2SD) was 94.6 % equivalent to 0.5 (± 0.01) nmol/l progesterone in plasma (n=26). All samples with a % binding greater than this were recorded as the mean limit of sensitivity.

Inter-assay precision was 19.0% at 0.9 nmol/l; 12.5% at 7.2 nmol/l, and 7.9% at 16.2 nmol/l (n=26). Intra-assay precision was 8.5% at 2.0 nmol/l; 8.7% at 3.3 nmol/l, and 7.2% at 7.3 nmol/l (n=10).

The mean (± s.e.m.) for non specific bindings (NSB/T) was 5.0 (± 0.1)%, (n=26). The mean (± s.e.m.) for maximum binding tubes ((B_0 - NSB)/T) was 33.1 ± 0.7% (n=16: M.R.C. tracer) and 54.3 ± 1.3% (n=10: NETRIA Ltd. tracer).

Comparison to previous progestrone assay

Levels of progesterone in samples estimated using this direct assay were compared to concentrations determined using a different assay carried out on solvent extracted samples (Curlewis *et al.*, 1988a). The results of this comparison are shown in Figure 2.12. Analysis of variance upholds the Null hypothesis that differences in estimates are due to chance ($P = 0.92$; equation of line, $y = -0.174 + 1.21x$, $r^2 = 94.8$ ($P<0.0001$).

S1508-10 ANTIBODY

Ovine anti-progesterone antibody S1508-10 (M.A.F. Cattle Breeding Unit, Reading, Surrey, U.K.). Used to determine plasma progesterone levels in experiment 3.

FIGURE 2.11: Specimen standard curves for the progesterone assay using (a) 361 antibody and (b) S1508-10 anibody. Concentration of standard is expressed on a logarithmic scale and plotted against % binding (B/B_0 x 100).

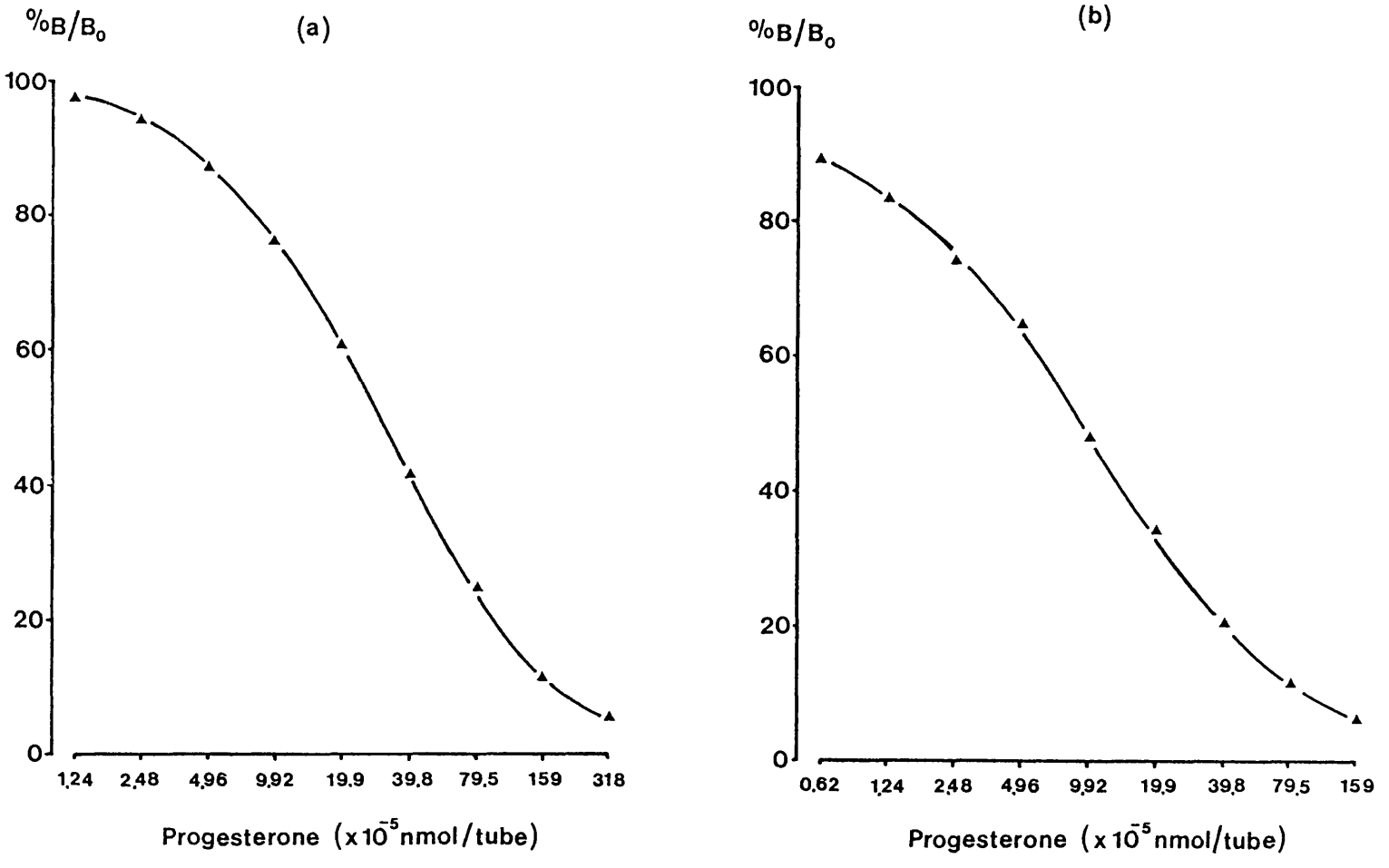
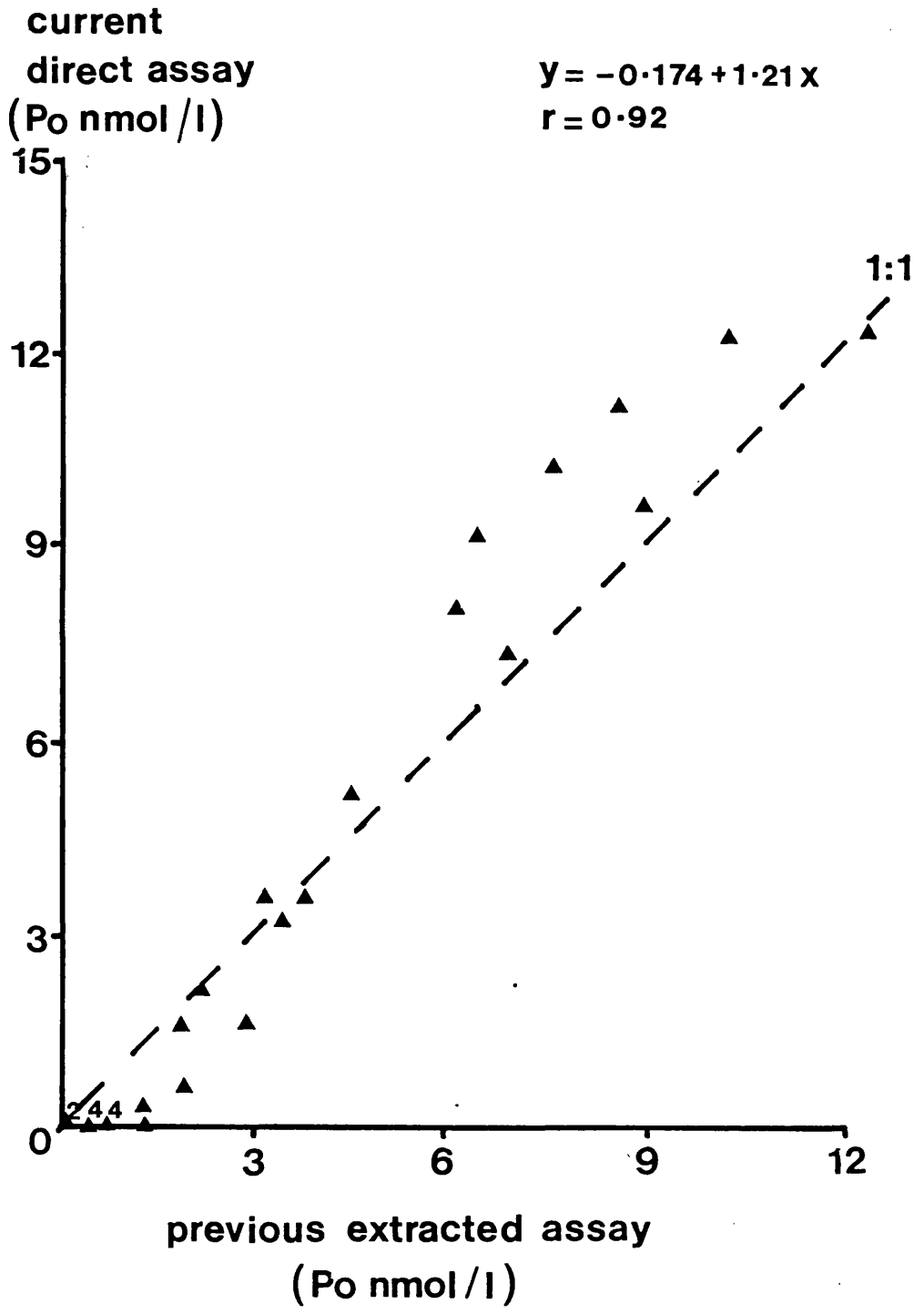


FIGURE 2.12: Comparison of progesterone levels in red deer plasma samples estimated using the current direct and previous solvent extraction assays.



The assay procedure was the same as described above with the following exceptions:

Materials and reagents

(i) **Antibody**

This was used at an initial dilution of 1:7,500 in PCBG. Stock was stored at -20°C.

(ii) Progesterone standard

This was serially diluted in PCBG from 15.9nmol/l to 0.057nmol/l in 9 dilutions.

(iii) **Non-detectable plasma**

Red deer stag blood was used as a source of progesterone free plasma. To ensure it was free of any possible progesterone the plasma was treated as follows: 10g/l activated, washed charcoal was added to the plasma and stirred for one hour at 4°C. This was then centrifuged and the supernatant decanted three times before filtering through a 0.2µm FlowPore D filter (Sartorius, Germany) into 2.5ml aliquots. Plasma was stored at -20°C until required. Samples of NDP were subject to assay procedure to confirm progesterone levels were non-detectable.

(iv) **Tracer**

Provided by NETRIA Ltd. (see above).

Assay protocol

Each assay consisted of up to 50 plasma samples with one standard curve.

DAY 1

LP4 tubes were set up as follows:

Order of tubes and contents (n)		Sample/ QC plasma	NDP	Standard	Buffer (PCBG)	Antibody	Tracer
Total counts	(3)	-	-	-	-	-	100
NSB	(4)	-	100	-	200	-	100
Max. binding	(5)	-	100	-	100	100	100
Standards	(3)	-	100	100	-	100	100
QC *	(2)	100	-	-	100	100	100
Samples	(2)	100	-	-	100	100	100
QC *	(2)	100	-	-	100	100	100
Total counts	(2)	-	-	-	-	-	100

All volumes are in µl.

'*' = at low, medium and high concentrations of progesterone (see text).

Assay range, sensitivity and precision

A typical standard curve is illustrated in Figure 2.11b.

The mean estimated concentration of standard at 20% binding was 4.4 (± 0.09) nmol/l; at 50% was 0.86 (± 0.02) nmol/l, and at 80% was 0.11 (± 6.1 x 10^{-3}) nmol/l, (n=31).

The mean limit of sensitivity (B_0 - 2SD) was less than the lowest concentration standard (plasma equivalent = 0.06 nmol/l). All samples with a % binding greater than this recorded as that of lowest concentration standard.

Inter-assay precision was 16.3% at 2.7 nmol/l;; 14.6% at 3.9 nmol/l, and 7.0% at 14.9 nmol/l (n=31). The mean (± s.e.m.) for non specific bindings (NSB/T) was 7.1 (± 0.3)%, (n=31). The mean (±

s.e.m.) for maximum binding tubes ((B_0 - NSB)/T) was 40.9 ± 0.6% (n=31).

2.6.3.4. OESTRADIOL 17β

Oestradiol 17β levels in plasma samples were generously assayed by Dr R. Webb (A.F.R.C., Institute of Animal Physiology and Genetics Research, Edinburgh, Roslin, Midlothian, EH26 9PS). The protocol used is described in Webb, Baxter, McBride, Nordblom and Shaw (1985).

2.6.4. ONSET AND TERMINATION OF BREEDING SEASON

Progesterone profiles in peripheral plasma were used to determine the onset and termination of the breeding season in the manner described by Loudon *et al.* (1989). The onset was defined as the date of the first increase in progesterone concentrations above 1.6 nmol/l for more than two consecutive blood samples (samples collected twice a week). The termination of the breeding season was taken as the last follicular phase of the breeding season. This was defined as the last period of low progesterone concentrations (< 1.6 nmol/l) followed immediately by progesterone concentrations in excess of 1.6 nmol/l for at least two consecutive samples. Anoestrous and follicular phase levels of progesterone are typically less than 0.3 nmol/l in red deer (Loudon *et al.*, 1989).

CHAPTER 3

THE ROLE OF THE ENVIRONMENT AND ENDOGENOUS SEASONAL STATE IN THE CONTROL OF HERBAGE INTAKE, REPRODUCTION AND PLASMA PROLACTIN CONCENTRATIONS IN RED DEER HINDS. (experiment 1)

3.1 INTRODUCTION

The existence of a seasonal appetite cycle is well documented for enhoused deer fed an *ad libitum* diet (see section 1.2.8. and Fig. 1.1.). The cycle synchronises nutritional requirements to natural patterns of herbage abundance. Its role in determining herbage intake in free-ranging deer, however, remains unclear. Annual cycles of body weight in wild deer (Mitchell *et al.*,1976) suggest a seasonal change in food intake, but fail to distinguish between the effects of appetite and herbage availability. In domestic sheep grazing agricultural pastures, foraging behaviour and herbage intake are strongly correlated to pasture characteristics (see section 1.2.12.1.). The latter suggests that intake primarily reflects food abundance rather than appetite. The objective of this study was to investigate the interaction between herbage availability and seasonal appetite changes in the control of herbage intake and foraging behaviour in red deer.

Daily administration of melatonin (10 mg at 1600 hr.) from 3 July, resulted in a two week advance in the seasonal decline in appetite of hinds maintained on an *ad libitum* diet (see Figure 3.1; Milne *et al.*, 1990). This phase-shift in response to exogenous melatonin was utilized during this study to permit comparison of animals in different appetite states under the same environmental conditions. The interaction between appetite and food availability was investigated by comparing herbage intakes of melatonin-treated and non-treated hinds grazing pastures providing different levels of food abundance during autumn, winter and spring.

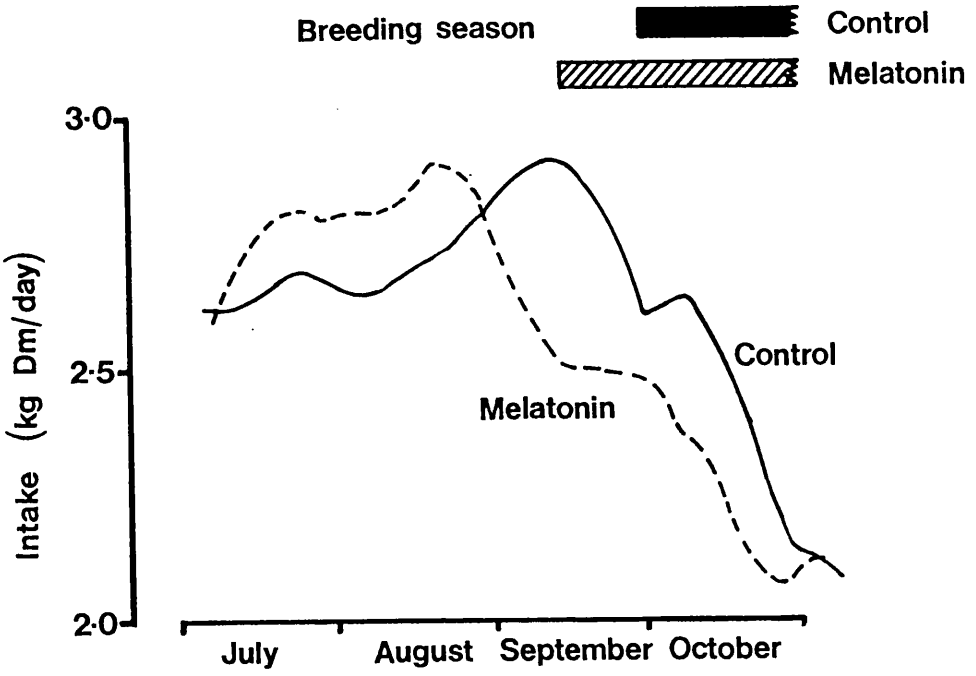


FIGURE 3.1: The effect of melatonin (10 mg administered orally once daily at 16.00 h from 3 July, – – –) and control (———) treatments on VFI of red deer hinds. From Milne *et al.*, 1990.

The seasonal appetite cycle in red deer exhibits a close temporal relationship with changes in reproductive condition, pelage and plasma prolactin concentrations (see section 1.2.6.). Melatonin administered from mid-summer until autumn not only advanced the decline in appetite, but also that of plasma prolactin concentrations (Adam *et al., 1987, 1989b; Milne et al.*, 1990), the onset of oestrous cyclicity (Adam *et al.*, 1986; Milne *et al.*, 1990) and winter coat growth (Webster and Barrell, 1985; Fisher *et al.*, 1988, 1990). The influence melatonin treatment exerted on seasonal changes later in the cycle, however, was not determined. It was the purpose of the second part of this study, to investigate the longer-term consequences of advancing the phase of seasonal cycles by

administering melatonin during summer. The influence on seasonal changes in appetite and plasma prolactin concentrations were investigated by comparing these parameters in treated and non-treated hinds during autumn, winter and spring. The influence on the breeding season was determined from the effect on timing of oestrous cyclicity. Unmated, Scottish red deer hinds will exhibit repeated oestrous cycles until March (Guinness *et al.*, 1971; Adam *et al.*, 1989a). The duration of cyclicity and the timing of seasonal anoestrus were compared in hinds exhibiting an advanced breeding season (following melatonin treatment) and in non-treated hinds. The purpose was to determine whether the time the breeding season commences influences the timing of anoestrus. The mechanism controlling the termination of the breeding season in deer remains unclear. In the ewe, evidence suggests the latter is primarily controlled by an endogenous mechanism rather than directly by prevailing environmental conditions. Anoestrus occurs spontaneously even in the absence of increasing spring daylengths (Worthy and Haresign, 1983; Robinson and Karsch, 1984). In addition, artifically shortened winter photoperiods (Malpaux *et al.*, 1988a,b) or melatonin treatment initiated towards the end of the breeding season (Nett and Niswender, 1982) can extend the period of ovarian activity, but treatments do not prevent its termination. In red deer however, recent evidence suggests lengthening photoperiods during spring may actively drive females into anoestrus. Adam *et al.* (1989a) gave hinds melatonin daily to simulate a short-day photoperiod from before the end of the breeding season for sixteen months. In contrast to untreated females which entered seasonal anoestrus in March, the treated animals continued exhibiting oestrous cycles throughout the period of melatonin treatment. This observation suggests that the termination of the breeding in red deer females is primarily determined by increasing daylengths in spring.

3.2 EXPERIMENTAL DESIGN

The experiment was performed at the Macaulay Land Use Research Institute's Glensaugh Research Station (Laurencekirk, Kincardineshire, U.K., 56°54'N) between July 1987 and June 1988.

Animals

Hinds were selected from the Institute's herd of farmed red deer, the origin of which is described by Blaxter *et al.* (1974). All animals were accustomed to handling.

Experimental treatments

Sixteen non-lactating hinds were treated orally with melatonin (as described in section 2.6.2.1.) from the 24 July to 8 October 1987 (group M). Sixteen untreated non-lactating hinds acted as controls (group C). Groups were balanced for hind live-weight at the onset of treatment and maintained on a common pasture. The mean live weights of melatonin-treated and control groups were 87.1 (± 1.7) kg and 89.7 (1.9) kg, respectively.

The response of hinds to either a low (LP) or a high (HP) herbage availability pasture was investigated on 3 occassions. These corresponded to the autumn decline, winter nadir and spring rise in appetite, observed in enhoused hinds fed *ad libitum* and maintained on natural photoperiods (Loudon *et al.*, 1989; see Figure 3.2).

Between comparisons animals were managed as a single group and maintained on a common pasture.

Experimental pastures

Different pastures were used in each comparison. During the autumn and spring comparisons herbage availability was defined by the sward surface height and herbage mass of perennial rye grass/white clover pastures. For the winter comparison it was determined by the availability of acid grass and heather dominated communities. Half of the hinds in each group were allocated to the

LP (groups LC and LM) and half to the HP (groups HC and HM) during each comparison. The method of allocation provided an orthogonal balanced design (i.e. balanced for live weight and previous treatment) which was applied as follows:

Autumn comparison: The 16 animals in each of the melatonin-treated or control groups were ranked according to liveweight. Adjacent pairs were allocated to either the low or high availability treatments.

Winter comparison: The two sets of 8 animals in each of the initial high and low availability treatments were ranked according to initial liveweight (i.e. before the start of the experiment). From adjacent pairs one animal was allocated to high and one to low availability treatments.

Spring comparison: The 4 sets of animals in each of the initial high and low availability treatments were ranked and allocated as described in the winter comparison.

Experimental Design

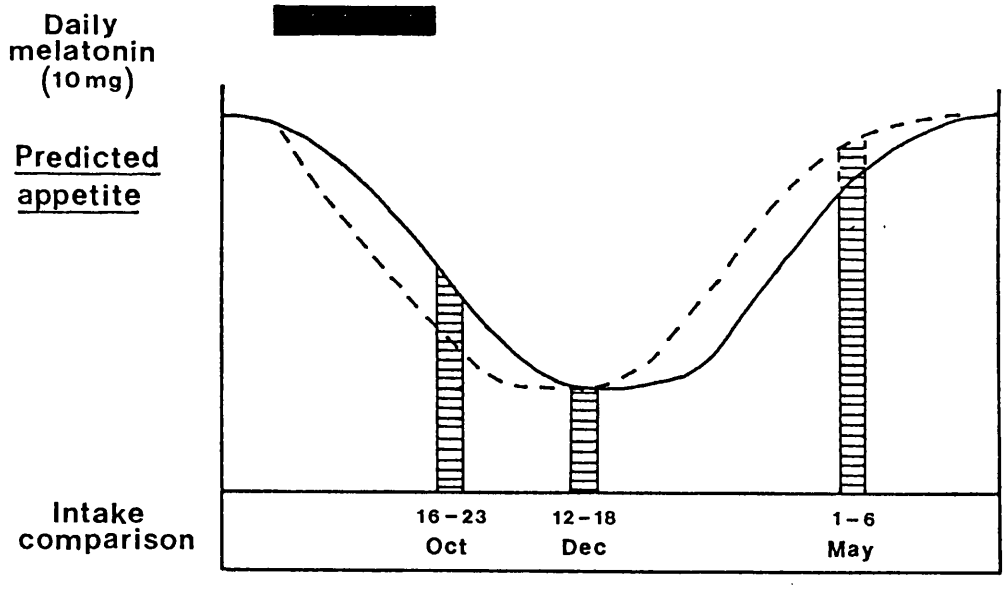


FIGURE 3.2: The predicted seasonal appetite changes melatonin-treated (– – –) and control (———) groups. Hinds were challenged with either 'high' or 'low' availability of herbage on 3 occasions (shaded bars).

MEASUREMENTS

Full details of the materials and methods outlined below are given in chapter 2.

Endocrine parameters

Blood samples were collected by jugular venipuncture at twice weekly intervals from September 1987 until May 1988, and thereafter once weekly until 20 June 1988. Plasma was assayed to characterise circulating levels of progesterone and prolactin. Plasma progesterone was monitored to determine the onset and termination of the breeding season (as described in section 2.6.4.).

Herbage intake

Herbage intake measurements consisted of a 1-2 week acclimatization period on experimental pastures, followed by a measurement of herbage intake using the n-alkane technique (see section 2.1.1.2.). This was measured over 7 days.

Hinds were weighed before and after each herbage intake determination.

Grazing activity

Observations of grazing activity were made during daylight hours (average length 850 min.) following each intake measurement to determine treatment effects on behaviour. Any hind exhibiting oestrus during the observation period was excluded due to the profound associated changes in behaviour (described in section 2.3.1.1). Activity was noted every 10 minutes during autumn and winter comparisons and every 5 minutes during the spring comparison (as described in section 2.3.1.2.). Bite rates were estimated from repeated determinations of the time taken for 20 bites. Behaviour was monitored from a discrete distance (30-100m) using a pair of 8x30 magnification binoculars.

3.3 STATISTICAL ANALYSIS

Analysis of variance was applied to the results, and the differences between means examined by the method of Least Significant Difference. Herbage intake was expressed in relation to metabolic live weight (Kleiber, 1961) to remove the effects of body size on herbage intake.

Prolactin profiles were compared by analysis of variance with repeated measures using log-transformed data so that an assumption of equal variances could be made. Analysis was carried out using the Genstat V program (Lawes Agricultural Trust). The onset of the spring rise in prolactin was defined as the first of 5 consecutive plasma samples with concentrations greater than twice the mean level for each hind between November and February (inclusive).

All data are expressed as mean ± s.e.m. (unless stated otherwise).

3.4 RESULTS

3.4.1 AUTUMN COMPARISON

Herbage availability

The mean (± s.e.m.) herbage masses of LP and HP were 701 (± 108) kg DM/ha and 1393 (± 446) kg DM/ha respectively ($P < 0.001$). The mean sward heights were 3.3 (± 0.12) cm for LP, and 5.0 (± 0.2) cm for HP ($P < 0.001$). The areas of LP and HP were 0.6 and 1.0 ha respectively.

TABLE 3.1
The responses of melatonin-treated and control hinds to variation in herbage availability during the October measurement period ($\bar{x}$ ± s.e.m.). Values with different superscripts are significantly different ($P < 0.05$).

Hind response	CONTROL		MELATONIN-TREATED	
	HP	LP	HP	LP
Herbage intake ($gOM/kg^{0.75}/day$)	90.0^{A} ± 5.95	51.2^{B} ± 3.39	70.9^{C} ± 2.66	51.3^{B} ± 2.82
Digestibility of diet	0.846^{E} ± 0.0031	0.766^{F} ± 0.0117	0.824^{G} ± 0.0062	0.774^{F} ± 0.0033
Liveweight change (kg) 5 - 23 Oct	1.50^{H} ± 0.57	-3.13^{H} ± 0.79	1.00^{H} ± 0.76	-0.88^{H} ± 1.08
Bite rate (bites/min)	57.8^{I}	54.1^{I}	55.1^{I}	56.5^{I}
Proportion of observed period				
Grazing	0.545^{J} ± 0.0290	0.600^{J} ± 0.0316	0.549^{J} ± 0.0207	0.611^{J} ± 0.0219
Active non-grazing	0.079^{K} ± 0.0180	0.174^{L} ± 0.0342	0.062^{K} ± 0.0103	0.132^{L} ± 0.0198
Inactive	0.374^{M} ± 0.0425	0.225^{N} ± 0.0210	0.387^{M} ± 0.0246	0.255^{N} ± 0.0135

Herbage intake and digestibility of diet

Herbage intakes for the 4 groups between 16 and 23 October are summarised in Table 3.1. Herbage availability profoundly influenced herbage intake. Intake was significantly greater in groups grazing the high (HP) than the low (LP) availability pasture ($P < 0.001$).

Melatonin-treated hinds grazing the HP consumed less herbage ($P < 0.05$) than non-treated females on the same pasture. There was, however, no effect of exogenous melatonin on the herbage intake in hinds grazing the LP.

The digestibility of the diet was greater for hinds grazing HP than LP ($P < 0.001$). In addition, melatonin treatment was associated with lower digestibility among hinds grazing the HP ($P < 0.05$) but not the LP.

Liveweight change

In general, hinds on LP lost weight while those on HP made small gains during the period of intake measurements. However, differences were not significant.

Melatonin-treatment did not significantly influence live weight.

Grazing behaviour

Observations of the daily activity-budget of hinds on both pastures were carried out over a period of 650 minutes, between dawn (0640 hrs) and dusk (1730 hrs). Three hinds, 1 from each treatment except the LP control group, exhibited behavioural oestrus during the observation period and were excluded from results (see section 3.2). Observations are summarized in Table 3.1.

The bite rate of grazing hinds was unaffected by herbage availability. However, deer on the LP spent a greater proportion of the observed period grazing, if values for all hinds on each pasture are pooled (0.606 *vs* 0.547; $P < 0.05$). Whilst active, however, the time spent grazing by LP groups was less than that of HP groups (0.811 ± 0.003 *vs* 0.906 ± 0.001, respectively; $P < 0.01$). As a result, LP groups spent a significantly longer time active than the HP groups (0.76 *vs* 0.62; $P < 0.01$). The greater non-grazing activity of LP hinds appeared to be associated with foraging activity.

Melatonin treatment exerted no significant effect on grazing behaviour.

3.4.2 WINTER COMPARISON

Herbage availability

The high and low herbage availability pastures (HP and LP) consisted of a 1:3 ratio by surface area of swards dominated by acid grass or heather separated into 3 clearly distinguished strips (heather:grass:heather). Pasture areas were 0.13 ha grass and 0.38 ha heather for LP, and 0.38 ha and 1.15 ha respectively for HP. There were no differences between HP and LP in the initial sward height of grass (HP, 4.9 ± 0.33 cm; LP, 4.2 ± 0.29 cm) or its herbage mass (HP, 3304 ± 536 kg DM/ha; LP, 3856 ± 394 kg DM/ha). The proportion (by weight) of green material in the grass sward was 30.5% (± 3.0). There were no differences between pastures in sward height of heather (HP, 22.5 ± 0.69 cm; LP, 18.44 ± 0.89 cm) or its mass (HP, 17,819 ± 1379 kg DM/ha; LP, 17,182 ± 1989 kg DM/ha).

Herbage intake and digestibility of diet

To calculate the relative proportions of heather and grass in the diet it was necessary to modify the procedure of the n-alkane technique. Normally, the n-alkane concentration profile of each dietary component is determined separately for each pasture (see section 2.1.1.2.). These profiles are estimated by analysis of the n-alkane content of herbage samples of each dietary component. The accuracy of the technique is dependent on these samples being representative of ingested herbage. There was some doubt during this study concerning how representative the samples of grass had been. The estimated n-alkane profiles for the grass community of the two pastures were significantly different, although the areas were in close proximity to each other and had a similar management history. Using the original HP alkane concentrations to calculate diet proportions of hinds grazing this pasture indicated that three hinds had consumed no grass. In fact, behavioural observations revealed that these animals spent longer grazing grass than heather. This

TABLE 3.2

The responses of melatonin-treated and control hinds to variation in herbage availability during the December measurement period ($\bar{x}$ ± s.e.m.). Values with different superscripts are significantly different ($P < 0.05$).

Hind response	CONTROL		MELATONIN-TREATED	
	HP	LP	HP	LP
Herbage intake ($gOM/kg^{0.75}/day$)				
Heather	47.5^{A} ± 1.57	54.8^{B} ± 3.22	41.1^{C} ± 0.75	57.6^{B} ± 3.98
Grass	17.7^{E} ± 2.21	11.6^{EF} ± 2.03	16.2^{E} ± 3.18	8.0^{F} ± 1.78
Total	65.3^{G} ± 2.83	66.4^{G} ± 3.28	57.3^{G} ± 3.45	65.6^{G} ± 4.57
Proportion of heather in diet	0.73^{H} ± 0.025	0.823^{I} ± 0.030	0.729^{H} ± 0.040	0.877^{I} ± 0.026
Digestibility of total diet	0.510^{J} ± 0.0046	0.567^{K} ± 0.0174	0.505^{J} ± 0.0243	0.544^{K} ± 0.0160
Liveweight change (kg) 3 - 21 Dec	-4.37^{L} ± 0.82	-5.19^{L} ± 0.65	-4.63^{L} ± 0.89	-2.81^{L} ± 0.89
Proportion of observed period				
Grazing heather	0.207^{M} ± 0.0140	0.274^{N} ± 0.0226	0.176^{M} ± 0.0192	0.298^{M} ± 0.0157
Grazing grass	0.371^{O} ± 0.0194	0.247^{MN} ± 0.0211	0.407^{O} ± 0.0192	0.232^{MN} ± 0.0163
Grazing total	0.578^{P} ± 0.0210	0.521^{Q} ± 0.0162	0.583^{P} ± 0.0124	0.53^{Q} ± 0.0198
Active non-grazing	0.055^{R} ± 0.0140	0.135^{S} ± 0.0200	0.057^{R} ± 0.0114	0.121^{S} ± 0.0307
Inactive	0.408^{O} ± 0.0140	0.382^{O} ± 0.0182	0.397^{O} ± 0.0100	0.387^{O} ± 0.0187

discrepancy may have been due to the high species diversity of the grass communities, and the method of herbage sample collection. This was carried out by observing deer grazing and collecting samples similar to that seen eaten. It appears that for diverse plant communities this may be a less reliable technique than using oesophageal fistulated individuals. For the purpose of analysis of diet composition, herbage samples for each pasture were pooled to provide a mean n-alkane profile for the grass and heather communities.

The mean herbage intakes between 12 and 18 December are summarized in Table 3.2. Herbage availability did not affect overall organic matter intake. On both pastures heather formed the major dietary component by dry weight ($P < 0.001$), although its relative proportion was greatest for hinds grazing the LP ($P < 0.05$).

Melatonin-treatment did not significantly influence total intake or the proportions of dietary components on either pasture. However, actual intake of heather was lower for melatonin-treated than control hinds on HP ($P < 0.01$) .

The digestibility of the total diet (i.e. heather and grass combined) was lower for deer grazing the HP than LP, but remained unaffected by melatonin treatment.

Liveweight change

All groups experienced similar reductions in live weight whilst maintained on experimental pastures.

Grazing behaviour

Observations of the daily activity budget of hinds on both pastures were carried for a period of 850 minutes, between dawn (c. 0800 hrs) and dusk (c. 1600 hrs) on 2 consecutive days (2 days necessary due to short length of daylight). During observations 4 hinds exhibited behavioural oestrus. Three of these were melatonin-treated animals (2 from LP and 1 from HP) whilst the remaining hind was from the HP control group.

Neither herbage availability nor melatonin treatment affected bite rates. The bite rate of heather (35.6 bites/min) was higher than that for grass swards (30.2 bites/min; $P < 0.05$).

The availability of herbage strongly influenced grazing behaviour. Both groups maintained on HP spent significantly longer grazing than those on LP (0.58 *vs* 0.526, respectively). However, the total time spent active was similar (HP, 0.654 *vs* LP, 0.637). This was the result of significantly greater non-grazing activity among LP hinds. The distribution of grazing activity between heather and grass communities was affected by their availability. Hinds on the HP grazed for significantly longer on grass than heather (0.67 *vs* 0.33, respectively). In contrast, on LP hinds spent longer grazing heather communities (grass, 0.457 *vs* heather, 0.544).

Melatonin treatment exerted little influence on grazing activity. The only significant effect observed, was a difference in the relative proportion of (but not actual) time spent grazing heather and grass communities by LP groups. Whereas control hinds spent a roughly equal time utilizing each community (grass, 0.475 *vs* heather, 0.525), melatonin-treated hinds spent less time grazing grass (grass, 0.438 *vs* heather 0.563; $P < 0.01$).

3.4.3 SPRING COMPARISON

For clinical reasons, two hinds (H31 and V4), both from the control group, were removed from the experiment prior to the spring comparison. H31 was suffering from scour, and V4 from Johne's disease. As a result HP and LP control groups each included 7 hinds.

Herbage availability

The mean herbage masses of LP and HP between 1-6 May were 753 (± 118) kg DM/ha and 1856 (± 235) kg DM/ha respectively ($P < 0.001$). The mean sward heights were 3.6 (± 0.11) cm for LP, and 8.5 (± 0.3) cm for HP ($P < 0.001$). The area of LP and HP were 1.0 and 1.5 ha respectively.

Herbage intake and digestibility of diet

The estimated herbage intake of one HP control hind (deer H7)

was nearly twice that of the group mean (101.3 *vs* 55.7 ± 8.1 g OM/d/$kg^{0.75}$). This animal may have had a greater appetite, or alternatively, the difference could have been due to unnoticed voiding of alkane pellets by the hind. If a pellet is spat out there is a decline in concentrations of the dosed alkane in the faeces. This leads to an over estimation of herbage intake. The second explanation is more probable as there was no substantial difference in the grazing time of hind H7 compared to the other animals on the same pasture (0.267 *vs* 0.215 ± 0.013 of observed period). As a result the herbage intake value for this hind was excluded.

The mean herbage intakes between 1 and 6 May are summarised in Table 3.3. The intake of LP hinds was greater than that of those grazing the HP ($P < 0.05$). There was however, no effect associated with melatonin treatment.

The digestibility of the diet was similar between all groups, irrespective of pasture or melatonin treatment.

Liveweight change

All groups experienced similar increases in live weight.

Grazing behaviour

Observations of the daily activity budget of hinds on both pastures were carried out for a period of 1050 min. between dawn (0415 hrs) and dusk (2210 hrs). Observations are summarized in Table 3.3.

Bite rates and grazing times were higher for LP than HP hinds ($P < 0.01$ and $P < 0.001$, respectively). In addition, LP animals spent a greater proportion of their active period grazing than HP (0.824 ± 0.013 *vs* 0.506 ± 0.024, respectively, $P < 0.001$).

Melatonin treatment had little influence on hind activity. The only significant difference related to treatment was a lower period of inactivity among treated hinds on LP.

TABLE 3.3

The responses of melatonin-treated and control hinds to variation in herbage availability during the spring comparison. ($\bar{x}$ ± s.e.m.). Values with different superscripts are significantly different ($P < 0.05$).

Hind response	CONTROL		MELATONIN-TREATED	
	HP	LP	HP	LP
Herbage intake ($gOM/kg^{0.75}/day$)	48.1^{A} ± 3.15	56.9^{B} ± 1.87	49.3^{A} ± 2.36	59.3^{B} ± 4.72
Digestibility of diet	0.746^{C} ± 0.0129	0.716^{C} ± 0.0081	0.722^{C} ± 0.0171	0.724^{C} ± 0.0142
Liveweight change (kg) 15 Apr - 10 May	2.57^{D} ± 0.72	2.86^{D} ± 0.94	3.25^{D} ± 0.94	3.5^{D} ± 0.96
Bite rate (bites/min)	51.3^{E}	66.7^{F}	52.3^{E}	69.7^{F}
Proportion of observed period				
Grazing	0.217^{G} ± 0.0145	0.426^{H} ± 0.0122	0.221^{G} ± 0.0205	0.46^{H} ± 0.0182
Active non-grazing	0.212^{G} ± 0.0149	0.093^{I} ± 0.0066	0.213^{G} ± 0.0147	0.094^{I} ± 0.0113
Inactive	0.571^{J} ± 0.0127	0.481^{K} ± 0.0083	0.567^{J} ± 0.0144	0.446^{HK} ± 0.0079

3.4.4 BREEDING SEASON

Onset of the breeding season in melatonin-treated hinds was a mean of 15.2 days earlier ($P < 0.001$) than in control hinds (see Table 3.4). Transitory rises in plasma progesterone which failed to satisfy the criteria used to determine the onset of the breeding season (see section 2.6.4) were observed in 6 melatonin-treated and 10 control hinds.

The termination of the breeding season was 10.2 days earlier in melatonin-treated hinds ($P < 0.05$). There was no significant difference in the duration of the breeding season. Results of individual hinds are illustrated in Figure 3.3.

TABLE 3.4

Effect of melatonin treatment on timing and duration of breeding season ($\bar{x}$ ± s.e.m.). Values with different superscripts are significantly different ($P < 0.05$).

Treatment	Onset (days from 1 January)	Termination (days from 1 January)	Duration (days)
MELATONIN -TREATED	5 Oct (16)^{A} (277.4 ± 1.17)	9 Feb (15)^{C}* (40.3 ± 3.19)	127.9 ± 3.58^{E}
CONTROL	21 Oct (16)^{B} (292.6 ± 2.08)	19 Feb (14)^{D}** (50.5 ± 3.27)	124.2 ± 4.04^{E}

Numbers in parentheses are group sizes.
* : 1 hind (R17) removed after conceiving to a wild stag
** : 2 hinds removed due to ill health.

3.4.5 PLASMA PROLACTIN CONCENTRATIONS

Plasma prolactin profiles of melatonin-treated and control groups are shown in Figure 3.4. Both groups exhibited low concentrations (1.8 - 2.8 µg/l) between October and the onset of a sustained rise in concentrations during late March. There was however, no difference in either the pattern of these profiles, or the timing of the spring rise in concentrations related to melatonin treatment (melatonin-treated: 18 April (± 4.0 days); controls: 23 April (± 3.1 days)).

FIGURE 3.3: The effect of 10 mg melatonin given orally at 16.00 hr from 24 July - 8 October on the onset (mean = ▼) and termination (mean = ▽) of the breeding season (defined by period of plasma progesterone cycles - see section 2.6.4). Two hinds were removed for clinical reasons (*) and one after conceiving to a wild stag (**).

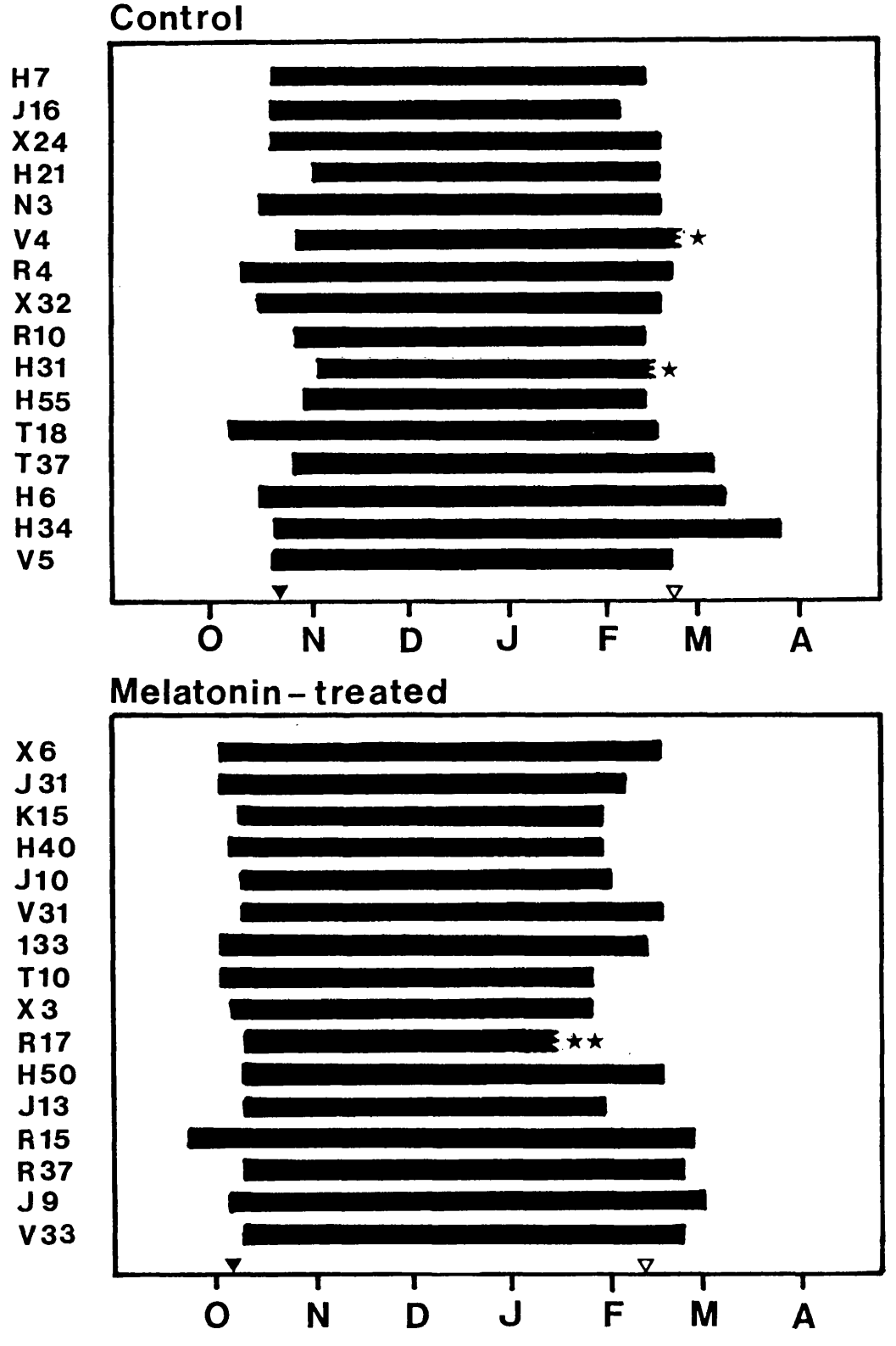
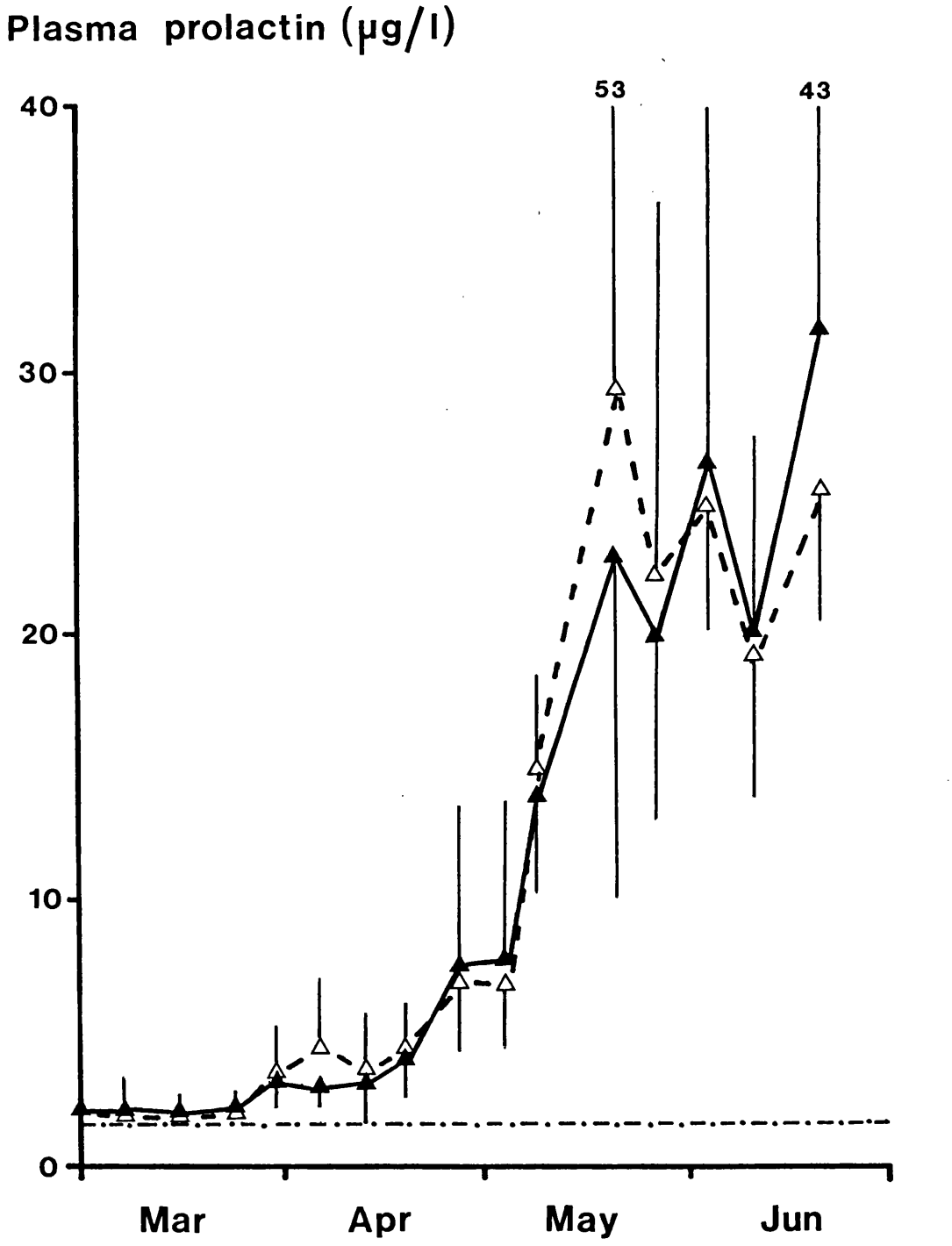


FIGURE 3.4: The spring rise in plasma prolactin concentrations ($\bar{x}$ + 95 % confidence limits) in melatonin-treated (△) and control (▲) non-pregnant hinds. The limit of assay sensitivity is indicated by (·-·-·-). Both groups exhibited a significant rise in plasma concentrations during spring ($P < 0.001$). The timing of the spring rise in concentrations and the pattern of changes was similar in both groups.



3.5. DISCUSSION

3.5.1. THE CONTROL OF HERBAGE INTAKE AND GRAZING BEHAVIOUR

3.5.1.1. The effect of herbage availability

(i) Autumn comparison

In autumn, herbage abundance was defined by the sward surface height and biomass of perennial rye-grass/white clover pastures. Herbage intake was greatest for hinds grazing the high availability pasture (HP). This is consistent with evidence in the ewe that herbage intake is positively correlated to sward surface height and plant biomass (Allden and Whittaker, 1970).

The hinds grazing the HP digested a greater proportion of the herbage they consumed than did animals on the low availability pasture (LP). As the digestibility of constant quality diets have been shown to decline as their intake increases (Kay and Goodall, 1976) it implies that the higher intakes of HP hinds were associated with more digestible plant material in the diet. This may have reflected differences in the relative proportion of stem and leaf material contained in each of the swards. Leaf material is more digestible than the stem. As the former comprised a larger component of the grass on HP, it is assumed animals grazing the latter were able to select a greater proportion of this more digestible material.

Hinds grazing the low availability pasture appear to have attempted to compensate for a lower rate of herbage intake during grazing by extending the duration of feeding activity (LP, 0.606 *vs* HP, 0.547 of observed period; $P < 0.05$). This is a typical response of grazing ungulates to food shortage (red deer: Clutton-Brock *et al.*, 1982a; Loudon *et al.*, 1984, reindeer: Trudell and White, 1981, sheep: Allden and Whittaker, 1970; Hodgson and Milne, 1978; Forbes and Hodgson, 1985). The inability of extended grazing times to fully compensate for reduced herbage availability has been commonly reported in sheep studies (Allden and Whittaker, 1970; Arnold, 1975; Hodgson and Milne, 1978; Jamieson and Hodgson, 1979) and is believed to reflect a ceiling limit to grazing time imposed by the need to ruminate. The lower proportion of activity devoted to grazing by

hinds kept on the LP was apparently associated with an increase in the effort required to locate suitable forage. The bite rates of deer grazing the low sward height were similar to those of hinds grazing the high sward height. This contrasts with evidence in sheep grazing similar pastures which has demonstrated a positive correlation between sward surface height and bite rate (Allden and Whittaker, 1970).

(i) Winter comparison

In December, herbage abundance was defined by the density of hinds grazing pastures comprising a 1:3 ratio, in surface area, of acid grassland and heather dominated communities. Hinds maintained at the lower density (i.e. high availability) spent longer grazing grass than heather areas. The reverse was true for deer kept at the higher density (i.e. low availability), although, even these also exhibited a preference for grass when differences in the relative availability of the two communities are taken into account. This variation in diet selection was reflected in the proportions of grass and heather in the diet. Hinds maintained at the lower density consumed relatively more grass, although their overall dry matter intakes were similar to those of hinds grazing at higher density. The animals at the higher density achieved a similar intake by consumimg a greater amount of heather.

Despite a better choice of available forage, dry matter digestibility of the diet was lower for animals kept at the lower density. This could reflect the greater proportion of grass in the diet of these animals. The grass contained only 30% green matter and may have been less digestible than the heather.

Typically ungulates increase the duration of grazing activity as herbage availability declines (red deer: Loudon *et al.*, 1984; reindeer: Trudell and White, 1981; sheep: Allden and Whittaker, 1970). During this comparison, however, the hinds maintained at lower density with greater apparent availability of herbage spent the longest grazing. As overal daily intakes were similar for hinds grazing both pastures, the difference in grazing time suggests a lower rate of herbage intake during grazing by deer at the lower density. Variation in intake rate may reflect the differences

observed in diet selection, in particular, the greater proportion of grass in the diet of animals kept at lower density.

Bite rates for both communities (grass, 30.2; heather, 35.6 bites/min) were relatively low compared to either the autumn (c. 55 bites/min) or spring comparisons (c. 50 - 70 bites/min). A similar difference between diverse indigenous plant communities and sown swards has been reported previously (Loudon *et al.*, 1984). This phenomenon probably reflects both: higher search times between bites, due to increased selection in diverse pastures, and increases in manipulative jaw movements associated with ingesting plants in structurally variable swards (Chambers *et al.*, 1981).

As observed in the autumn comparison, hinds grazing the lower herbage availability pasture in the present study spent a greater proportion of time active but not grazing. Visual observations suggested this was related to an increase in time spent locating suitable feeding sites on this heavily grazed pasture.

(i) Spring comparison

During spring hinds grazed perennial rye grass/white clover pastures of different sward height and biomass. Both bite rate and grazing times were greater in hinds kept on the low availability pasture. This response was consistent with the relationship that has been reported between sward surface height and grazing strategy in sheep (Allden and Whittaker, 1970; Jamieson and Hodgson, 1979; Black and Kenny, 1984 - see Fig. 1.3). It suggests that animals attempt to compensate for the reduced weight of herbage per bite associated with low sward heights (sheep, Allden and Whittaker, 1970; Black and Kenny, 1984; illustrated in Fig. 1.3) by increasing bite rate and the duration of grazing activity. In contrast to the autumn comparison (see above), herbage intake during spring was greatest in hinds grazing the low availability pasture. The increased bite rate and extended grazing times of these hinds were clearly sufficient to compensate for the reduced abundance of herbage. The fact that herbage intake was greater in deer grazing the low availability pasture may have been associated with longer grazing activity of these animals. This would have resulted in higher energy expenditure and hence elevated nutritional requirements. Unlike the autumn

comparison, it appears that the intake of hinds grazing the low availability pasture was not restricted by herbage abundance and was primarily determined by appetite state. The ability of the hinds to compensate for a low availability of herbage by modifying grazing activity may have reflected a relatively low appetite. This is implied by the low proportion of time spent grazing by hinds kept on the high availability pasture (0.219 of observed period). The duration of their daily grazing activity was equivalent to about 5.3 h/day which is considerably less than the maximum reported grazing times for female red deer (11.8 h/day, Clutton-Brock *et al.*, 1982a; 11.7 h/day, Loudon *et al.*, 1984).

The digesibility of the diet was similar for hinds grazing both pastures. This contrasts with the autumn comparison in which digestibility was greatest for hinds grazing the high sward surface height. This may reflect differences in the growth phase of the swards. During October, plant growth was negligible and as a result grazing pressure would have progressively increased the proportion of less digestible stalk material in the diet. In May, however, growth was at a peak. As a result the availability of digestible leaf matter was high even at low sward heights.

3.5.1.2. The effect of seasonal appetite state

The influence of seasonal appetite state on the herbage intake of deer was investigated by administering melatonin to a group of hinds between July and October. The purpose was to advance the phase of seasonal changes in appetite.

The lower herbage intakes of melatonin-treated hinds grazing the high availability pasture during autumn are similar to the effects observed in enhoused deer (Milne *et al.*, 1990; see Figure 3.1). It suggests that an advance in the timing of the seasonal appetite decline resulted in an early fall in herbage consumption. These data provide the first direct evidence that the seasonal appetite cycle demonstrated in enhoused animals, and illustrated in Figure 1.1 (Kay, 1979; Loudon *et al.*, 1989) can restrict the intake of grazing deer.

The autumn measurement of herbage intake, however, coincided with the onset of the breeding season. As a result variation in intake may simply reflect the different reproductive states of melatonin-treated and control hinds. All the treated hinds had commenced their first oestrous cycle (defined as the start of the first luteal phase) prior to the intake measurement period. In contrast, this was the case in only 5 of 16 control animals. The ovarian steroid oestradiol-17β can suppress food intake (sheep, Forbes, 1972; see section 5.1). Thus, differences in circulating steroid hormone concentrations between cycling and non-cycling hinds may explain the lower intake of melatonin-treated deer. It is significant, however, that while 5 out of 16 control hinds started cycling during the measurement period, on average, melatonin-treated hinds were in their first luteal phase at this time. Plasma oestradiol-17β concentrations, believed to peak shortly before ovulation, are important in initiating oestrus (ewe, Hauger *et al.*, 1977). Oestrus is associated with a substantial decline in food intake (ewe, Tartellin, 1968; Argo, 1986) which is illustrated by the 35.5 (± 11.2)% reduction in the grazing time of 3 hinds which exhibited oestrus during the present study. The fact that 5 out of 16 control hinds started cycling (i.e. exhibited oestrus) during the intake measurement period implies that the difference in intake between these and melatonin-treated hinds may have been underestimated, and not the reverse.

The lower herbage intake of the melatonin-treated hinds grazing the high availability pasture presumably reflected a reduction in nutritional requirements compared to the untreated hinds. Studies of enhoused hinds fed *ad libitum*, have observed that during autumn liveweight gains cease as appetite declines (Loudon *et al.*, 1989). Consequently an advance in the phase of seasonal rhythms following melatonin treatment could explain a reduction in energetic requirements. In addition, treated hinds may have experienced reduced maintenance energy expenditure due to the advanced growth of their winter coat providing relatively better heat insulation. The mean length of primary fibres at the time of the comparison was 8% greater in melatonin-treated than control hinds. This represented

about 2 weeks difference in the phase of primary fibre growth (Swift, 1988).

Whatever reason for the difference in herbage intake, the critical point is that melatonin-treated hinds maintained intakes below the maximum feasible under prevailing pasture conditions. This supports an hypothesis that appetite primarily determines intake.

There is no clear explanation for the small but significant ($P < 0.05$) difference in the digestibility of diet of melatonin-treated and control hinds grazing the high availability pasture. The lower digestibility of herbage by melatonin-treated hinds could reflect a faster gut passage rate. In view of their lower herbage intake this is unlikely. Current evidence suggests that passage rate normally declines as the level of herbage intake falls (Kay and Goodall, 1976). A faster gut passage rate in melatonin-treated hinds also conflicts with evidence that seasonal changes in VFI (at least in spring) are not accompanied by changes in digestibility (Milne *et al.*, 1978). Possibly the difference in diet digestibility may have resulted from the selection of a poorer quality diet by melatonin-treated hinds.

Behavioural observations failed to determine how melatonin treatment modified grazing strategy to bring about the observed differences in herbage intake among hinds grazing the high availability pasture. Variation observed in the duration of grazing activity between lactating and non-lactating ruminants suggests appetite differences may be expressed via the duration of grazing activity (red deer: Clutton-Brock *et al.*, 1982a,b; sheep: Arnold and Dudzinski, 1967). The failure to demonstrate any difference in grazing times between high pasture groups during the present study may be a consequence of insufficient length of observation, particularily at night. Loudon *et al.* (1984) studying animals from the same herd, grazing similar pastures, reported an absence of nocturnal grazing activity during July and August. Observations during this and the second experiment (chapter 4), however, suggest significant night-time activity. Low night temperatures are likely to cause a preference for diurnal grazing, in which case it is

during the night, that differences in motivation to forage are most likely to find expression.

Melatonin treatment did not result in a reduction in the herbage intake of treated hinds compared to the controls for groups grazing the low availability pasture (LP). Both control and melatonin-treated hinds on the LP consumed less than animals from *either group* grazing the high availability pasture (HP), and thus clearly failed to meet their energetic requirements. Observations of foraging activity (discussed above) indicate that both groups kept on the LP attempted to compensate for the limited abundance of herbage by extending grazing times. These data suggest that the absence of a reduced herbage intake accompanying melatonin treatment of hinds grazing the LP was the result of inadequate herbage resources to meet even the reduced appetite of melatonin-treated hinds.

Expression of melatonin's phase shift of the appetite cycle in hinds grazing the high, but not low availability pasture is an important observation. It suggests a dual role for appetite and herbage availability in the control of food intake in seasonal ruminants. Potential appetite differences failed to influence herbage intake when animals were faced with restricted food abundance. Thus, it appears that herbage availability may be the prime determinant of intake in impoverished habitats and that deer would attempt to maximize intake under such conditions. Reported grazing times of red deer feeding on indigenous pastures in Scotland are near the maximum recorded for ungulates, providing indirect support for this conclusion (stags, summer: 10.4 h/day; winter: 12.88 h/day, Clutton-Brock *et al.*, 1982a; lactating hinds: 11.7 h/day, Loudon *et al.*, 1984). When food is relatively plentiful, however, there is a greater predicted role for appetite in determining intake and behaviour.

Although melatonin treatment reduced herbage intake in some hinds during the autumn comparison there was no observed effect of treatment on intake during winter or spring measurements. Possibly the melatonin induced phase shift in the appetite cycle was

insufficient at these times to result in a significant difference in herbage intake. Alternatively, the effects of melatonin administered from July to October may be shortlived, with hinds having re-synchronized their seasonal appetite cycle by the December measurement period.

3.5.2. THE EFFECT OF EXOGENOUS MELATONIN ON REPRODUCTION

Melatonin-treatment successfully advanced the onset of the breeding season. The time of onset was 73 days after initiation of treatment representing an advance of 15 days compared to untreated hinds. This value is less than previously reported in studies where melatonin has been administered daily to non-lactating hinds (35 days: Adam *et al.*, 1986; 28 days: Adam *et al.*, 1987; 33 days: Adam *et al.*, 1989b; 19 and 22 days, Milne *et al.*, 1990). This could reflect the late onset of treatment in this study (24 July *vs* May/June: Adam *et al.*, 1986,'87,89b; early July: Milne *et al.*, 1990). The significance of this observation in interpreting the action of exogenous melatonin on the timing of the breeding season, is discussed further in chapter 4 (section 4.5.3.3.).

The onset of seasonal anoestrous in spring was also advanced in treated hinds, by mean of 10 days. Thus, the duration of the potential breeding season in treated and control hinds was similar at approximately 7 or 8 oestrous cycles in total. The advance in the time of anoestrus could reflect the persistence of the phase shift effect of melatonin-treatment on the reproductive cycle. Such an effect was not, however, observed for seasonal changes in herbage intake or plasma prolactin concentrations, which suggests no long-term effect of melatonin treatment. Alternatively, red deer hinds may exhibit a fixed length breeding season, which having started early, also ends early. As the difference in the onset of anoestrus was less than one oestrous cycle in length, however, it is equally possible that spring photoperiods directly drove the hinds into anoestrus.

3.5.3. THE EFFECT OF EXOGENOUS MELATONIN ON PLASMA PROLACTIN CONCENTRATIONS

The timing of the spring rise in plasma prolactin concentrations occurred during the same period as previously reported in red deer hinds reflecting the spring increases in daylength (Loudon *et al.*, 1989). There was no difference in the timing of the onset of the rise associated with melatonin treatment the previous summer. This suggests there was no long-term influence of treatment on the phase of this rhythm.

3.6. SUMMARY

Melatonin treatment between July and October resulted in a reduction in herbage intake by non-lactating hinds grazing a high herbage availability pasture during autumn. This provides the first direct evidence that the seasonal appetite changes observed in enhoused deer fed an ad libitum diet (Loudon *et al.*, 1989) can influence the food intake of grazing deer. The absence, however, of differences between melatonin-treated and control hinds grazing the low availability pasture indicates that seasonal appetite changes can only be expressed when herbage resources are adequate.

Variation in herbage abundance profoundly influenced the intake and grazing behaviour of hinds. During the autumn and spring comparisons low grass height was associated with extended grazing times and in spring, also increased bite rates. These responses to herbage availability are consistent with observations reported in sheep grazing comparable pastures (e.g. Allden and Whittaker, 1970). These changes to grazing behaviour were able to compensate for the effects of low herbage availability during the spring but not autumn comparison. This indicates that the ability to compensate for variation in herbage availability by modifying foraging behaviour is limited.

The December comparison demonstrated that the availability of different plant communities influenced diet selection, although a preference for grass was maintained irrespective of its relative

abundance. Diet choice was associated with differences in the duration of daily grazing activity and bite rate.

Melatonin treatment between late-July and October did not result in any observed effect on herbage intake or plasma prolactin concentrations during spring. Only the reproductive axis exhibited evidence of a long-term influence of treatment. Possibly, the advanced termination in oestrous cyclicity may have simply reflected the response of a fixed duration breeding season to an early onset, rather than the persistence of a phase shift in seasonality.

CHAPTER 4

THE EFFECT OF HERBAGE AVAILABILITY ON FOOD INTAKE, GRAZING BEHAVIOUR, PELAGE AND REPRODUCTION IN HINDS WITH DIFFERENT NUTRITIONAL REQUIREMENTS. (experiment 2)

4.1 INTRODUCTION

The first experiment (chapter 3) demonstrated that the herbage intake and foraging behaviour of hinds during autumn are determined by the interaction between seasonal appetite state and herbage availability. The study showed that appetite changes influence food intake when herbage resources are abundant. It also demonstrated that deer are able to compensate for some variation in herbage availability by modifying grazing strategies. The first experiment, however, only examined the interaction between herbage abundance and appetite state in hinds with relatively low energetic requirements. In addition, the study did not investigate the long-term effects of variation in food resources on grazing strategies, or consider the consequences for body condition and reproductive success. In this second experiment, the interaction between herbage abundance and appetite state was examined in lactating hinds over a four month period during summer.

Temperate zone habitats are characterized by seasonal variation in daylength, temperature and, consequently, plant growth. In response to these selection pressures deer have evolved numerous physiological and morphological adaptations related to survival in seasonal environments (see chapter 1). These include timing conception in autumn such that parturition coincides with increasing temperatures and plant growth during early summer. This ensures that the high energy demands of lactation (Arman *et al.*, 1974) occur at a time when food is abundant. Failure to synchronize reproduction appropriately with environmental changes can exert a heavy penalty on reproductive success and survival. In wild red deer, birth even one day after the median birth date results in a 1% increase in calf mortality (Clutton-Brock *et al.*, 1987; also see Guinness *et al.*,

1978), and a 1% reduction in the hind's probability of being fertile the next year (Clutton-Brock, et al., 1983). Whilst, however, the pattern of climatic changes is generally consistent, there can be substantial annual variation in temperature and rainfall. This should have the most influence on deer in over-populated or nutritionally impoverished habitats. Red deer populations in Scotland provide a good example. At the northern extremity of the species latitudinal range, many populations live in exposed moorland habitats. This contrasts with the open woodland and forest-edge habitats occupied by red deer throughout most of their range (Kay and Staines, 1981). Moorland areas are characterized by impoverished acidic soils with a relatively low annual production of plant biomass (Kay and Staines, 1981). In addition, population densities in Scotland are often high, due to to low predation and occassional successions of mild winters.

With the exception of the first experiment in this thesis, there have been no published studies examining the influence of herbage resources on food intake in red deer. Current understanding is based largely on the differences in foraging behaviour observed between deer grazing different plant communities (see section 1.2.12). Loudon *et al.* (1984), for example, reported that lactating hinds in summer grazing an indigenous pasture with high plant diversity, grazed for an average of 11.7 h/day. Comparable animals kept on a sown pasture, in contrast, grazed for only 6.0 h/day. The long grazing times of hinds on the indigenous pasture were associated with low bite rates. This probably reflected a high search time/bite associated with grazing a pasture of relatively high species diversity. It suggests that these animals attempted to compensate for a lower rate of intake by extending the duration of daily foraging activity. In addition, the lower live weight and milk production of hinds grazing the indigenous pasture provides further indirect evidence that pasture characteristics influence herbage intake (Loudon *et al.*, 1984). Studies of sheep grazing uniform pastures have demonstrated that a close correlation exists between herbage availability, and the individual's intake and foraging behaviour (see section 1.2.12.1). Currently however, there are no comparable data in deer detailing this relationship.

Variation in food intake potentially affects both current and future reproductive success. Reduced milk production results in lower calf growth rates (Loudon *et al.*, 1983,1984). This can reduce calf survival as lighter animals suffer higher mortality (Guinness *et al.*, 1978). In addition, evidence from studies of both wild and farmed mammals indicates that fertility is strongly corrrelated with hind body condition (Hamilton and Blaxter, 1980; Albon *et al.*, 1983, 1986). Hamilton and Blaxter (1980) observed that hinds failed to conceive if their live weight at the previous rut had been below 52 kg. At 60 kg and 80 kg the probability of calving increased from 0.49 to 0.91. There is also evidence indicating that poor nutrition delays the onset of the breeding season (deer, Loudon *et al.*, 1983; sheep, Gunn and Doney, 1975). Loudon *et al.* (1984) reported a delay of 6.2 days in lactating hinds grazing an impoverished hill pasture compared to a sown pasture. This is important as delayed conception (and hence calving) not only reduces calf survival but future hind fertility (Clutton-Brock *et al.*, 1983. 1987). The mechanism(s) by which nutrition influences the duration of anoestrus and fertility remains unclear (see section 1.2.11.3.). Body condition may act by influencing gonadotrophin secretion as reported in cattle and sheep (Wright *et al.*, 1987; Thomas *et al.*, 1989). Lactating females may be subject to an additional influence related to calf suckling activity and prolactin secretion. In Brahmin cattle, artifically reducing suckling frequency results in higher pregnancy rates (Bastidas *et al.*, 1984). It has been shown in red deer that poor nutrition was associated with elevated suckling frequencies which were accompanied by increased prolactin secretion and a delayed breeding season (Loudon *et al.*, 1983).

Ryder (1977) reported differences of about a month in the timing of coat growth between grazing and enhoused red deer. It was suggested that this may have reflected the better food quality of enhoused animals. The secretion of prolactin, which has been implicated in the regulation of pelage growth (see section 1.2.7.) is sensitive to nutrition in lactating animals (Loudon *et al.*, 1983; Suttie and Kay, 1985). In poorly nourished lactating hinds plasma concentrations are elevated (Loudon *et al.*, 1983). This may be due to the increase in calf suckling frequency which accompanies low

milk production. Normally, the moult of the summer coat and growth of the winter coat occur as plasma prolactin concentrations fall in autumn (Loudon *et al.*, 1989). Thus, delaying the seasonal decline in plasma concentrations may delay the onset of the autumn moult.

The impact of variation in herbage availability depends partly on the seasonal appetite state of the individual. During winter when food is normally scarce, appetite is low (see Fig. 1.1). Studies of enhoused deer provided with *ad libitum* access to food, have indicated that intake during this period remains low irrespective of food availability (Kay, 1979; Loudon *et al.*, 1989). In contrast, evidence from the first experiment (chapter 3) suggests that during summer when appetite peaks, prevailing herbage abundance has a marked influence on food intake.

Seasonal appetite changes are modified by lactation. Enhoused lactating hinds fed to appetite consume up to 2.6 times the maintenance ration of non-lactating females (Arman *et al.*, 1974). Herbage intake has not previously been measured in grazing lactating deer, however, higher intakes have been observed in lactating ewes (Arnold and Dudzinski, 1967; Arnold, 1975; Doney *et al.*, 1981). The longer daily grazing activity of lactating hinds on the Isle of Rhum, suggests greater herbage consumption (Clutton-Brock, 1982b). In addition, there is evidence that lactational demands modify diet selection (Clutton-Brock *et al.*, 1982b).

Prolonged anoestrus and reduced fertility are commonly associated with lactation in wild (Mitchell, 1973; Guinness *et al.*, 1978; Albon *et al.*, 1983) and captive (Adam *et al.*, 1985; Milne *et al.*, 1987) red deer. Hamilton and Blaxter (1980) reviewing data collected by Mitchell (1973) proposed that the lower live weights of lactating hinds indicate this may be partly due to the effects of lactational energetic demands on body condition when food resources are limited. Lactation also results in elevated plasma prolactin concentrations (Adam *et al.*, 1987, 1989b) suggesting there may be an effect on the timing of winter coat growth.

Objectives

The aim of this study was to investigate the interaction of the endogenous seasonal state and herbage adundance, in the control of herbage intake, grazing behaviour, reproduction and pelage growth in red deer hinds at the normal time of lactation. This was tackled in two ways. Firstly, by manipulating the food supply of grazing hinds and, secondly, by examining how differences in nutritional requirements, due to lactation and manipulation of the phase of the seasonal cycle by melatonin, influence the hind's response to limited food resources.

To examine the effects of herbage availability, lactating hinds were maintained on pastures providing either abundant or limited herbage resources throughout the summer.

The influence of lactation was investigated by comparing lactating and non-lactating hinds grazing the limited availability pasture. The influence of seasonal appetite state was investigated by advancing the seasonal decline of appetite in lactating hinds using exogenous melatonin. Administering melatonin from around mid-summer advances the phase of seasonal appetite, reproductive and pelage changes in deer (see sections 1.2.3.5. and 1.2.6). In particular, melatonin treatment has been shown to result in an advanced decline in the food intake of enhoused hinds fed to appetite in the autumn (Milne *et al.*, 1990 - see Fig. 3.1). Experiment 1 (chapter 3) demonstrated that similar melatonin treatment also reduces the herbage intake of grazing deer during autumn.

4.3. EXPERIMENTAL DESIGN

The experiment was performed at the Macaulay Land Use Research Institute's Glensaugh Research Station (Laurencekirk, Kincardineshire, U.K., 56°54'N) between July and November 1988.

Experimental treatments

Twenty-four lactating red deer hinds with calves, and 8 non-lactating hinds were allocated to 4 treatment groups as follows:

The 8 non-lactating hinds (group LNL) and 16 of the lactating animals were maintained on a low herbage availability pasture. The lactating hinds were split into two groups of 8 animals (group LL and LLM). Group LLM were given two 0.5 g sub-cutaneous melatonin implants between the 23 June - 8 November (see section 2.6.2. for implant details).

The remaining 8 lactating hinds (group HL) were maintained on a pasture providing an abundant availability of herbage.

A summary of the experimental treatments is given in Figure 4.1.

Experimental pastures

The pastures were part of a two-year-old perennial rye grass/white clover reseed sub-divided for this experiment. The area of the high and low availability pastures were 0.81 and 1.76 ha respectively. The surface height of the grass was manipulated to provide either low (4-5 cm) or high (8-10 cm) herbage availability. To achieve and maintain the desired sward surface height on low availability pasture, hind density was altered using additional hinds or temporary fencing to limit pasture area. An agricultural mower was used as required to prevent the grass height on high availability pasture exceeding approximately 10 cm. Both pastures received equal levels of a nitrogen fertiliser.

This simple grazing system was chosen in preference to a more complex indigenous pasture for two principal reasons. Firstly, the n-alkane technique used to measure intake is best suited to pastures

with a low species diversity. Secondly, the relative uniformity of the rye grass/clover pasture made it easier to quantify the effects of herbage characteristics on foraging behaviour than would have been possible using a more complex pasture.

Experimental comparisons

(i) The effects of herbage availability

These were investigated in lactating hinds by comparing group HL maintained on the high surface height sward, and group LL maintained on the low surface height sward.

(ii) The effects of nutritional status

The role of nutritional requirements in determining the response of hinds to limited herbage availability was investigated as follows:

(a) The role of lactation

This was examined by comparing lactating (group LL) and non-lactating (group LNL) hinds maintained on the low surface height sward.

(b) The role of seasonal status

This was investigated by comparing lactating hinds treated with melatonin implants (group LLM) with the untreated lactating hinds (group LL) on the low surface height sward. The purpose of melatonin treatment was to advance the seasonal decline in appetite (see Introduction) and permit comparison of animals in different appetite states maintained under the same pasture conditions. Melatonin implants were chosen in preference to a daily dosing regime to reduce handling, and permit undisturbed day-long behavioural observations.

Animals

Hinds used in this study were selected from the Institute's herd of farmed red deer, the origin of which is described by Blaxter *et al.* (1974). Hinds were accustomed to handling and had previously reared calves successfully. Seven of the non-lactating animals had

been part of the control group in experiment 1. Prior to this experiment, hinds and calves were maintained on similar perennial rye grass/white clover pastures to those used in this study. Calves were kept with their mothers until the end of the experiment.

Hinds were allocated to appropriate pastures on 22 June 1988. Lactating groups were balanced for hind liveweight, calf sex, date of birth and birth weight (see Table 4.1). Non-lactating hinds, none of whom had conceived at the previous rut, were chosen to reflect the typical live weight and condition of non-lactating animals in the herd (see Table 4.1).

TABLE 4.1
Allocation details (mean ± s.e.m.)

Treatment group	Hind weight (kg) (22 June)	Calf sex ratio ♀:♂	Date of birth	Birth weight (kg)
LL	84.0 ± 2.1	1:1	1 Jun ± 1.2	7.9 ± 0.3
HL	86.7 ± 2.8	1:1	1 Jun ± 1.4	8.1 ± 0.5
LNL	89.1 ± 2.2	-	-	-
LLM	85.1 ± 2.2	1:1	1 Jun ± 1.3	8.0 ± 0.3

MEASUREMENTS

Measurements commenced on 1 July and continued until 25 October 1988.

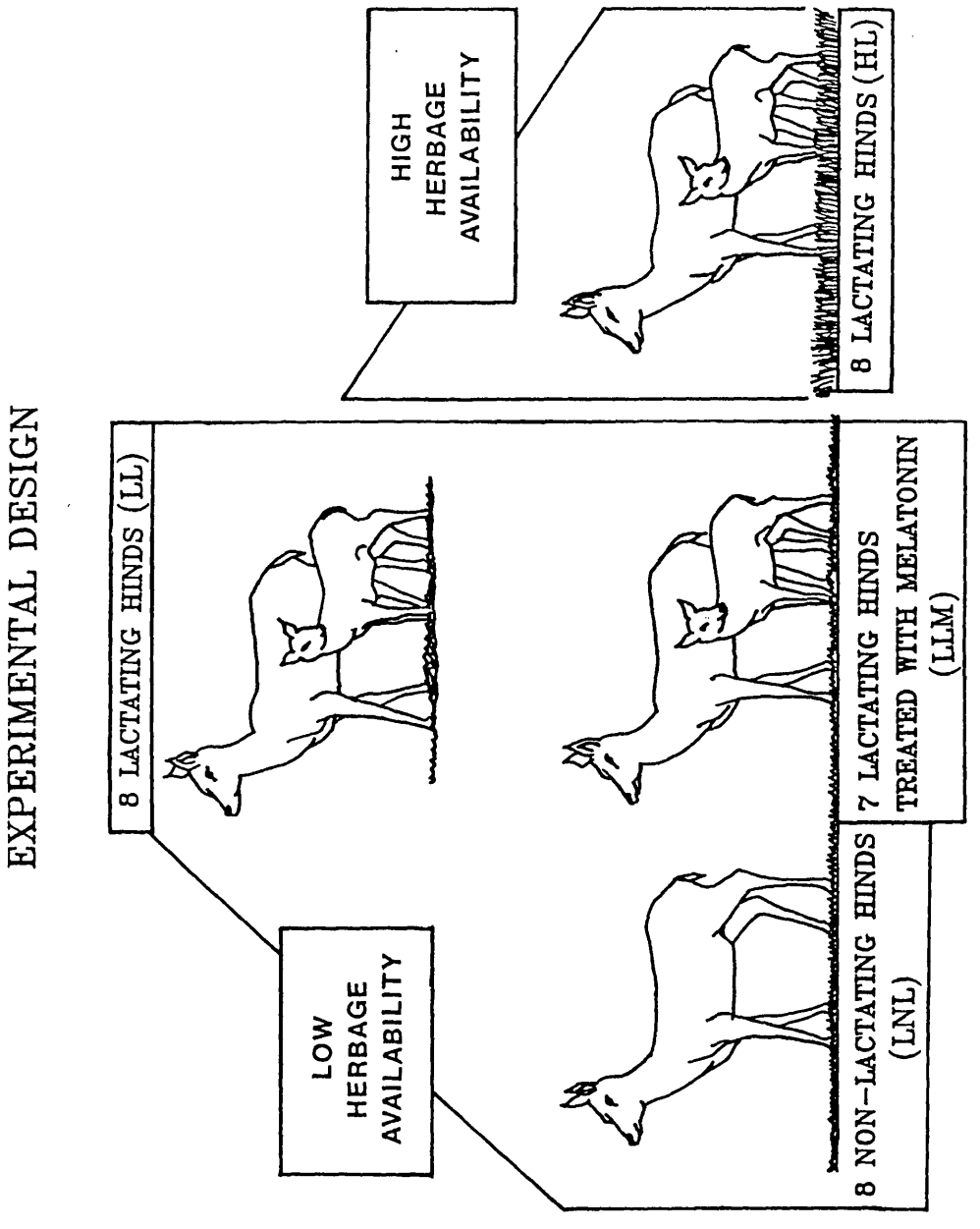
The effects of experimental treatment on nutrition, reproduction and pelage changes were assessed as follows:

Nutritional measurements

(a) Herbage intake, digestibility of diet and live weight of hinds

Daily herbage intake and the digestibility of the diet was measured on 7 occasions between July and October using the n-alkane technique (see Table 4.2). The method of intake measurement is described in section 2.1.1.2. Live weight was recorded weekly and

FIGURE 4.1


EXPERIMENTAL DESIGN
LOW HERBAGE AVAILABILITY
HIGH HERBAGE AVAILABILITY
8 LACTATING HINDS (LL)
8 LACTATING HINDS (HL)
8 NON-LACTATING HINDS (LNL)
7 LACTATING HINDS TREATED WITH MELATONIN (LLM)

body condition scores estimated at fortnightly intervals (as described in sections 2.1.2 and 2.1.3. respectively).

(b) Hind grazing behaviour

Following each estimation of herbage intake hind grazing activity was recorded. This was determined over a single continuous period of about 20 hours, between approximately 0200 and 2200 hrs (GMT). Details are given in Table 4.2. Individual hind activity categorized (as described in section 2.3.2) at 5 minute intervals during daylight and every 10 minutes at night. Observations were made from outside the experimental paddocks using 10x50 magnification binoculars during daylight, and an image intensifier (light magnification x50,000) at night. Bite rates were recorded from the same animals the following day.

TABLE 4.2

Dates and lengths of herbage intake and behavioural comparisons.

Comparison	Intake measurement period	Grazing activity observations		
		Date	Times (GMT)	Duration (min)
1	4 - 9 Jul	11 Jul	0310 - 2355	1245
2	21 - 26 Jul	28 Jul	0230 - 2135	1145
3	7 - 12 Aug	15 Aug	0200 - 2200	1200
4	24 - 29 Aug	31 Aug	0130 - 2235	1225
5	11 - 16 Sep	19 Sep	0130 - 2210	1235
6	28 Sep - 3 Oct	6 Oct	0545 - 2315	1050
7	16 - 20 Oct	24 Oct	0140 - 2140	1205

(c) Calf growth and behaviour

Live weight of calves was recorded twice weekly and used as an index of lactational performance since growth rate is linearly related to milk yield (Loudon and Kay, 1984; Robbins *et al.*, 1987)). Suckling activity was recorded between 0600 and 1600 h (GMT) during each adult behavioural observation. Every suckling bout observed was

noted to produce an estimate of daily bout frequency. In addition, bout length was measured whenever possible.

Eight-hour observations of calf grazing activity were carried out during daylight hours on 14 August, 9 September and 10 October. These recorded the number of animals from each treatment group grazing at 5-minute intervals. It was not possible to distinguish individual calves within groups. A single measurement of herbage intake was made during mid-September using the n-alkane technique.

Reproductive parameters

The onset of the breeding season was determined by monitoring plasma progesterone concentrations, measured in twice weekly blood samples as described in section 2.6.4. Hinds were monitored until they commenced oestrous cycles (up to January 1989). Calves were not weaned until sampling had ceased.

Luteinizing hormone was measured in plasma from blood samples taken at weekly intervals throughout the study. The pituitary LH response to a dose of 1 µg GnRH was determined at the beginning of each intake measurement trial (except the first). The challenge procedure is described in section 2.6.2.4. The dose was based on that used by Curlewis *et al.* (1991) in Pere David's deer, scaled for differences in live weight. The purpose was to determine treatment effects on seasonal changes in pituitary responsiveness to GnRH. Sampling frequencies necessary to characterize LH pulse frequency and amplitude would have caused unacceptable disruption to grazing activity and calf suckling.

Seasonal changes in pelage

To determine treatment effects on pelage changes the proportion of summer and winter fibres in the hind's coat was estimated visually at weekly intervals on a 5-point scale (as described in section 2.4.). In addition, the growth of the new winter coat was recorded by taking weekly *in situ* measurements of primary fibre length until late October. A further measurement was made in December and in January to determine if treatments influenced coat

length in mid-winter. In January a sample from a fixed area (4 x 4 cm) was clipped to compare the growth of winter coat by weight. This was taken from half-way up the third from last rib, on the hind's flank.

Plasma prolactin concentrations

Plasma prolactin concentrations were measured from blood samples collected at twice-weekly intervals.

4.3. STATISTICAL ANALYSIS

Analysis of variance was applied to the results, and differences between means were examined by the method of Least Significant Difference. This was carried out using the Genstat V program (Lawes Agricultural Trust, 1984). Analysis of variance of prolactin, LH, bite weight and winter coat growth data were conducted on log-transformed values so that an assumption of equal variances could be made.

Analysis of herbage intake was carried out on actual organic matter intakes, and intakes in relation to metabolic liveweight (Kleiber, 1961). The latter were necessary to distinguish between intake differences resulting from live weight effects on food requirements, and those due to experimental treatment (e.g. appetite or food availability).

The weight of herbage/bite was calculated from the mean daily dry matter intake, and an estimate of the total number of bites taken per day. Total bites/day was derived from the bite rate during grazing periods and the length of daily grazing activity. To estimate grazing time it was consumed hinds grazed for the same proportion of time during the unobserved as during the observed period.

χ^2 tests (using the Yates correction for small sample size) were used to compare the presence or absence of ovarian activity by hinds by January 1989.

All data are expressed as mean ± s.e.m. (unless stated otherwise).

Two calves in group LLM died during the study. Calf 271 (hind T3) contracted louping-ill and died on 22 September. Calf 271 (hind Z23) suffered a leg injury and died on 26 September. Data from these hinds have been excluded after the loss of their calves.

4.4.1. HERBAGE AVAILABILTY

The herbage mass and sward height of both pastures declined progressively during the study, although consistent differences were maintained between low and high availability pastures throughout ($P < 0.01$; Fig. 4.2).

4.4.2. HERBAGE INTAKE AND THE DIGESTIBILITY OF THE DIET

Herbage intake of hinds

The effect of treatments on herbage intake are shown in Figure 4.3. and 4.4. There was a significant effect of time of year on both absolute herbage intake and intake relative to metabolic liveweight. In all groups herbage intake was greatest in July, exhibiting a significant decline between July and 26 August. Intake subsequently increased between 26 August and 13 September. From 13 September until the end of the study there was a further decline in intake, although this change was only significant for lactating groups on the low availability pasture.

(i) Effects of herbage availability

There was a significant effect of herbage availability on the food intake of lactating hinds. Animals grazing the high availability pasture (group HL) maintained significantly greater intakes (absolute and relative to metabolic weight) than those on the low availability pasture (group LL) during all but the first comparison. On average, HL hinds consumed 1.2 kg OM/day more than those of group LL ($P < 0.001$).

(ii) Effects of lactation

The intakes of non-lactating hinds on low sward pasture (group

FIGURE 4.2: The mean (± s.e.m.) sward surface height and herbage masses of low (▲) and high (△) pastures during experiment 2.

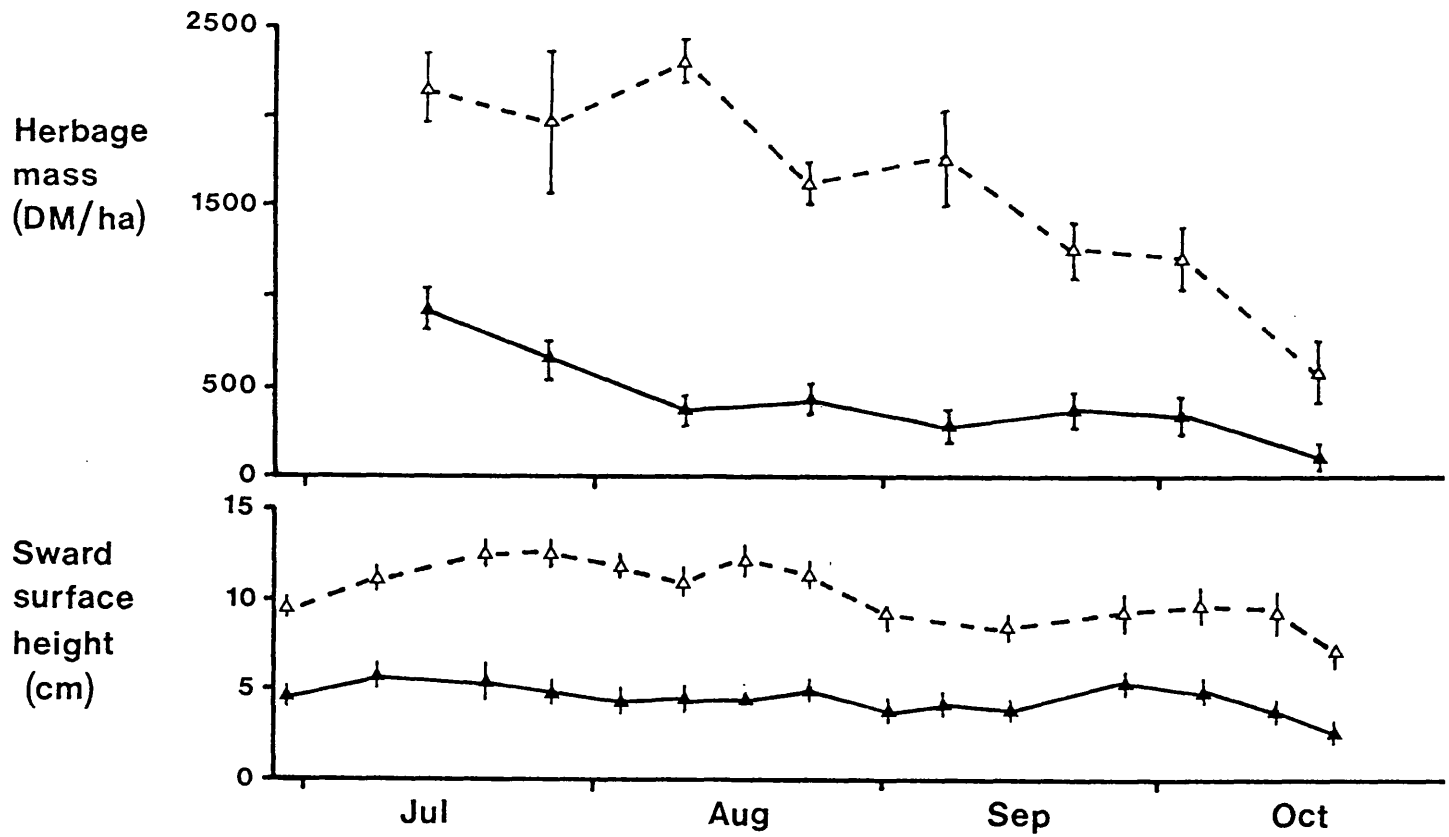


FIGURE 4.3: Mean (± s.e.m.) herbage intake relative to metabolic live weight (g OM/kg$^{0.75}$/day) for groups lactating (LL, ●); lactating with melatonin implants (LLM, ○) and non-lactating (LNL, ■) on a low availability pasture, and lactating on a high availability pasture (HL, □). In all groups, intake declined between 6 Jul and 26 Aug ($P < 0.05$), and then increased between 26 Aug and 13 Sept ($P < 0.05$). Between 13 Sept and 18 Oct there was a decline in groups LL and LLM ($P < 0.05$). LL *vs* HL, $P < 0.05$ for all time points except 6 Jul; LL *vs* LNL, $P < 0.05$ for all time points except 9 Aug and 18 Oct; LL *vs* LLM, differences not significant.

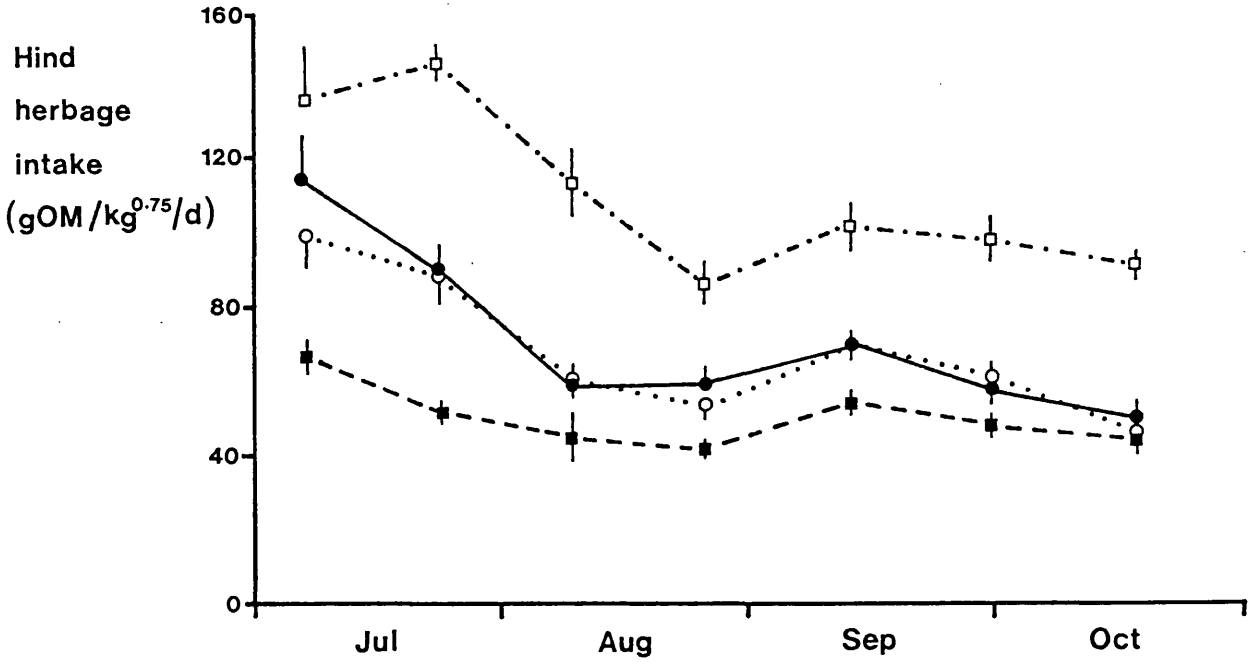
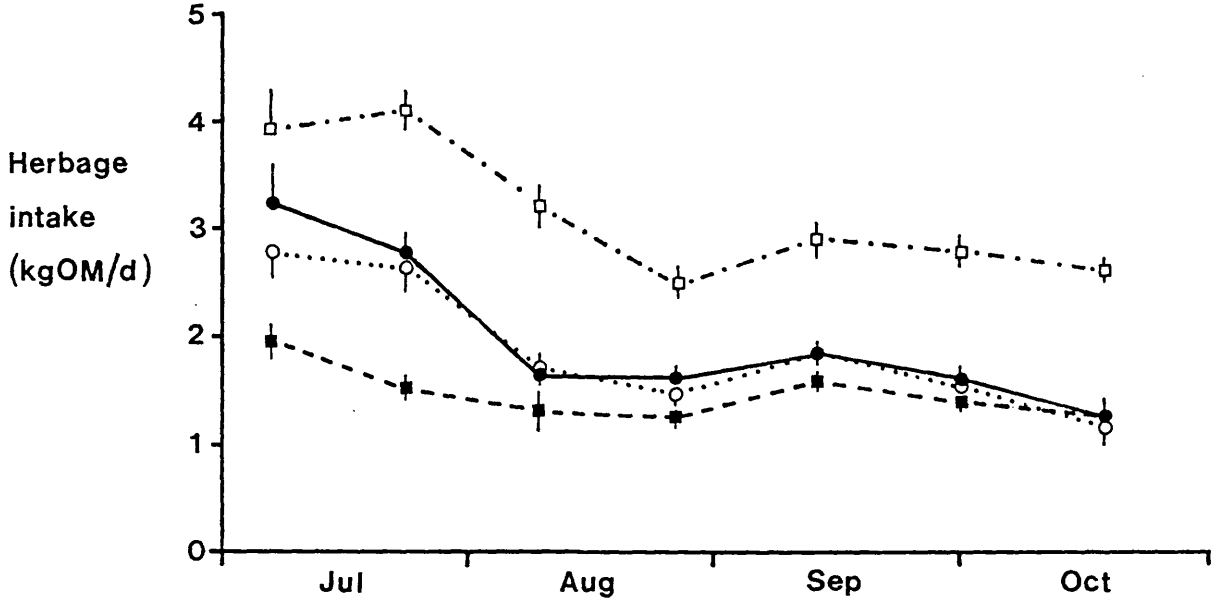


FIGURE 4.4: Mean (± s.e.m.) absolute herbage intake (kg OM/d) for groups lactating (LL, ●); lactating with melatonin implants (LLM, ○) and non-lactating (LNL, ■) on a low availability pasture, and lactating on a high availability pasture (HL, □). Significance of differences same as for Fig. 4.3, except: LL *vs* LNL, $P < 0.05$ for all time points before 13 Sept. Remaining time points not significantly different.



LNL) were significantly less than those of the lactating hinds on the low availability pasture throughout most of the summer (see Fig. 4.3 and 4.4). Differences between these groups were greatest in July, declining as the summer progressed.

(iii) Effects of melatonin treatment

Melatonin treatment of lactating hinds (group LLM) showed no significant effects on herbage intake over the course of the study.

Digestibility of diet

The effect of treatments on the digestibility of the diet are shown in Table 4.3.

(i) Effects of herbage availability

The digestibility for hinds grazing the high availability pasture was greater than that of animals on the low availability pasture during all but the second comparison (23 July). Digestiblity remained relatively constant on both pastures during the study except for the final comparison, when digestibility declined significantly for animals in group LL.

TABLE 4.3
The proportion of the diet digested by groups during each herbage intake comparisons ($\bar{x}$ ± stratum s.e.m.)
For values with different superscripts $P < 0.05$.

Comparison	Dates	GROUP LL	HL	LNL	LLM	s.e.m.
1	4-9 Jul	0.703^{A}	0.780^{B}	0.735^{A}	0.720^{A}	0.0309
2	21-26 Jul	0.752^{B}	0.786^{B}	0.763^{B}	0.723^{A}	0.0239
3	7-12 Aug	0.687^{A}	0.758^{B}	0.727^{A}	0.687^{A}	0.0581
4	24-29 Aug	0.668^{A}	0.771^{B}	0.697^{A}	0.680^{A}	0.0303
5	11-16 Sep	0.678^{A}	0.753^{A}	0.711^{A}	0.690^{A}	0.0356
6	28 Sep-3 Oct	0.695^{A}	0.779^{B}	0.728^{A}	0.714^{A}	0.0262
7	16-20 Oct	0.490^{C}	0.771^{B}	0.596^{C}	0.548^{C}	0.0204

(ii) Effect of lactation

The overal mean digestibility for group LNL during the study

was significantly higher than that of group LL (0.727 *vs* 0.697, $P < 0.01$).

(iii) Effect of melatonin treatment

The digestibility of the diet was unaffected by melatonin treatment. However, the digestibility of the diet of one LLM hind (R31) was substantially lower than the remainder of this group from comparisons 3 to 6, inclusive (0.442 *vs* 0.700). Although there was no visible indication at the time this hind may have been ill. Inclusion of R31 in statistical analysis did not affect the relationship between groups LL and LLM.

4.4.3. HIND GRAZING BEHAVIOUR

Bite rate

The pattern of bite rate changes are shown in Table 4.4. The bite rates of hinds grazing the high availability pasture were significantly less than those of animals kept on the low availability pasture. The bite rates of the 3 groups grazing the low availability pasture were similar throughout the study.

Activity budget

Changes in the pattern of hind behaviour between July and October are detailed in Table 4.4. Changes in grazing times are also illustrated in Figure 4.5.

(i) Effects of herbage availability

The proportion of the observed period spent grazing by hinds in group LL rose significantly from 0.428 on 11 July to 0.535 on 28 July. Thereafter it remained constant until 15 August after which it declined again to 0.438 of the observed period on 19 September. Between mid-September and the end of the study daily grazing activity increased to 0.592 of the observed period. Throughout the study animals in group HL spent significantly less time grazing, and inactivity was greater for hinds kept on the high availability pasture. There were no observed differences in the duration of suckling activity between the pastures. For hinds in both group LL and HL, however, the proportion of time calves suckled during the

FIGURE 4.5: Proportion of observed period spent grazing ($\bar{x}$ ± s.e.m.) by groups lactating (LL, ●); lactating with melatonin implants (LLM, ○) and non-lactating (LNL, ■) on a low availability pasture, and lactating on a high availability pasture (HL, □). For all groups there was a significant increase in grazing time between 11 Jul. and 28 Jul. ($P < 0.05$). Between 15 Aug and 19 Sep. there was a decline in all groups except HL ($P < 0.05$). Between 19 Sep. and 24 Oct. grazing increased in all groups ($P < 0.05$). LL *vs* HL, $P < 0.05$ for all time points; LL *vs* LNL, $P < 0.05$, for all time points except 6 Oct. and 24 Oct; LL *vs* LLM, $P < 0.05$ for 19 Sep. only.

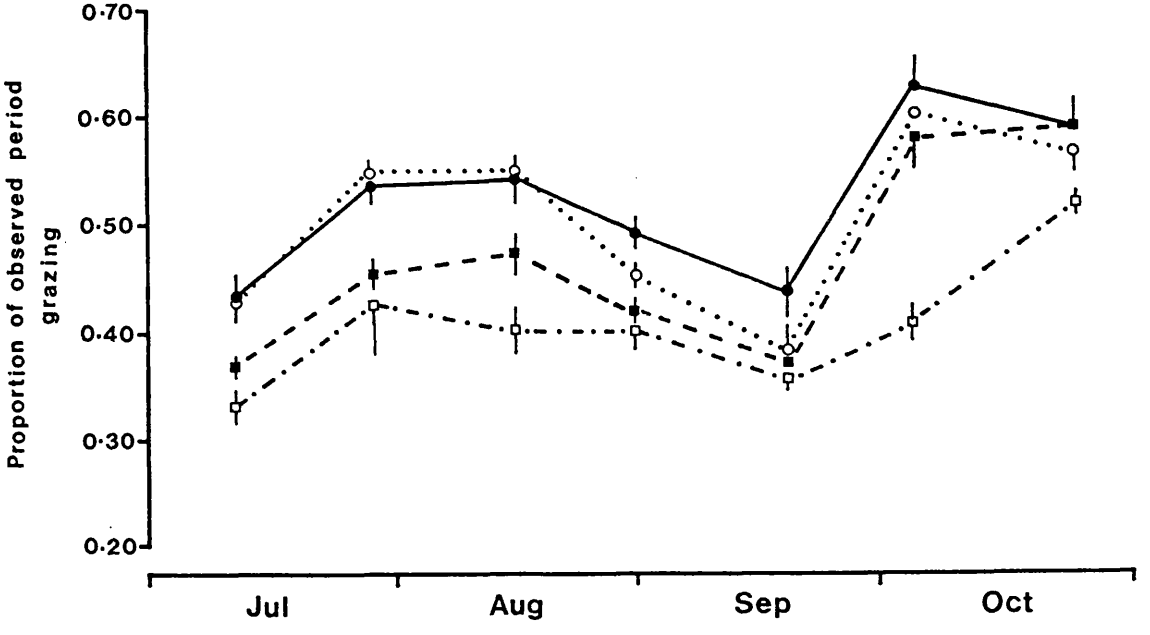


TABLE 4.4

Hind bite rate (bites/min) and activity profiles during experiment 2 ($\bar{x}$ ± stratum s.e.m.) For each comparison values with different superscripts $P < 0.05$.

Comparison		Bite rate (bites /min)	Proportion of observed period			
Date Length	Group		Grazing	Active non-grazing	Suckling (x 10^{-3})	Non-active
C1	LL	72.8^{A}	0.428^{C}	0.067^{F}	8.5^{H}	0.496^{I}
11 July	HL	63.0^{B}	0.330^{D}	0.134^{G}	8.5^{H}	0.528^{I}
1245 min	LNL	72.1^{A}	0.368^{D}	0.086^{F}	-	0.546^{J}
	LLM	71.1^{A}	0.426^{C}	0.070^{F}	4.6^{H}	0.499^{I}
	s.e.m.	4.90	0.0446	0.0342	5.13	0.0421
C2	LL	74.0^{A}	0.535^{C}	0.067^{F}	7.8^{H}	0.389^{I}
28 July	HL	69.8^{A}	0.440^{D}	0.098^{F}	8.0^{H}	0.454^{JD}
1145 min	LNL	75.7^{A}	0.459^{D}	0.142^{G}	-	0.400^{ID}
	LLM	75.0^{A}	0.550^{C}	0.062^{F}	4.4^{H}	0.384^{I}
	s.e.m.	8.17	0.0591	0.0251	4.56	0.0503
C3	LL	82.1^{A}	0.542^{C}	0.053^{F}	9.4^{H}	0.395^{I}
15 Aug	HL	61.6^{B}	0.404^{D}	0.130^{G}	7.3^{H}	0.528^{J}
1200 min	LNL	83.0^{A}	0.475^{E}	0.080^{F}	-	0.446^{KE}
	LLM	81.8^{A}	0.552^{C}	0.051^{F}	10.7^{H}	0.386^{I}
	s.e.m.	8.10	0.0543	0.0912	5.49	0.0482
C4	LL	80.4^{A}	0.490^{C}	0.025^{F}	7.1^{H}	0.477^{IC}
31 Aug	HL	63.6^{B}	0.407^{D}	0.052^{F}	5.1^{H}	0.538^{J}
1225 min	LNL	82.5^{A}	0.424^{D}	0.059^{F}	-	0.517^{IJ}
	LLM	84.6^{A}	0.448^{CD}	0.019^{F}	4.7^{H}	0.530^{J}
	s.e.m	9.79	0.0398	0.0186	4.67	0.0400

(continued)

TABLE 4.4. (continued)

Comparison		Bite rate (bites /min)	Proportion of observed period			
Date Length	Group		Grazing	Active non-grazing	Suckling (x 10^{-3})	Non-active
C5	LL	76.4^{A}	0.438^{C}	0.050^{F}	5.6^{H}	0.513^{I}
19 Sept	HL	67.8^{B}	0.360^{D}	0.081^{F}	2.5^{H}	0.556^{J}
1235 min	LNL	77.4^{A}	0.374^{D}	0.068^{F}	-	0.558^{J}
	LLM	74.1^{A}	0.381^{D}	0.038^{F}	3.5^{H}	0.581^{J}
	s.e.m.	6.39	0.0460	0.0210	3.18	0.0425
C6	LL	78.9^{A}	0.628^{C}	0.052^{F}	1.9^{H}	0.317^{I}
6 Oct	HL	71.7^{B}	0.410^{D}	0.121^{G}	1.9^{H}	0.467^{JD}
1050 min	LNL	82.4^{A}	0.583^{C}	0.125^{G}	-	0.290^{K}
	LLM	81.1^{A}	0.609^{C}	0.069^{F}	6.8^{H}	0.314^{I}
	s.e.m.	12.10	0.0659	0.0681	3.73	0.0455
C7	LL	71.4^{A}	0.592^{C}	0.017^{F}	0.5^{H}	0.391^{I}
24 Oct	HL	75.5^{A}	0.519^{D}	0.026^{F}	2.1^{H}	0.453^{J}
1205 min	LNL	73.3^{A}	0.593^{C}	0.030^{F}	-	0.378^{I}
	LLM	74.5^{A}	0.567^{C}	0.018^{F}	2.1^{H}	0.407^{I}
	s.e.m.	5.80	0.0563	0.0164	2.43	0.0530

final 2 comparisons was significantly less than during the first 5 comparisons ($P < 0.05$).

(ii) Effects of lactation

There was a significant effect of lactation on the grazing times of hinds kept on low availability pasture. From the start of the study in July until 19 September hinds in group LNL spent significantly less time grazing than those in group LL (see Fig. 4.5). Throughout most of the study non-lactating animals spent longer inactive (see Table 4.4).

(iii) Effects of melatonin treatment

There was little effect of exogenous melatonin on the activity budget of lactating hinds kept on the low availability pasture (see Table 4.4). The proportion of time spent grazing was similar between group LL and LLM except for 19 September, when the period of grazing was lower in melatonin-treated hinds.

Estimated bite weight

Changes in estimated bite weight (mg DM/bite) between July and October are summarized in Figure 4.6. For all groups there was a significant effect of time on bite weight ($P < 0.001$). The pattern of changes was similar in the 4 groups. Bite weight declined significantly between 6 July and 31 August. This was followed by an increase in estimated bite weight between 31 August and 19 September ($P < 0.05$). From the 19 September until the end of the study there was a further decline in bite weight ($P < 0.05$).

(i) Effect of herbage availability

The estimated weights of herbage/bite of hinds in group HL were approximately twice that of those in group LL throughout the study (see Fig. 4.6).

(ii) Effects of lactation

During July the estimated weights of herbage/bite were significantly lower for hinds in group LNL than LL. For the remainder of the study there was no variation associated with lactation.

(iii) Effects of melatonin

Melatonin treatment exerted no influence on the bite weight of lactating hinds grazing the low sward pasture.

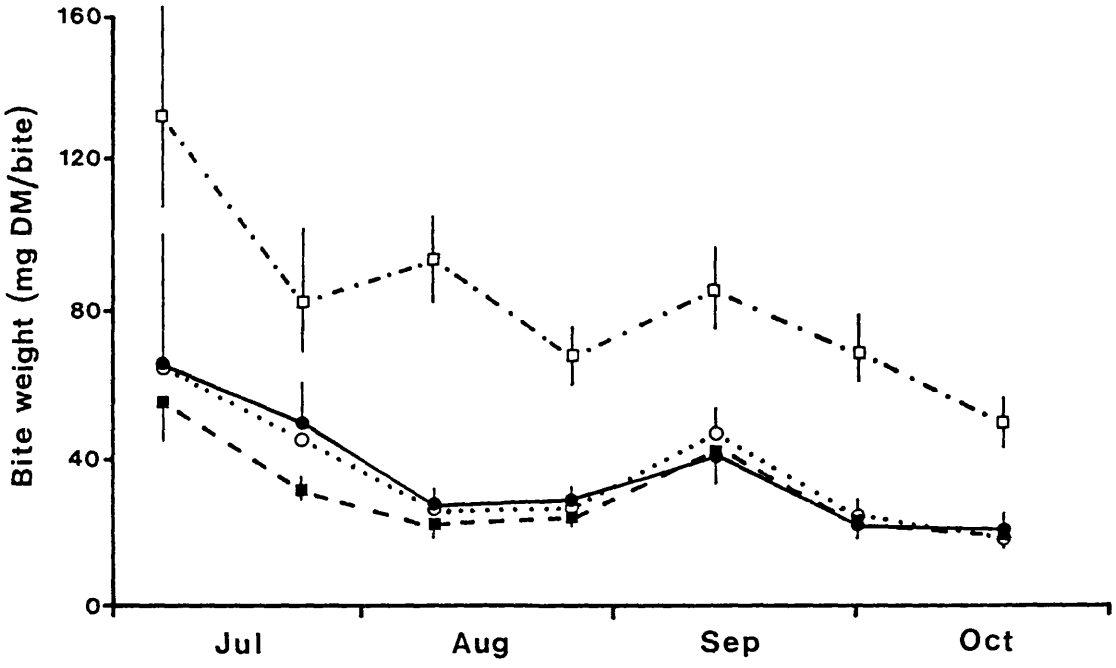
4.4.4. LIVE WEIGHT OF HINDS

The effects of treatment on live weight are shown in Figure 4.7a.

(i) Effects of herbage availability

The live weight of hinds in group LL declined significantly from a peak of 87.0 (± 2.3) kg at the start of the study to 77.3 (±

FIGURE 4.6: Estimated bite weight ($\bar{x}$ + 95% confidence limits), for groups lactating (LL, ●); lactating with melatonin implants (LLM, ○) and non-lactating (LNL, ■) on a low availability pasture, and lactating on a high availability pasture (HL, □). LL *vs* HL, $P < 0.01$ for all time points; LL *vs* LNL, $P < 0.05$ for 6 and 23 July; LL *vs* LLM, no significant difference.



1.5) kg at the end of October (Fig. 4.7a). This contrasts with hinds in group HL, which after an initial decline up until 22 July, exhibited a significant and sustained increase in live weight from the beginning of August. From 26 August the live weights of hinds in group HL were therefore greater than those of group LL.

(ii) Effects of lactation

There was a significant effect of lactation on live weight. In contrast to animals in group LL, non-lactating hinds (group LNL) maintained a relatively constant live weight during the study. From the 16 July the average weight of hinds in group LNL was significantly greater than that of group LL.

(iii) Effects of melatonin treatment

There was no significant effect of exogenous melatonin on the live weight of lactating hinds.

Hind body condition score

The effects of treatments on body condition score are shown in Figure 4.7b. See section 2.1.3. for a description of the scores.

(i) Effects of herbage availability

Herbage availability had a significant impact on body condition score. The condition score of hinds in group LL declined progressively during the summer, whereas, in group HL hind body condition remained relatively constant. As a result from 9 September the condition score of animals in group HL was greater than that of animals in group LL hinds.

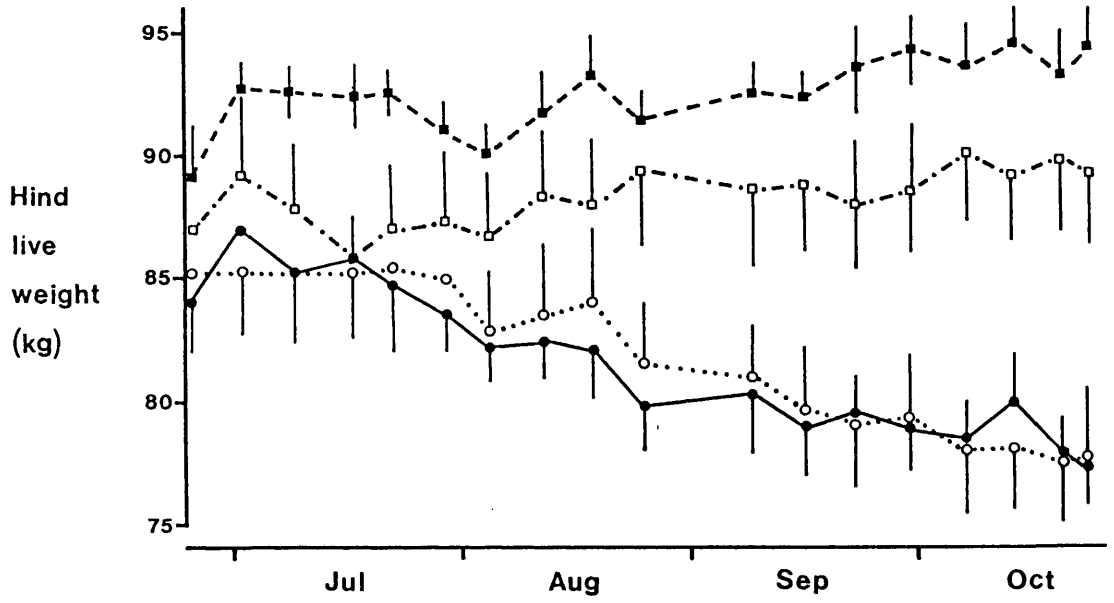
(ii) Effects of lactation

In contrast to the lactating hinds (group LL), non-lactating deer exhibited a significant increase in body condition score between 28 June and 26 July. Thereafter condition remained unchanged for hinds in group LNL. From 12 July onwards the body condition of hinds in group LNL was higher than that of those in group LL, and from 12 August it was also higher than that of animals in group HL.

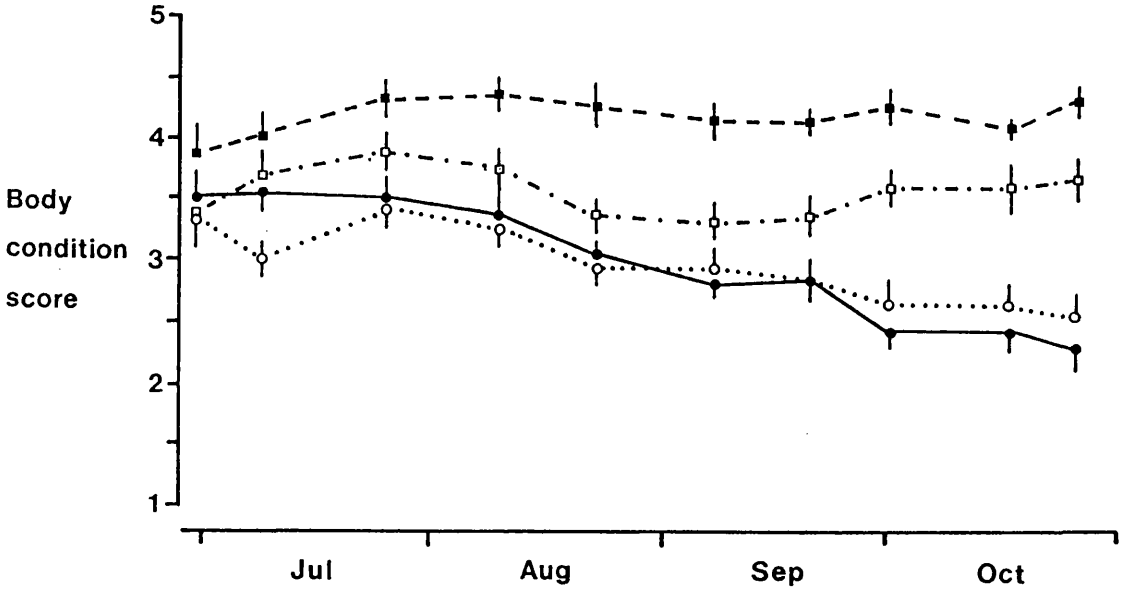
(iii) Effects of melatonin treatment

There was no observed effect of melatonin on body condition score of lactating hinds grazing the low availability pasture.

FIGURE 4.7: (a) Changes in hind live-weight ($\bar{x}$ ± s.e.m.) for groups lactating (LL, ●); lactating with melatonin implants (LLM, ○) and non-lactating (LNL, ■) on a low availability pasture, and lactating on a high availability pasture (HL, □). LL *vs* HL, $P < 0.05$ all time points from 26 Aug; LL *vs* LNL, $P < 0.05$ all time points from 16 July; LL *vs* LLM not significantly different.



(b) Changes in body condition ($\bar{x}$ ± s.e.m.) of hinds (same groups as above). LL *vs* HL, $P < 0.05$ for all time points from 9 Sept. LL *vs* LNL, $P < 0.05$ for all time points from 12 Jul. LL *vs* LLM, differences not significant.



4.4.5. CALF GROWTH AND BEHAVIOUR

Live weight

The pattern of calf growth is shown in Figure 4.8.

(i) Effect of herbage availability

Calves on the high availability pasture were significantly heavier than those on the low availability pasture from 20 August until the end of the study. The mean live weights at the end of the study (25 October) were, for calves of hinds in group LL, 43.2 (± 1.04) kg and for HL, 52.1 (± 1.78) kg.

(ii) Effects of melatonin treatment

Melatonin treatment of hinds had no effect on the growth of their calves. The live weight of calves of hinds in group LLM was 43.3 (± 2.32) kg on 25 October.

Suckling activity

There were no treatment differences in suckling bout frequency during the 10 hour observations. Bout frequency was highest during July (equivalent to 4.5 ± 0.61 bouts/day; Fig. 4.9) declining from August until the end of the study (equivalent to 1.9 ± 0.43 bouts/day).

(i) Effects of herbage availability

The average suckling bout length of calves during the period between day 40 - 90 of lactation was significantly longer ($P < 0.05$) for calves on the high availability pasture (HL, 55.5 ± 2.60 sec.; n=31) than for the two low availability pasture groups combined (LL, 49.0 ± 2.40 sec.(n=45) and LLM, 49.4 ± 2.58 sec.(n=35)).

(ii) Effects of melatonin treatment

There was no significant effect related to melatonin treatment of hinds.

Calf herbage intake

Absolute herbage intakes (kg OM/day) during mid-September were similar for all groups (see Table 4.5).

(i) Effects of herbage availability

Corrected for metabolic live weight, the combined average herbage intake of the two low availability pasture groups was

significantly greater than that of calves on the high availability pasture. The digestibility of the diet (including milk) was marginally less for calves grazing the low availability pasture (see Table 4.5).

(ii) There were no significant differences associated with melatonin treatment.

TABLE 4.5
Calf herbage intakes between 11-16 September
($\bar{x}$ ± s.e.m.)

Group	HERBAGE INTAKE kg OM/day	HERBAGE INTAKE g OM/kg$^{0.75}$/day	Digestibility of the diet
LL	0.509 (± 0.0350)	34.1 (± 2.28)^{A}	0.714 (± 0.0192)^{D}
HL	0.503 (± 0.0286)	30.1 (± 1.06)^{B}	0.764 (± 0.0059)^{E}
LLM	0.542 (± 0.0391)	37.1 (± 2.63)^{C}	0.737 (± 0.0136)^{F}

(A + C) *vs* B ($P < 0.05$) (D + F) *vs* E ($P < 0.05$)

Grazing behaviour

The pattern of changes in calf day-time grazing activity are summarized in Figure 4.9. At the time of the first measurement in August there were no differences between treatments groups.

(i) Effects of herbage availability

From September calves of hinds in group LL spent a greater proportion of the observed period grazing than those of hinds in group HL. This was despite a progressive increase in the grazing activity of both groups.

(ii) Effects of melatonin treatment

Melatonin treatment had no significant effect on grazing behaviour.

4.4.6. REPRODUCTION

The effects of treatment on reproduction are summarized in Table 4.6.

Onset of the breeding season

(i) Effects of herbage availability and lactation

The onset of the breeding season was unaffected by herbage

FIGURE 4.8: Changes in the mean (± s.e.m.) calf liveweight of hinds, lactating (LL, ●) and lactating with melatonin implants (LLM, ○) on a low availability pasture and lactating on a high availability pasture (HL, □). LL *vs* HL, $P < 0.05$ for all time points from 20 Aug. LL *vs* LLM, no significant differences observed.

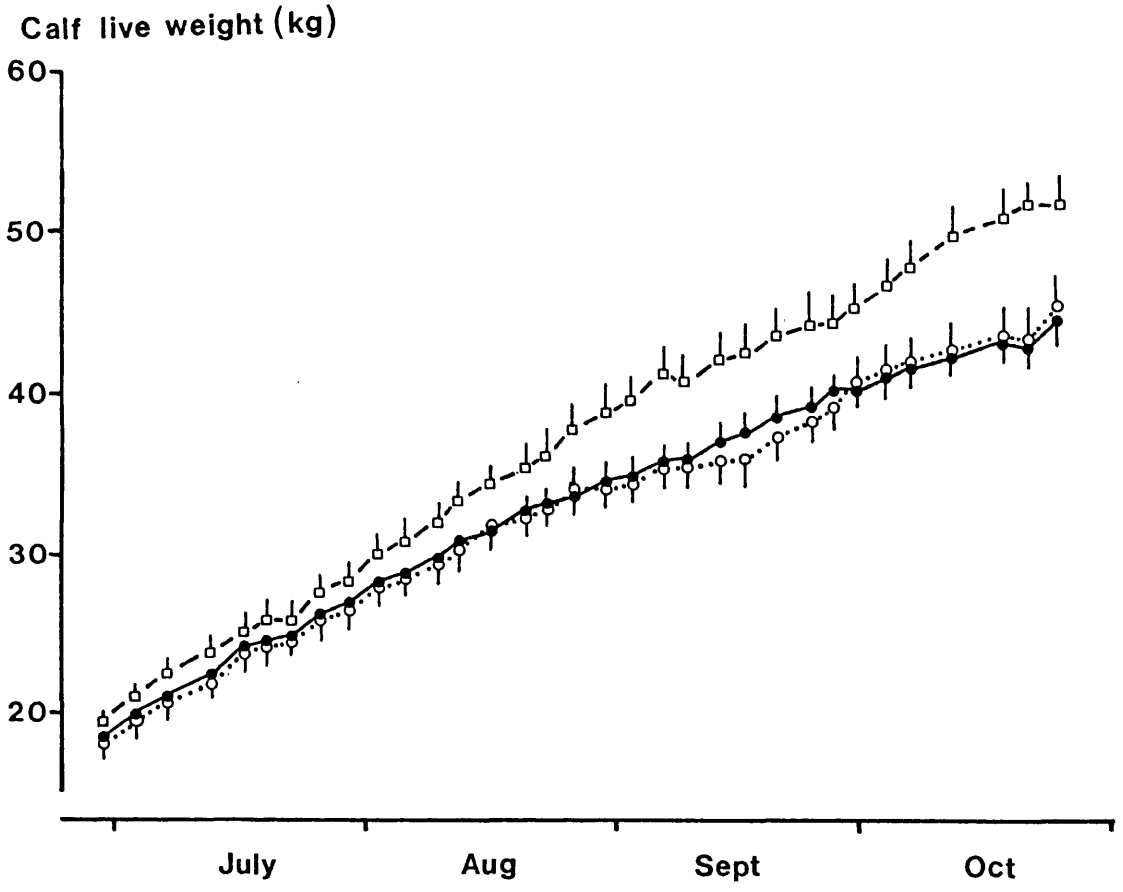
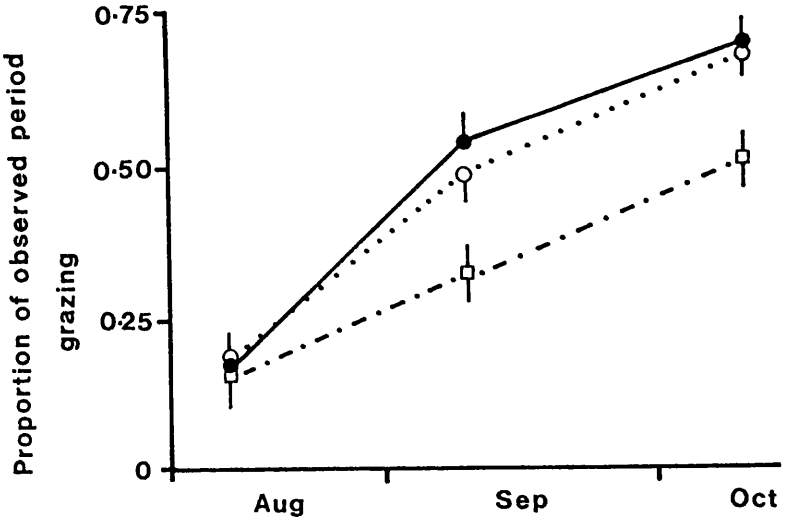
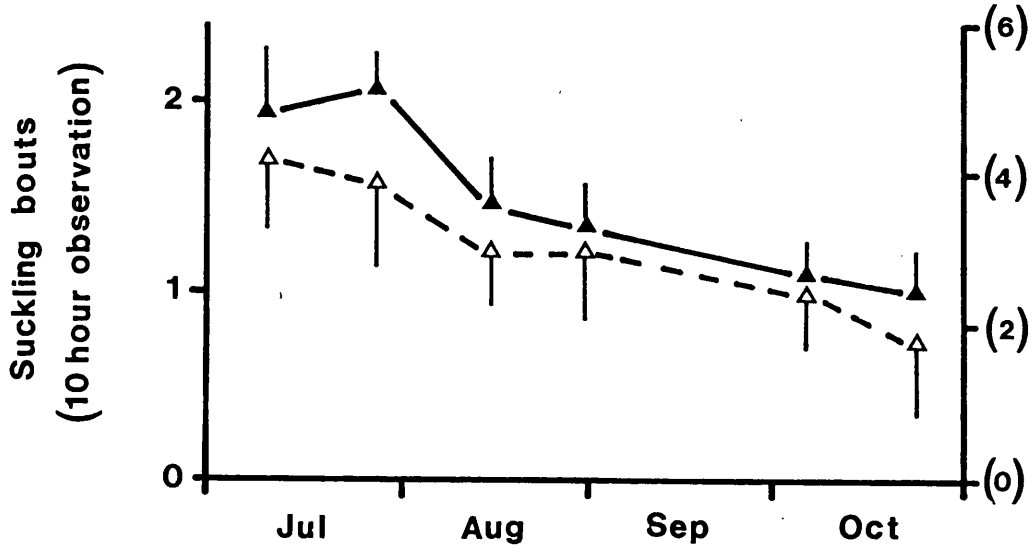


FIGURE 4.9: (a) Calf day-time grazing activity. Mean (± s.e.m.) proportion of 8 hour observation period spent grazing by calves of hinds, lactating (LL, ●) and lactating with melatonin implants (LLM, ○) on the low availability pasture and lactating on a high availability pasture (HL, □). Grazing times increased significantly with time in all groups ($P < 0.001$). LL *vs* HL, $P < 0.01$ except August measurement. LL *vs* LLM, no significant differences.



(b) Suckling bout frequency. Number of bouts (⧖ ± s.e.m.) during 10 hour day-time observation period for calves on low (▲) and high (△) availability pastures. There were no treatment effects on bout frequency. In all groups frequency declined significantly as the summer progressed ($P < 0.05$).



availability or lactation (see Table 4.6). Significantly fewer hinds in group LL, however, exhibited raised plasma progesterone concentrations by January 1989 than in either groups HL or LNL.

(ii) Effects of melatonin treatment

The onset of the breeding season commenced 18 days earlier in melatonin-treated hinds on the low availability pasture. In addition, only one hind in group LLM (R31) failed to exhibit raised progesterone concentrations by January 1989.

Luteinizing hormone

There were no significant changes observed in the weekly mean plasma LH concentrations within the four groups during the study. There were, however, significant differences between the overall mean plasma concentration for each of group, for the period July to October (see Table 4.6).

(i) Effect of herbage availability

The overall mean concentration was lower in animals in group LL than group HL.

TABLE 4.6

The effect of melatonin, lactation and nutrition on the onset of the breeding season and mean plasma LH concentrations ($\bar{x}$ ± stratum s.e.m.). Values with different superscripts are significanty different ($P < 0.01$).

Group	Onset of breeding season (days from 1 January)	Number of cycling deer	Mean plasma LH (µg/l)
LL	20 Oct (293.6 ± 5.4)^{A}	5/8^{C}	0.49 ± 1.069^{E}
HL	17 Oct (291.1 ± 1.7)^{A}	8/8^{D}	0.65 ± 1.054^{F}
LNL	23 Oct (296.8 ± 2.5)^{A}	8/8^{D}	0.44 ± 1.059^{E}
LLM	2 Oct (275.5 ± 3.6)^{B}*	7/8^{CD}	0.69 ± 1.049^{F}

* mean excluding animal T3 (calf died prior to first oestrus) = 2 Oct (275.9 ± 3.3 days).

(ii) Effect of lactation

There was no significant effect of lactation on plasma LH concentrations.

(iii) Effect of melatonin treatment

Melatonin treatment of lactating hinds was associated with an elevation of plasma LH concentrations.

The LH response of hinds to 1 µg exogenous GnRH is summarized in Figure 4.10. There was no significant effect of treatment or time on this response. In addition, there was no difference between the response of hinds that exhibited oestrus during the study and those which did not (data not presented).

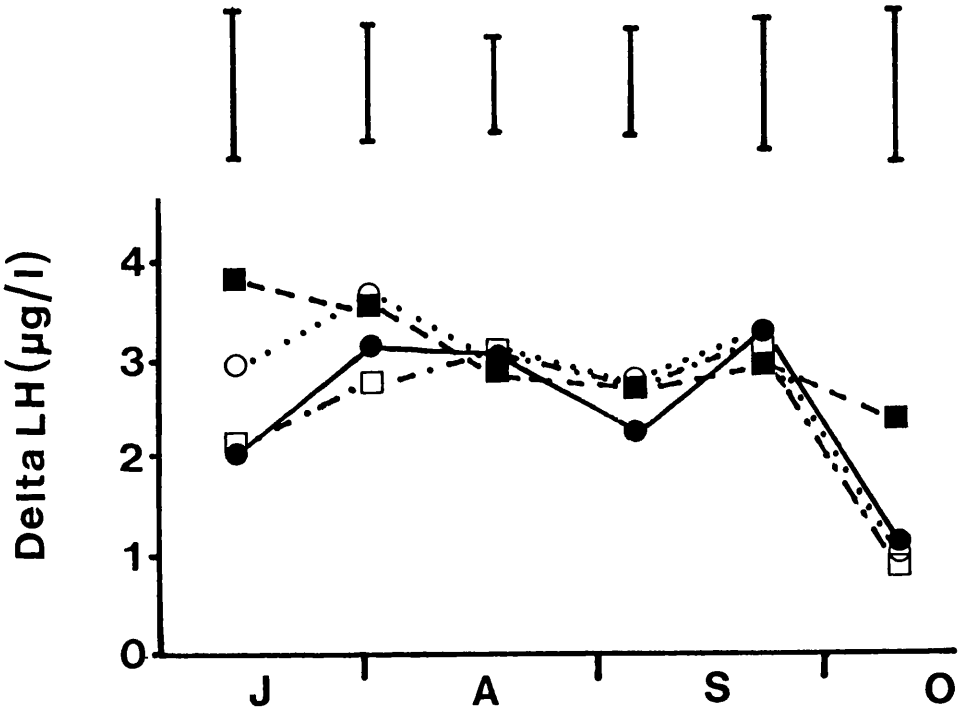


FIGURE 4.10: Response of hinds to a challenge of 1 µg GnRH. Values are ($\bar{x}$ ± s.e.m.) delta LH concentrations (t20 - t0 µg/l) of both doses of GnRH for hinds in groups LL (●), HL (□), LNL (■) and LLM (○). There were $\overset{ns}{\wedge}$ significant effects of treatment.

4.4.7. PELAGE AND PLASMA PROLACTIN CONCENTRATIONS

Changes in winter primary fibre growth and coat score are shown in Table 4.7 and in Figures 4.11 and 4.12. Changes in plasma prolactin concentrations are shown in Figures 4.13 and 4.14.

Growth of winter coat

There was substantial variation in the date of onset but not rate of winter hair growth. All groups exhibited a significant increase in winter primary fibre length from the onset of growth in late summer until the end of weekly measurements on 25 October. Coat growth commenced significantly later for hinds in group LL than in either groups HL or LNL or LLM. Primary fibre length was significantly less for hinds in group LL until the measurement on 16 January. The mass of hair (mg/cm^2) at this time was significantly lower in group LL hinds despite similar lengths implying a long-term effect of treatment on hair density (see Table 4.7).

TABLE 4.7.
Onset of winter coat growth and the mass of fibres per unit area by mid-winter ($\bar{x}$ ± s.e.m.). Values with different superscripts are significantly different ($P < 0.05$).

Group	DATE OF ONSET (days after 1 Jan 1988)	Mass of fibres on 16 Jan 1989 (mg/cm^2)
LL	5 Sept^A (248.9 ± 3.12)	127.0 ± 11.72^E
HL	27 Aug^B (239.6 ± 3.30)	166.7 ± 7.53^F
LNL	16 Aug^C (228.8 ± 3.33)	196.6 ± 8.23^F
LLM	1 Aug^D (214.1 ± 3.03)	177.0 ± 19.22^F

Coat condition score

Changes in the proportion of summer and winter fibres were strongly influenced by experimental treatment ($P < 0.001$). For all groups, the pelage consisted of predominately summer fibres at the start of the study in July (see Fig. 4.12). There were no significant differences between the groups until the onset of growth of the new winter coat. This first appeared later in hinds of group LL than those in either group HL or LNL or LLM. By late October only traces of summer coat remained in any of the groups.

FIGURE 4.11: Changes in winter coat length ($\bar{x}$ + 95% confidence limits) of hinds July and January for groups lactating (LL, ●); lactating with melatonin implants (LLM, ○) and non-lactating (LNL, ■) on a low availability pasture and lactating on a high availability pasture (HL, □). All groups are significantly different until 25 Oct ($P < 0.05$). For 6 Dec: LL *vs* HL or LNL or LLM, $P < 0.05$. For 16 Jan. no significant differences.

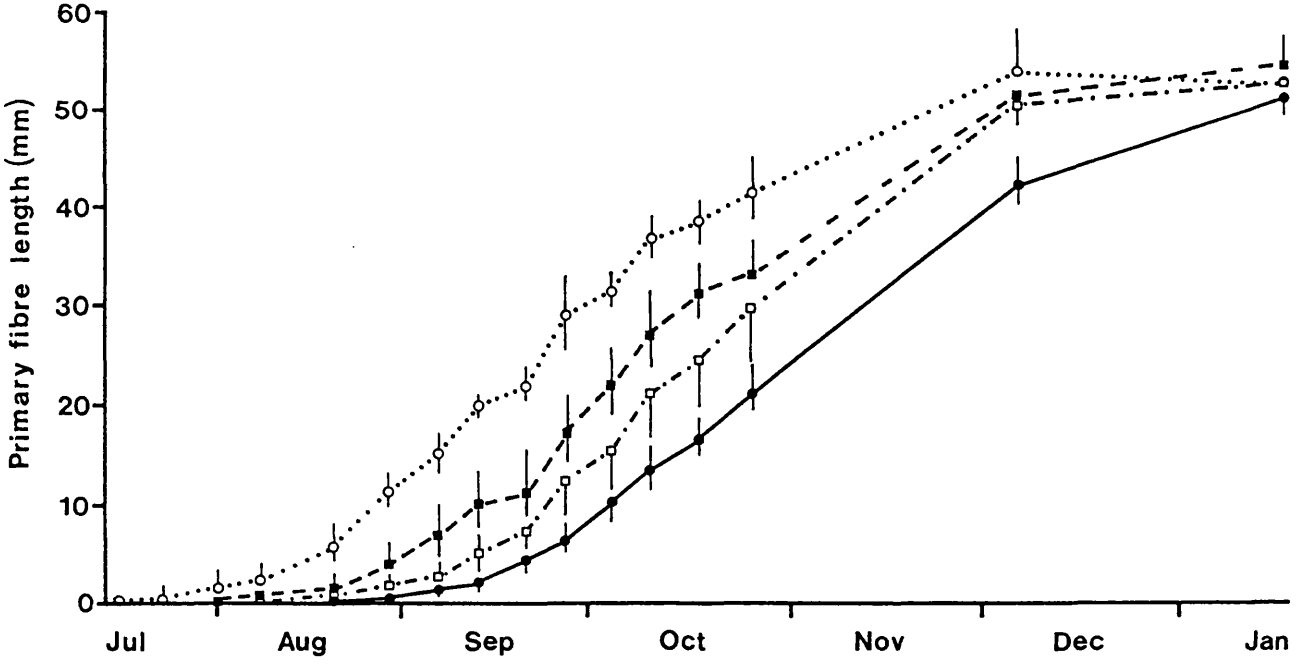
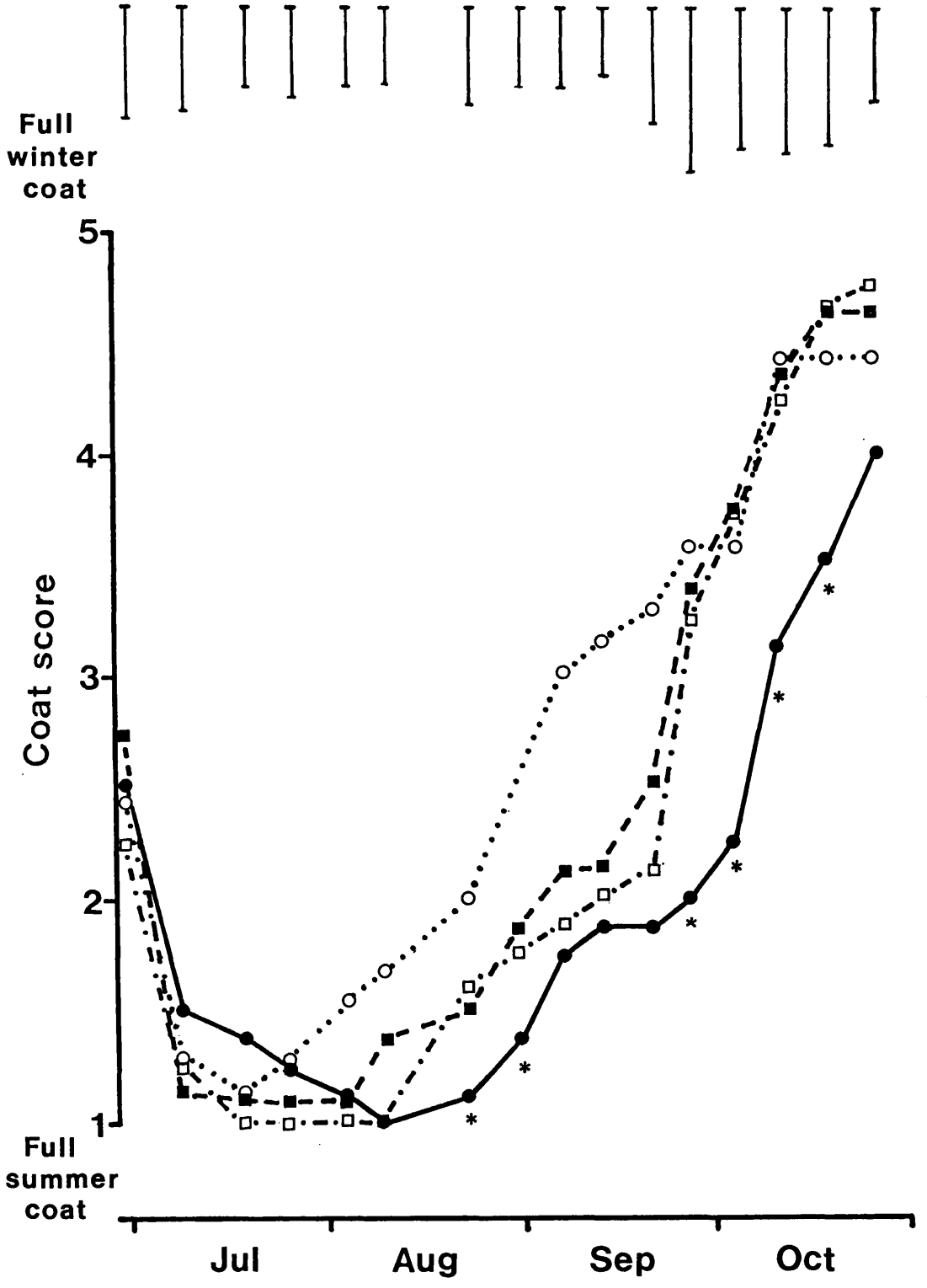


FIGURE 4.12: Changes in coat score ($\bar{x}$ ± pooled s.e.m., n=8) of hinds between July and October for groups lactating (LL, ●); lactating with melatonin implants (LLM, ○) and non-lactating (LNL, ■) on a low availability pasture and lactating on a high availability pasture (HL, □). LL *vs* HL or LNL, $P < 0.05$ for dates marked by '*'; LL *vs* LLM, $P < 0.05$ for all dates from 2 Aug. until 25 Oct.



Plasma prolactin concentrations

All groups exhibited a significant decline in plasma prolactin concentrations during the study (see Fig. 4.13).

(i) Effects of herbage availability and lactation

The mean plasma prolactin concentration for group LL between the onset of the study and August was significantly higher than that of either group HL or LNL (LL = 20.2 µg/l; HL = 14.5 µg/l; LNL = 14.6 µg/l). From August until the end of the study plasma concentrations were similar between these groups.

(ii) Effects of melatonin treatment

The plasma prolactin concentrations of melatonin-treated hinds declined from 8.4 µg/l at the onset of the study (5 days after implanting), to 0.98 µg/l by the beginning of August. These concentrations were significantly lower than those present in all other groups by the 4 July and remained so until early September.

The relationship between plasma prolactin concentrations and winter primary fibre length is illustrated in Figure 4.14. In all groups the increase in primary fibre length coincided with declining concentrations of this hormone. This is mostly clearly illustrated by comparing the melatonin-treated and non-treated lactating hinds on the low availability pasture. In both groups, substantial differences in the length of winter primary fibre were associated with similar differences in the decline of plasma prolactin concentrations. In all groups, except LLM, the onset of winter coat growth clearly preceded the fall to 'basal' concentrations.

FIGURE 4.13: Plasma prolactin profiles ($\bar{x}$ ± pooled s.e.m., n=8) between July and October for groups lactating (LL, ●); lactating with melatonin implants (LLM, ○) and non-lactating (LNL, ■) on a low availability pasture and lactating on a high availability pasture (HL, □). LL *vs* HL or LNL, $P < 0.05$ during July. LLM *vs* LL, HL or LNL, $P < 0.05$ for all dates until September.

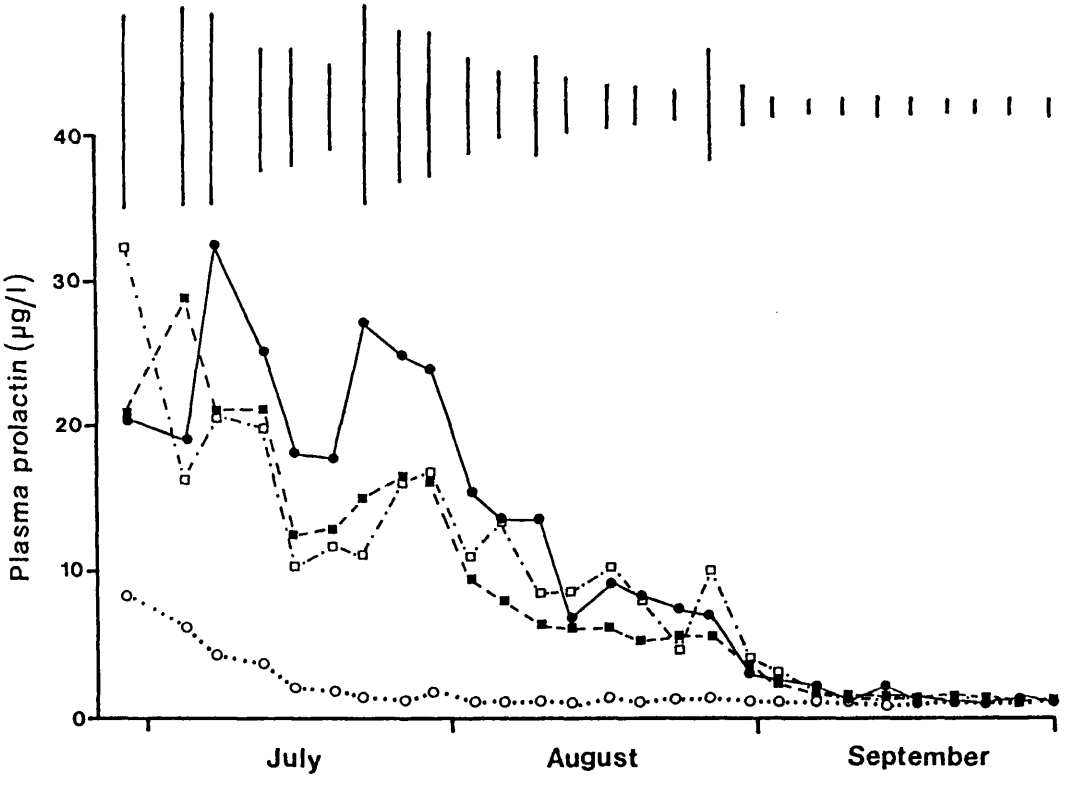
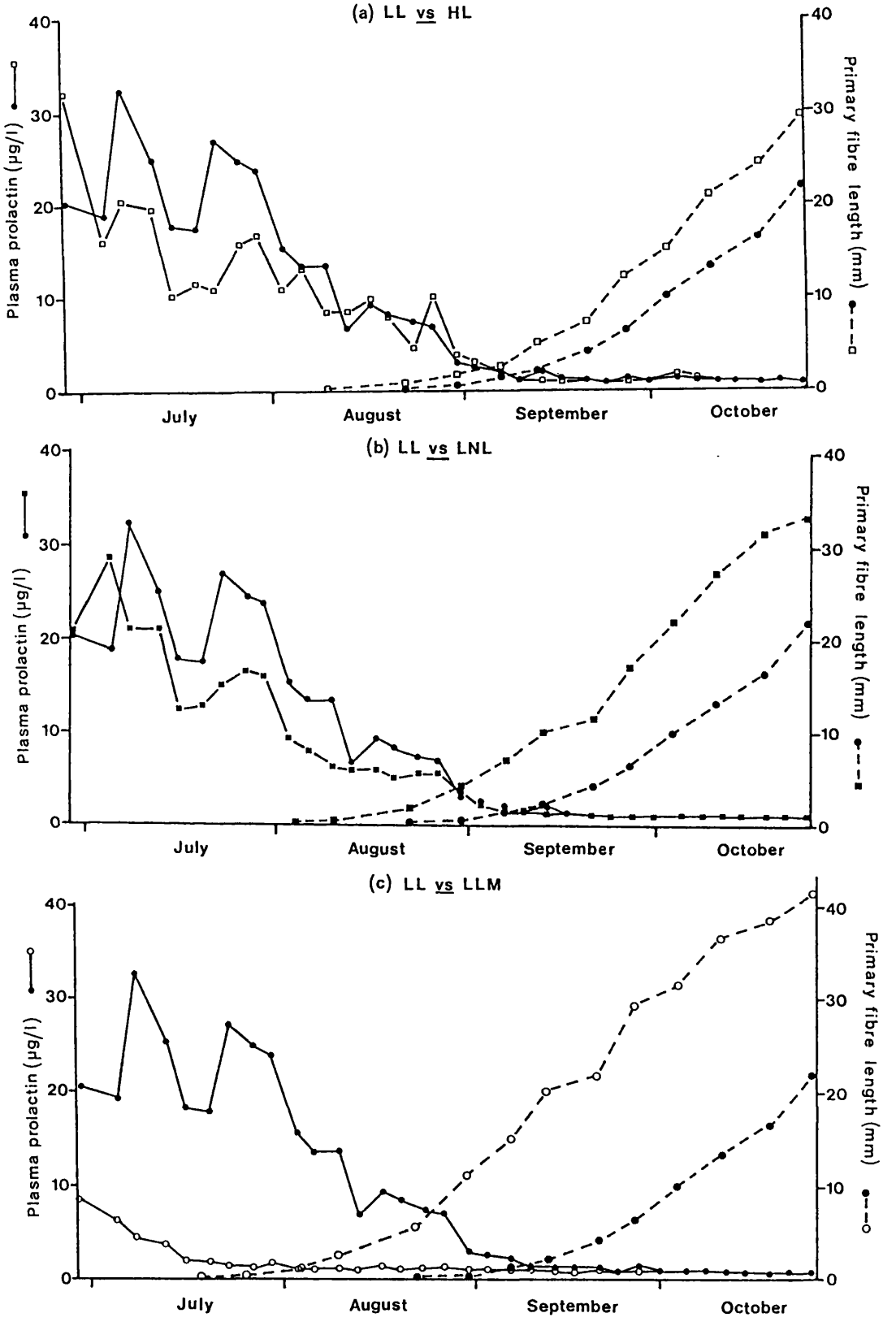


FIGURE 4.14: Comparison of winter coat growth and plasma prolactin concentrations of hinds during experiment 2. (a) group LL (●) *vs* group HL (□); (b) group LL *vs* group LNL (■) and (c) group LL *vs* group LLM (○).



4.5. DISCUSSION

4.5.1. CONTROL OF HERBAGE INTAKE AND GRAZING BEHAVIOUR

4.5.1.1. Effects of herbage availability

Thoughout the study herbage intake was greater in lactating hinds grazing the high (group HL) than the low (group LL) availability pasture. Herbage availability rather than appetite appears to have primarily determined the level of intake in hinds of group LL. This is consistent with the positive correlation observed between level of intake and herbage availability of ewes grazing pastures similar to those used in this study (Allden and Whittaker, 1970 - see Fig. 1.3).

Herbage intake is determined by a number of factors including grazing time, bite rate and bite weight (see section 1.2.12.). Although the hinds in group LL consumed 33% less herbage than those in group HL, their bite rates and daily grazing times were significantly higher. This indicates that these animals attempted to compensate for the lower sward surface height and herbage biomass by modifying grazing behaviour. Increases in bite rate accompanying reduced sward surface height have been reported in ewes and are believed to reflect a reduction in handling time associated with smaller bite sizes (Allden and Whittaker, 1970 - Fig. 1.3). The average proportion of time spent grazing by hinds in group LL was equivalent to 12.6 h/day (if it is assumed they grazed for the same proportion of the unobserved as observed periods). This is comparable with the maximum daily grazing times reported in a variety of deer species and sheep. These include, for red deer hinds 11.8 h/day (Clutton-Brock *et al.*, 1982a), 11.7 h/day (Loudon *et al.*, 1984); for reindeer, 12 h/day (Skogland, 1984), 12.7 h/day (Trudell and White, 1981) and for ewes 11.2 h/day (Arnold and Dudzinski, 1967) and 13 h/day (Allden and Whittaker, 1970). Collectively these data suggest there is an upper limit to the duration of daily grazing activity of about 12 h/day which may be imposed by the need to ruminate. It suggests that hinds in group LL were grazing for the maximum duration possible. Thus, their failure to achieve a similar

level of herbage intake to hinds in group HL reflected an inability to further extend grazing times.

The fact that group HL hinds maintained higher herbage intakes than those in group LL, despite lower bite rates and daily grazing times, demonstrates the importance of bite weight in determining the level of intake. The estimated weight of herbage/bite was significantly higher for hinds in group HL throughout the study. In ewes grazing similar pastures bite weight is positively correlated with both sward surface height and herbage biomass (Allden and Whittaker, 1970). This suggests that the difference in herbage intake between hinds grazing the high and low availability pastures was primarily due to the effect of sward surface height and biomass on the weight of herbage/bite.

Thus, consistent with the conclusion of the first experiment (chapter 3) it is clear that expression of appetite effects on food intake are dependent on the availability of herbage, and that the ability to compensate for reductions in herbage availability by modifying grazing behaviour are limited.

4.5.1.2. Effects of lactation

Lactation significantly elevates the energetic requirements of the hind. Arman *et al.* (1974) demonstrated that enhoused lactating deer consume up to 2.6 times the maintenance ration of non-lactating females when fed to appetite. In this study, lactating (group LL) and non-lactating (group LNL) hinds were maintained on a low availability pasture to determine the influence of limited food abundance on expression of these appetite differences. Evidence discussed in the previous section (4.5.1.1.) indicated that the food intake of the lactating hinds was restricted by herbage abundance. Despite this, the intake of non-lactating hinds was significantly less than that of the lactating females (mean 1.48 *vs* 1.99 kg OM/day). The difference in intake due to lactation was greatest at the start of the study and was no longer significant by September. This is consistent with decline in milk production and hence lactational energy requirements reported in grazing red deer (Loudon *et al.*, 1983). The similarity of herbage intakes between groups LL and LNL from September may also be a consequence of the progressive

decline in herbage biomass that occurred during the summer further restricting the intake of group LL. Thus, non-lactating hinds during the early stages of the study did not maximize herbage intake under the prevailing pasture conditions, indicating that appetite rather than food availability primarily determined the level of their intake. This provides the first direct in deer evidence that lactation increases herbage intake under grazing conditions. This has been assumed based on differences in grazing behaviour between lactating and non-lactating deer (Clutton-Brock *et al.*, 1982a,b) and from direct measurements in sheep (Arnold and Dudzinski, 1967; Arnold, 1975; Doney *et al.*, 1981).

The lower herbage intakes in non-lactating hinds were associated with shorter grazing times indicating that the duration of feeding activity is an important component in the expression of appetite differences. A similar relationship has been reported in ewes (Arnold and Dudzinski, 1967). There was also evidence for a lower herbage intake rate during grazing in non-lactating hinds. The estimated average weight of herbage/bite for non-lactating hinds was 13% less than that of lactating animals ($P < 0.05$) which suggests increased selectivity of herbage. The experimental pasture had a low species diversity and a fairly uniform sward height. It would be expected, however, that there would have been a greater proportion of less digestible stem material in the lower horizon of the sward. The larger estimated bite weight of the lactating deer implies that they consumed a greater amount of lower horizon plant material in each bite. This is reflected in a reduction in overall diet digestibility (LL, 0.70 *vs* LNL, 0.73, $P < 0.01$). In addition, digestibility tends to decline with increasing food consumption (Kay and Goodall, 1976). In sheep (Allden and Whittaker, 1970) bite rate increases as bite weight falls with reduced herbage availability. In this study a difference in bite rate between the lactating hinds with the larger estimated bite weight and the non-lactating hinds with the smaller bite weight was observed, although the difference was not significant (LL 76.6 *vs* LNL 78.9 bites/min). Less discriminative grazing by lactating hinds may have occured because of their high energetic requirements and the limited availability of herbage. Variation in diet selection relating to

lactation has also been reported in free-ranging red deer (Clutton-Brock *et al.*, 1982a,b - see section 1.2.12.2.).

The data reported here indicates that deer modify both the duration of feeding activity and the rate of herbage intake during grazing, in the attempt to meet nutritional requirements.

4.5.1.3. Effects of melatonin treatment

The phase of seasonal rhythms of lactating hinds was manipulated by administering continuous release melatonin from mid summer. In enhoused non-lactating hinds fed to appetite, daily melatonin treatment from this time has been demonstrated to result in an early decline in food intake (Milne *et al.*, 1990, see Fig. 3.1). During this study, however, there was no evidence that treatment influenced either food intake or grazing behaviour. There are two possible explanations for this: Firstly, the absence of an effect of melatonin may have been related to limited food availability. This is consistent with observations in experiment 1 (chapter 3). During that study, expression of melatonin effects on the appetite of non-lactating hinds were dependent on adequate herbage availability. Secondly, the elevated nutritional demands associated with lactation may have been superimposed on the normal seasonal decline in appetite thus masking its effect. In lactating Soay ewes maintained on a 6-month daylength cycle, voluntary food intake and lamb growth remained the same as in ewes under natural photoperiods (Argo, 1986). In well-fed deer, the calf growth rates of melatonin-treated hinds were not different from those of control hinds (Adam *et al.*, 1986). The implication from these studies is that lactational demands take precedence over the seasonal appetite decline.

4.5.2. HIND BODY CONDITION AND CALF GROWTH

4.5.2.1. Effects of herbage availability

Lactating hinds grazing the high availability pasture maintained a fairly constant live weight and body condition throughout the study. This indicates that herbage intake was sufficient to satisfy maintenance and lactation energy requirements.

There was also evidence of a small but significant increase of 3 kg in live weight between August and late October. As herbage intake was lower during this period than earlier in the study, it suggests maintenance and/or lactational energy requirements had declined permitting deposition of fat reserves. This is consistent with evidence that milk yield in red deer declines progressively after about 40 days of lactation (Loudon *et al.*, 1983). In hinds from the same herd grazing a similar pasture, milk yield fell by about 50% from the peak at day 30 and day 100 of lactation (Loudon *et al.*, 1983). In contrast, hinds grazing the low availability pasture (group LL) exhibited a progressive loss of live weight and body condition. In these animals herbage consumption was clearly insufficient to meet the energy demands of lactation and maintanance resulting in the utilisation of stored energy reserves.

Despite using fat reserves to supplement herbage intake the smaller liveweight gains of calves in group LL indicates that milk yield of their mothers was less than that of hinds in group HL. This conclusion is supported by the briefer duration of suckling bouts by calves on the low availability pasture. Bout length has been shown to be related to milk yield, as at any one suckling time there is a greater amount of milk available in the mammary gland of a high yielding hind (Loudon *et al.*, 1983). There was, however, no significant elevation in suckling bout frequency associated with shorter bout length (Loudon *et al.*, 1983). It has been suggested that calves of low yielding mothers attempt to obtain sufficient milk intake to maintain growth at its maximum genetic potential by suckling more frequently (Loudon *et al.*, 1983).

Loudon *et al.* (1983) noted that in association with low milk yield and increased suckling bout frequency there was an elevation in plasma prolactin concentrations. Although there were no differences in suckling frequency observed during this study and inspite of shorter suckling bout length, the plasma prolactin concentrations of hinds in group LL were higher than those of group HL during July.

Calves on both pastures exhibited a gradual reduction in bout frequency as lactation progressed. Similar, although less marked, trends have been reported previously (Kelly and Drew, 1976; Loudon

et al., 1983). This presumably reflects the decline in milk yield which occurs as lactation progresses (Loudon *et al.*, 1983). This is associated with declining dependence on milk as a source of calf nutrition. In red deer, from about day 30 - 40 of lactation milk no longer provides the total energy requirement of the growing calf and it becomes increasingly dependent on herbage intake (Loudon and Kay, 1984). As expected, a gradual increase in grazing activity by calves on both pastures was observed during this study. At about day 70 of lactation this accounted for only 0.17 of the observed period, but by day 130 this had risen to an average of 0.63 and was comparable with maximum grazing times by adults. By mid-September (day 102 - 108) herbage intake of calves on the high availability pasture was nearly a third of that of their mothers, (30.1 *vs* 100.7 g OM/$kg^{0.75}$/day). Herbage intake by calves grazing the low availability pasture was higher (35.6 g OM/$kg^{0.75}$/day, $P < 0.05$). It appears the calves reared on the low availability pasture attempted to compensate for their lower milk consumption by increasing herbage intake. Despite the increased herbage intake the calves of hinds in group LL were an average of 9 kg lighter than the HL group calves at 4 months of age, demonstrating that this increase in herbage intake was insufficient to compensate for the effects of lower milk consumption.

4.5.2.2. Effect of lactation

On a low availability pasture lactating hinds consumed nearly 40% more herbage than non-lactating hinds per unit metabolic body weight. From early July, however, both the live weight and body condition of lactating hinds on the low availability pasture were significantly lower than that of non-lactating females. These parameters exhibited a progressive decline in lactating hinds during the study but remained stable in those not lactating reflecting the heavy energetic cost of lactation. This is reflected in the similarity in live weight between hinds in group HL and LNL despite the HL hinds consumimg 2.1 times the amount of herbage.

4.5.2.3. Effect of melatonin treatment

Advancing the underlying seasonal appetite cycle of lactating

hinds on the low availability pasture using melatonin did not result in an observed affect on hind live weight or calf growth compared to hinds in group LL.

4.5.3. CONTROL OF REPRODUCTION

4.5.3.1. Effects of herbage avilability

At the beginning of the breeding season there were large differences in herbage intake (90 *vs* 49 g OM/kg$^{0.75}$/day), live weight (89.7 *vs* 77.7 kg) and body condition (score 3.7 *vs* 2.3) between the lactating hinds grazing the high (group HL) and low availability (group LL) pastures. Despite this, there was no significant difference in the time of onset of the breeding season of these two groups (17 *vs* 20 October for groups HL and LL, respectively). This contrasts with evidence of a small, but significant delay of 6.2 days reported in hinds grazing an indigenous hill pasture compared to a perennial ryegrass pasture (Loudon *et al.*, 1983). Based on evidence in humans (McNeilly, 1979; Howie and McNeilly, 1982) it was suggested by Loudon *et al.* (1983) that the high calf suckling frequency of hinds grazing the hill pasture (associated with low milk yields, see section 4.5.2.1.) was responsible for delaying the onset of the breeding season. This is supported by evidence of a 33% increase in pregnancy rates in Brahmin cattle (*Bos indicus*) following experimental restriction of calf suckling activity (Bastidas *et al.*, 1984). In red deer increased suckling frequency is associated with elevated plasma prolactin concentrations (Loudon *et al.*, 1983). Suppressing (using the dopamine agonist bromocriptine) or stimulating (using the dopamine antagonist domperidone) plasma prolactin concentrations from mid-summer, however, failed to alter the timing of the breeding season in non-lactating hinds (Milne *et al.*, 1990). Whether this hormone is involved in mediating the effects of the suckling stimulus on reproduction is unclear. During this study the higher plasma prolactin concentrations of hinds in group LL during July were not associated with a delay in the onset of the breeding season.

The absence of an effect of nutrition on the timing of the breeding season during this study, and the relatively small delays, if any, reported previously (e.g. 6.2 days, Loudon *et al.*, 1983) imply that the timing of reproductive activity is relatively insensitive to prevailing nutritional status. This could be related to the importance for highly seasonal species like red deer of conceiving (and thus calving) during specific periods of the year. Failure to synchronize parturition with optimum environmental conditions in early summer exerts a heavy penalty on hind reproductive success (see section 4.1.). The selection pressures related to timing of calving have thus resulted in the evolution of an annual reproductive cycle which times conception to a relatively circumscribed period in autumn. This is entrained by the annual photoperiod cycle (see chapter 1 for evidence). Social factors may also have a role in fine tuning reproductive synchronization. Evidence indicates that social contact results in greater synchrony of oestrus in hinds (deer: Iason and Guinness, 1985; sheep: Wayne *et al.*, 1989; Sunderland *et al.*, 1990) and that early cycling hinds can advance reproductive activity in males (M.W. Fisher, *unpublished data*). Social factors may explain the absence of any influence of nutrition on the timing of the breeding season in this study. Both groups LL and HL grazed adjoining pastures and were exposed to regular contact during handling procedures. In contrast, the hill and permanent grass pastures used by Loudon *et al.* (1983) were 600m apart. Thus, in the present study, a relatively small potential influence of nutrition could have been masked.

Although there was no effect on the timing of the breeding season related to herbage availability there was an influence on the proportion of hinds exhibiting oestrus. Only 5/8 hinds in group LL had exhibited oestrous cyclicity by January. In contrast, all hinds in group HL commenced cycling during October. The most important effect of herbage availability may therefore be to determine whether hinds breed rather than when they do so. Evidence from wild (Albon *et al.*, 1983) and farmed (Hamilton and Blaxter, 1980) red deer has demonstrated a clear relationship between hind liveweight and fertility. Farmed hinds failed to calve if their live weight at the

previous rut had been less than 52 kg, but at 60 and 80 kg the probability increased to 0.49 and 0.91 respectively (Hamilton and Blaxter, 1980). In all probability body weight itself is not important, but rather some aspect of body condition. In humans evidence of a critical weight associated with fertility (Frisch and Revelle, 1971) is believed to reflect a critical proportion of body fat to lean body mass (Frisch and McArthur, 1974). This suggests that the influence of herbage abundance on oestrous cyclicity observed in this study was related to its impact on stored energy reserves. It remains unclear, however, whether the effects are directly related to body condition, or indirectly by reduced milk production increasing the intensity of suckling activity (Loudon *et al.*, 1983, see above).

Differences in live weight and body condition score between hinds in groups LL and HL were not associated with variation in the pituitary LH response to exogenous GnRH. Recent evidence in ewes suggests this may reflect the fact that pituitary GnRH receptor number (Tatman *et al.*, 1990) and LH content (Thomas *et al.*, 1989) are relatively unaffected by declining body condition. The low body condition of hinds in group LL was, however, associated with a reduction in mean plasma LH concentrations. A similar relationship between body condition and mean plasma LH concentrations has been reported in ewes in some studies (Thomas *et al.*, 1989; Tatman *et al.*, 1990) but not in others (Rhind and McNeilly, 1986; Rhind *et al.*, 1989). This disparity may reflect differences in the extent of nutritional restriction between studies. Tatman *et al.* (1990) noted that a decline in LH release was only associated with reductions in body condition, if condition score deteriorated below a particular threshold. The reduced mean plasma LH concentrations were associated with a reduction in LH pulse frequency (Thomas *et al.*, 1989). Poor body condition in ewes also results in an elevated GnRH content within the pituitary stalk median eminence of the hypothalamus (Tatman *et al.*, 1990). These observations led Tatman *et al.* (1990) to propose that secretion of GnRH was chronically inhibited in ewes in very poor condition. This suggests that the lower mean plasma LH concentrations and the reduced incidence of oestrous cyclicity in

hinds of group LL, may have resulted from a suppression of GnRH pulse frequency.

4.5.3.2. Effects of lactation

By the onset of the breeding season there were substantial differences between lactating (group LL) and non-lactating (group LNL) hinds in live weight (77.7 kg *vs* 93.1 kg) and body condition (score 2.3 *vs* 4.4). Despite this, and the presence of the calf suckling stimulus, there was no difference in the timing of the breeding season between the groups. This is in contrast to some studies in which earlier reproductive activity occurred in non-lactating animals (19 days, Adam *et al.*, 1989b) or when weaning was advanced (16 days, Adam *et al.*, 1985; 8 days, Milne *et al.*, 1987). However, the study by Adam *et al.* (1986) did not demonstrate an effect of lactation. There is no obvious explanation for this variation.

There were differences in the proportion of hinds exhibiting oestrous cyclicity. Oestrous cycles were exhibited by all hinds in group LNL but in only 5/8 lactating hinds in group LL. In wild Scottish populations lower ferility is commonly associated with previous lactation (Lowe, 1969; Mitchell, 1973; Guinness *et al.*, 1978) particularily when population densities are high (Albon *et al.*, 1983). On the Isle of Rhum the calving rate of hinds barren the previous year was 20-26% higher than that of hinds previously lactating (Mitchell, 1973). Hamilton and Blaxter (1980) using their asymptotic regression, relating fertility to live weight in farmed deer, were able to reasonably predict the calving rates observed by Mitchell (1973) from hind body weights. This suggests that the primary effect of lactation on reproduction maybe related to its impact on hind body condition. If this is the case then the influence of lactation should be dependent on food availability (see section 4.5.3.1.). This was in fact the case and the incidence of oestrous cycles were significantly higher in lactating deer that were grazing the high availability pasture. Perhaps, when herbage is abundant hinds are able to increase food intake sufficiently to meet the energetic demands of lactation. However, when resources are limited, as on the low pasture, intake cannot be raised sufficiently

to meet both maintenance and lactational requirements. This results in depletion of energy reserves and a reduction in fertility.

Lactation, and the associated decline in body condition did not affect either pituitary sensitivity to exogenous GnRH, or mean plasma LH concentrations. In addition, the plasma LH concentrations for hinds in groups LL and LNL were both significantly lower than those in group HL. Collectively these data imply that the lower proportion of group LL hinds exhibiting oestrous cycles compared to either group LNL or HL may have been unrelated to LH secretion. Rhind *et al.* (1989) observed, however, that poor nutrition in ewes reduced LH pulse frequency without influencing mean plasma concentrations of the hormone. Clearly, a better understanding of the effects of lactation and nutrition on the LH secretory mechanism and oestrous cyclicity awaits a detailed investigation into the influence of these factors on LH pulse frequency and amplitude.

4.5.4.3. Effect of melatonin treatment

Melatonin treatment successfully advanced the timing of first oestrus in lactating hinds maintained on the low availability pasture. Clearly nutritional restriction was unable to prevent expression of the advanced reproductive status of group LLM hinds. A summary of published data for the effect of melatonin treatment on the timing of the breeding season in red deer hinds is presented in Table 4.8. The timing of the breeding season in group LLM was similar to that observed in other studies (see Figure 4.15). Collectively, these data indicate that there was relatively little effect of the date of commencement of melatonin treatment on the shift in the timing of the breeding season. This was for a range from 59 days before the summer solstice to 33 days after for the commencement of treatemnt. The only two studies comparing date of treatment commencement have demonstrated no significant effect related to the onset of treatment (Fisher *et al.*, 1990, and 'in press'). The data presented in Figure 4.15 also suggest that method of melatonin treatment did not influence the observed advance in the breeding season (see section 1.2.3.5.).

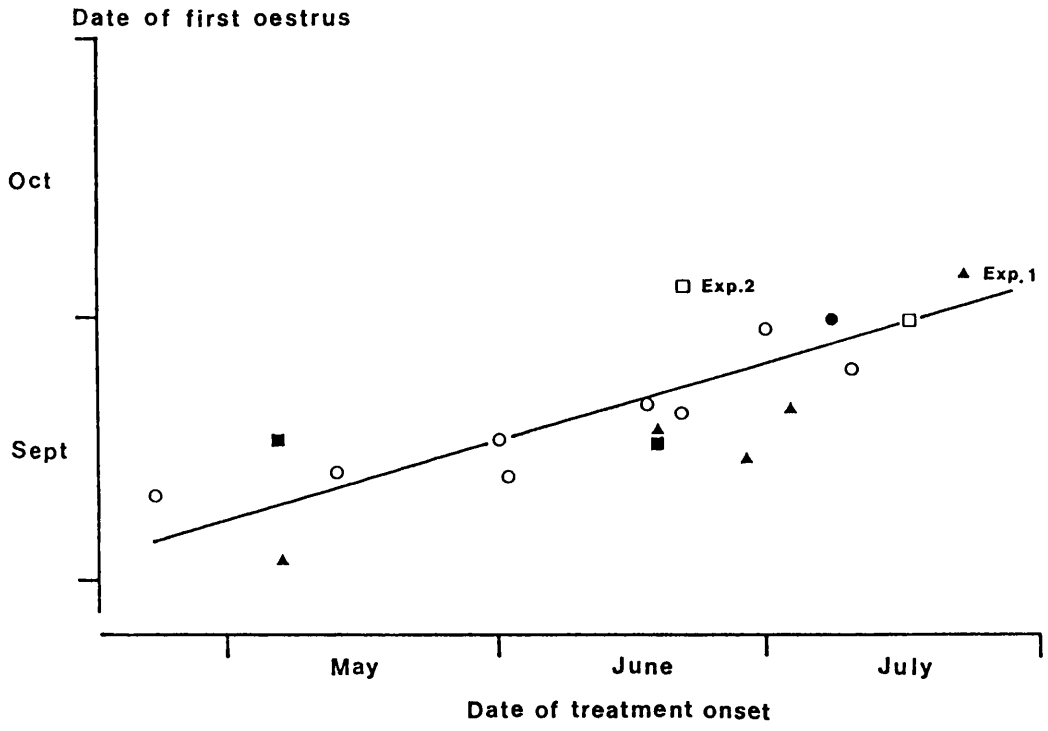
TABLE 4.8

Summary of published data for the advancement of the onset of the breeding season by melatonin treatment in red deer hinds, by daily injection (DI); daily oral dosing (DO), and slow release implants (IMP). Expanded version of review by Loudon and Brinklow (in press)

Physiological status	Onset of breeding season		Advance (days)	Interval (days)[A]	Treatment method
	Control	Treated			
a) Prepubertal	24 Apr[C]	29 Mar	26	81	DI
b) "	>11 Apr[BC]	11 Mar	>31	142	IMP
c) "	>11 Apr[BC]	13 Mar	>29	124	IMP
d) "	>11 Apr[BC]	12 Mar	>30	103	IMP
e) "	>11 Apr[BC]	19 Mar	>23	90	IMP
f) "	>11 Apr[BC]	22 Mar	>20	73	IMP
g) "	>13 Apr[BC]	17 Mar	>27	107	IMP
h) "	>13 Apr[BC]	30 Mar	>14	120	IMP
i) "	4 Apr[C]	24 Mar	11	100	IMP
j) Lactating	17 Oct	15 Sep	32	88	DO
k) "	24 Oct	16 Sep	38	133	DO
l) "	**20 Oct**	**2 Oct**	**18**	**96**	**IMP**
m) "	8 Apr[C]	28 Mar	11	73	IMP
n) Non-lactating	21 Oct	16 Sep	35	89	DO
o) "	5 Oct	2 Sep	33	119	DO
p) "	8 Oct	19 Sep	19	78	DO
q) "	5 Oct	13 Sep	22	76	DO
r) "	21 Oct	5 Oct	16	71	DO

A: Interval from onset of treatment to onset of breeding. B: None of controls cycling by end of monitoring on this date. C: Southern hemisphere dates. D: Breeding season as determined from progesterone profiles. E: Breeding based on mating dates. F: Breeding season determined from calving dates (- 234 days). *References:* (a) Webster and Barrell (1985)[E]; (b - f) Fisher *et al.* (1990)[D]; (g - h) Fisher *et al.* (in press)[D]; (i) and (m) Fisher *et al.* (1988)[C,F]; (j) + (n) Adam *et al.* (1986)[D]; (k)+(o) Adam *et al.* (1989b)[D]; (l) expt. 2, this thesis,[D]; (p - q) Milne *et al.* (1990)[D]; (r) expt. 1, this thesis,[D].

FIGURE 4.15: Relationship between date of onset of the breeding season and time of treatment with melatonin, for pre-pubertal (●,○); lactating (■,□), and non-lactating (▲,△) red deer hinds. Results for daily dosing (oral or injection) and slow release implant treatments are represented by solid or clear symbols respectively. Details are given in Table 4.7. For comparative purposes, data from the southern hemisphere (New Zealand) are presented with respect to northern hemisphere dates. 'Exp. 1' and 'Exp. 2' represent the results for experiments 1 and 2 of this thesis. The relation is described by the equation: $y = 215 + 0.297 x$ ($r^2 = 0.63$, $P < 0.001$), where 'y' represents the onset of the breeding season and 'x' represents the date of treatment commencement. Values are days after 1 January.



Although body condition and food intake were similar between both melatonin-treated (group LLM) and non-treated (group LL) hinds grazing the low availability pasture, there was a tendency for a greater proportion of hinds in group LLM to exhibit oestrus (7/8 *vs* 5/8). Thus, there may have been a diminished effect of poor nutrition on reproductive activity associated with melatonin treatment. This might have been related to the influence of melatonin on timing of the breeding season. As live weight progressively declined during the summer an earlier breeding season induced by melatonin treatment may have coincided with relatively better body condition. Neither live weight nor condition score, data, however, suggest an 18 day displacement in the onset of the breeding season would make much difference. Possibly body condition interacted with level of herbage intake shortly before the onset of the breeding season. In sheep, the level of pre-mating food intake has been shown to influence embryo mortality irrespective of body condition (Gunn and Doney, 1975). During this study herbage intake declined progressively and was signficantly lower at the time of breeding season onset in group LL than in group LLM.

The one melatonin-treated hind that failed to cycle (R31) possessed very low diet digestibility (0.44 *vs* mean of 0.71 for remaining group LLM hinds). Clearly this hind was obtaining less nutritional value from the food it consumed than other animals (as intakes were similar).

4.5.4. CONTROL OF COAT GROWTH

4.5.4.1. Effects of herbage availability

Winter primary fibre growth of hinds in groups LL and HL became evident in August as plasma prolactin concentrations declined. An association between coat growth and plasma prolactin concentrations has been reported previously in red deer (Loudon *et al.*, 1989) and sheep (Linoln, 1990). There was a 9 day delay in the onset of winter hair growth of hinds in group LL compared to those in group HL. This may have been related to the significantly higher plasma prolactin concentrations detected in group LL during July. In the Djungarian hamster, prolactin injections prevented a short-day induced winter

moult and maintained the summer coat (Duncan and Goldman, 1984b). In mink, delaying the autumn moult by maintaining females on long photoperiods was correlated with a delay in the seasonal decline in plasma prolactin concentrations (Martinet *et al.*, 1984). The higher plasma concentrations of prolactin in hinds of group LL may have been due to an effect of poor maternal nutrition on calf suckling activity (see section 4.5.2.1.).

In addition to exhibiting a delayed onset of primary fibre growth, the mass of hair fibres per unit area in January was less in group LL than group HL, although fibre length at this time was similar. Thus, it appears that the level of food intake during summer can have an important influence on the insulatory quality of the winter coat. Poorly nourished hinds are therefore disadvantaged not only by reduced energy reserves (see section 4.5.2.1.) but by increased susceptibility to heat loss. Inevitably the survival chances of these hinds through a harsh winter would be reduced.

4.5.4.2. Effects of lactation

The onset of winter primary fibre growth occurred earlier in the non-lactating hinds (group LNL) than in either non-melatonin treated lactating groups (group LL and HL). This difference is suggestive of the involvement of prolactin in the control of coat growth as lactation is accompanied by an elevation of plasma prolactin concentrations (Adam *et al.*, 1989). During the present study, the 20 day delay in the onset of winter fibre growth of the hinds in group LL was associated with elevated plasma prolactin during July. The 11 day separation in the timing of coat growth between groups LNL and HL, however, was not correlated with a measurable effect of lactation on plasma prolactin concentrations.

4.5.4.3. Effects of melatonin treatment

Treating lactating hinds with continuous release melatonin from late June resulted in a 35 day advance in the onset of winter primary fibre growth. A similar one month advance in the winter moult has since been reported in lactating (Fisher *et al.*, 1988) and yearling (Fisher *et al.*, 1990) hinds following melatonin treatment. Poor body condition and low herbage intake during this study were

clearly unable to prevent expression of seasonal manipulation by melatonin.

Associated with the early winter coat growth in melatonin-treated hinds was an advance in the seasonal decline in plasma prolactin. In both treated and non-treated hinds winter coat growth commenced as plasma prolactin concentrations approached basal levels. In mink, inducing an early decline in plasma prolactin using either exogenous melatonin (Allain *et al.*, 1981), maintenance on short photoperiods, or bromocriptine treatment (Martinet *et al.*, 1984) resulted in an advance in winter coat growth. These data and the results of the present study are suggestive of a direct role for seasonal changes in prolactin concentrations in the induction of winter coat growth in mammals, or the maintenance of the summer coat.

4.6. SUMMARY

The objective of this study was to investigate how herbage availability and nutritional requirements interact to control herbage intake, grazing behaviour, reproduction and winter coat growth.

The herbage intake of lactating hinds was strongly influenced by sward surface height and herbage biomass. Over the range of herbage availability investigated, the ability of hinds to compensate for reductions in estimated bite weight by increasing bite rate and daily grazing time was apparently limited. This appeared to be due to a ceiling on the duration of daily grazing activity of about 12 hours. The higher nutritional requirements of lactating hinds were associated with greater herbage intakes compared to non-lactating females. This difference was predominately expressed via extended grazing activity and an apparent change in diet selection. Advancing the phase of the underlying seasonal appetite rhythm using melatonin did not lead to an early decline in herbage intake of lactating hinds grazing a low herbage availability pasture. This may have been due to a combination of lactation elevating nutritional requirements and low herbage availability restricting intake in melatonin-treated and non-treated hinds alike.

Herbage availability influenced both hind body condition and calf growth rates. There was evidence that calves of hinds grazing the low availability pasture attempted to compensate for lower milk consumption by weaning earlier. The effect of herbage availability on the body condition of hinds was related to their nutritional requirements. Under the same pasture conditions non-lactating hinds were able to maintain a high body condition, whereas, lactating hinds exhibited a progressive decline.

Lactation and low hind live weight did not prevent melatonin implants advancing the timing of the breeding season. The principal effect of lactation and nutrition on the breeding season was apparently related to the ability to exhibit oestrous cyclicity, and not on the timing of the breeding season. The absence of variation in the onset of oestrous cyclicity and the relatively small delays, if any, reported previously (6.2 days, Loudon *et al.*, 1983) implies the timing of the onset of the breeding season is relatively insensitive to prevailing nutritional status. The results of the present study suggest that lactation only prevents oestrous cycles if it is associated with a sufficient loss of body condition. The impact of lactation is therefore dependent on whether or not prevailing food resources are sufficient to enable hinds to achieve the raised nutritional requirements.

Differences in the timing of winter primary fibre growth between treatment groups were associated with similar variation in the timing of the seasonal decline in plasma prolactin concentrations. These data provide support for an hypothesis that prolactin is involved in the control of seasonal pelage changes. Both lactation and poor nutrition resulted in a delay in the onset of winter fibre growth. There was also a long-term effect on the density of hairs fibres associated with lactating on the low availability pasture.

CHAPTER 5

THE ROLE OF OVARIAN STEROIDS IN DETERMINING SEASONAL CHANGES IN VOLUNTARY FOOD INTAKE AND GROWTH OF ADULT AND PRE-PUBERTAL HINDS (experiment 3)

5.1 INTRODUCTION

Both mature and pre-pubertal female red deer exhibit seasonal changes in voluntary food intake (VFI) when maintained on constant quality *ad libitum* diets (Loudon *et al.*, 1989; Loudon and Brinklow, unpublished observations). Food intake peaks in mid-summer and declines to a nadir in winter, before rising again in spring (see Figure 1.1). Although the general pattern of change is similar in mature and pre-pubertal animals, there is evidence for differences in the phase of these seasonal changes. Loudon *et al.* (1989) reported that in mature animals, the spring rise in appetite commenced in late March, with food intake peaking on the 10 June. However, in pre-pubertal females both the onset of the increase, and the peak of appetite occured earlier (late January, peaking on 23 May). Differences were also observed in the pattern of live weight changes which corresponded closely with the changes in VFI. No liveweight gains were exhibited by mature animals during late autumn and winter. In fact, these hinds lost weight during winter despite access to abundant high-quality food. In contrast, the period of reduced growth during winter months was less prolonged in pre-pubertal animals (see also, Milne *et al.*, 1987). Further differences were observed in plasma prolactin profiles of mature and pre-pubertal females. The onset of the spring rise in plasma concentrations was approximately two weeks earlier in the pre-pubertal than mature females.

These differences could be related to either the effects of photoperiodic history, or some aspect of maturity. Pre-pubertal animals have experience of fewer seasonal daylength changes than mature hinds and this difference might explain age related variation in the phase of seasonality. However, evidence suggests pre-pubertal

deer respond to photoperiod changes in a similar manner to mature animals. Webster and Barrell (1985) reported that at 13 months of age, transferring red deer from long summer photoperiods to a short 8L:16D photoperiod, resulted in an advance in the onset of puberty. More recent evidence indicates that deer are able to respond to photoperiod changes by at least six months of age. Loudon and Brinklow (1990) transferred animals to summer solstical photoperiods at their first winter solstice. This resulted in advance in the timing of the spring rise in VFI and plasma prolactin concentrations. Studies carried out on sheep and rodents (discussed in section 1.2.9.) suggest that, in fact, seasonal mammals may be capable of responding to daylength changes even *in utero*. Thus, while photoperiodic history cannot be dismissed as contributing to variation in the phase of seasonal cycles, pre-pubertal deer are clearly responsive to photoperiod.

Alternatively, the variation between mature and pre-pubertal animals may be related to maturity of the reproductive system and differences in ster d hormone secretion. In mature non-pregnant deer, the reduction in appetite, observed in animals fed *ad libitum*, coincides with the breeding season (defined as the period during which hinds exhibit repeated oestrous cycles). Puberty in this species does not occur until at least the second autumn of life (Mitchell, 1973). In impoverished habitats, this can be delayed for 3-4 years (Mitchell, 1973; Clutton-Brock *et al.*, 1982a). Thus, the delay observed in the timing of spring increases in VFI, live weight and plasma prolactin in mature females may be a consequence of reproductive activity. This influence could be due to gonadal steroids modulating underlying seasonal changes as there are believed to be substantial differences in ovarian steroid secretion between cycling and pre-pubertal deer.

The breeding season of red deer hinds is characterized by repeated oestrous cycles of approximately 18-21 days which commence in early autumn and continue through to spring if there is no conception (Guinness *et al.*, 1971; experiment 1, this thesis). Typical steroid profiles for a cycling hind are summarized in Figure 5.1. (see also Fig. 1.2). Plasma progesterone concentrations (the solid line) rise sharply with the formation of the *corpus luteum*

after ovulation. Concentrations during the luteal phase range between 3-10 nmol/l (data from experiments 1 and 2). These remain elevated for approximately two weeks declining following luteolysis to a follicular phase concentration of < 1.0 nmol/l. Data for oestradiol-17β concentrations in red deer are currently lacking. The profile of this hormone reported in the ewe is summarised in Fig. 1.2. (data from Hauger *et al.*, 1977). Briefly, concentrations of oestradiol-17β peak at approximately 33 pmol/l during the follicular phase. There are, however, also 1-2 smaller peaks present during the luteal phase. Average mid-luteal concentrations are between 10-15 pmol/l (Hauger *et al.*, 1977).

In pre-pubertal females, plasma progesterone concentrations remain at about 0.3 nmol/l throughout the period of the adult breeding season (Loudon *et al., 1989;* A.S.I. Loudon and B.R. Brinklow, *unpublished data*). These concentrations resemble those present in mature hinds during anoestrus (Loudon *et al.*, 1989; experiment 1, this thesis). As already stated oestradiol-17β concentrations have yet to be characterized for red deer. In the pre-pubertal ewe, they are reported to be between 3-10 pmol/l (Foster and Ryan, 1981; Foster *et al.*, 1986).

In red deer relatively little is known about the role of gonadal steroids in the control of VFI and growth. In stags testosterone has a significant impact on the amplitude of the appetite cycle. Normally plasma testosterone concentrations peak at, or slightly before the nadir of VFI (Suttie and Kay, 1985). Following castration the amplitude of the cycle is approximately halved (Kay, 1979) and resembles that of mature females (Loudon *et al.*, 1989). This is due both to a reduction in the level of peak food intake, normally associated with pre-rutting live weight gains, and the absence of rut inappetance (Kay, 1979). Whilst rutting, stags reduce the duration of their feeding activity from 44% to 5% of the day (Clutton-Brock *et al.*, 1982a). Thus, testosterone modulates the underlying appetite cycle by directly or indirectly increasing its amplitude.

In female deer, the influence that gonadal steroids exert on food intake and growth has yet to be determined. As already stated, the breeding season in non-pregnant deer coincides with the seasonal

reductionof appetite. Data from sheep suggests that, at least, one of the ovarian steroids, oestradiol-17β, can influence food intake. A marked transient decline in VFI in Cheviot (Tartellin, 1968) and Soay (Argo, 1986) ewes has been reported 0-2 days before the date of oestrus. This ephemeral inappetance corresponds to the period of maximal oestradiol-17β secretion during the follicular phase of the oestrous cycle (Hauger *et al.*, 1977). The decline in VFI observed in ewes following injection of oestradiol-17β into the brain clearly demonstrates the ability of this steroid to suppress appetite centrally (Forbes, 1972). There is also evidence in the rat that while oestradiol reduces VFI and adiposity, progesterone causes an increase (Wade and Gray, 1979). The role of ovarian steroids, however, is complex. While high concentrations of oestradiol reduce VFI and body weight, low exogenous doses have been shown to increase growth in castrate male sheep (Sillence *et al.*, 1987). This evidence suggests that the secretion of steroid hormones by cycling females might explain the delay observed in the timing of spring increases in VFI and growth of mature compared to pre-pubertal hinds (Loudon *et al.*, 1989).

Objective

The main objective of this study was to test the hypothesis that 'ovarian steroids secreted during the breeding season modulate the expression of an underlying seasonal rhythm, and thereby delay the onset of spring changes in VFI, live weight, coat growth and plasma prolactin'.

This was investigated in two ways, (i) by suppressing the breeding season in a group of mature females, to simulate the steroid hormone secretion of pre-pubertal females, and (ii) by simulating the steroid profiles of cycling hinds in pre-pubertal animals for the duration of the breeding season observed in non-pregnant deer. The influence of steroid manipulation on the timing of seasonal changes was determined by monitoring VFI, live weight, coat growth and plasma prolactin.

The breeding season was suppressed using the GnRH agonist Buserelin (HOE 766, donated by Dr J. Sandow, Hoechst

Aktiengesellschaft, Frankfurt, Germany). Administered as a continuous release implant this hormone analogue has been shown to suppress pulsatile secretion of LH in a variety of female mammals including cats, dogs, monkeys (J. Sandow, *pers. comm.*), sheep (Robinson *et al.*, 1989) and cattle (McLeod *et al.*, 1990). In the absence of pulsatile LH secretion ovulation cannot occur and oestrous cyclicity is prevented. In cattle, plasma LH concentrations initially exhibited a ten-fold increase following administration of buserelin. Within a day, however, these returned to and remained at concentrations similar to the previous baseline (cattle, McLeod *et al.*, 1990). Pulsatile secretion of LH was curtailed resulting in suppression of plasma progesterone for at least 28 days. The influence of buserelin on plasma oestradiol-17β has not been investigated.

The steroid hormone profiles of hinds exhibiting oestrous cycles were simulated in pre-pubertal animals using sub-cutaneous implants containing the ovarian steroids progesterone and oestradiol-17β (described in section 2.6.2.2. and Fig. 2.3). These were used to produce eight 21 day simulated 'oestrous cycles'. The number and duration of these was based on observations from experiment 1 (chapter 3) and from Guinness *et al.* (1971) and Adam *et al.* (1989a).

5.2 EXPERIMENTAL DESIGN

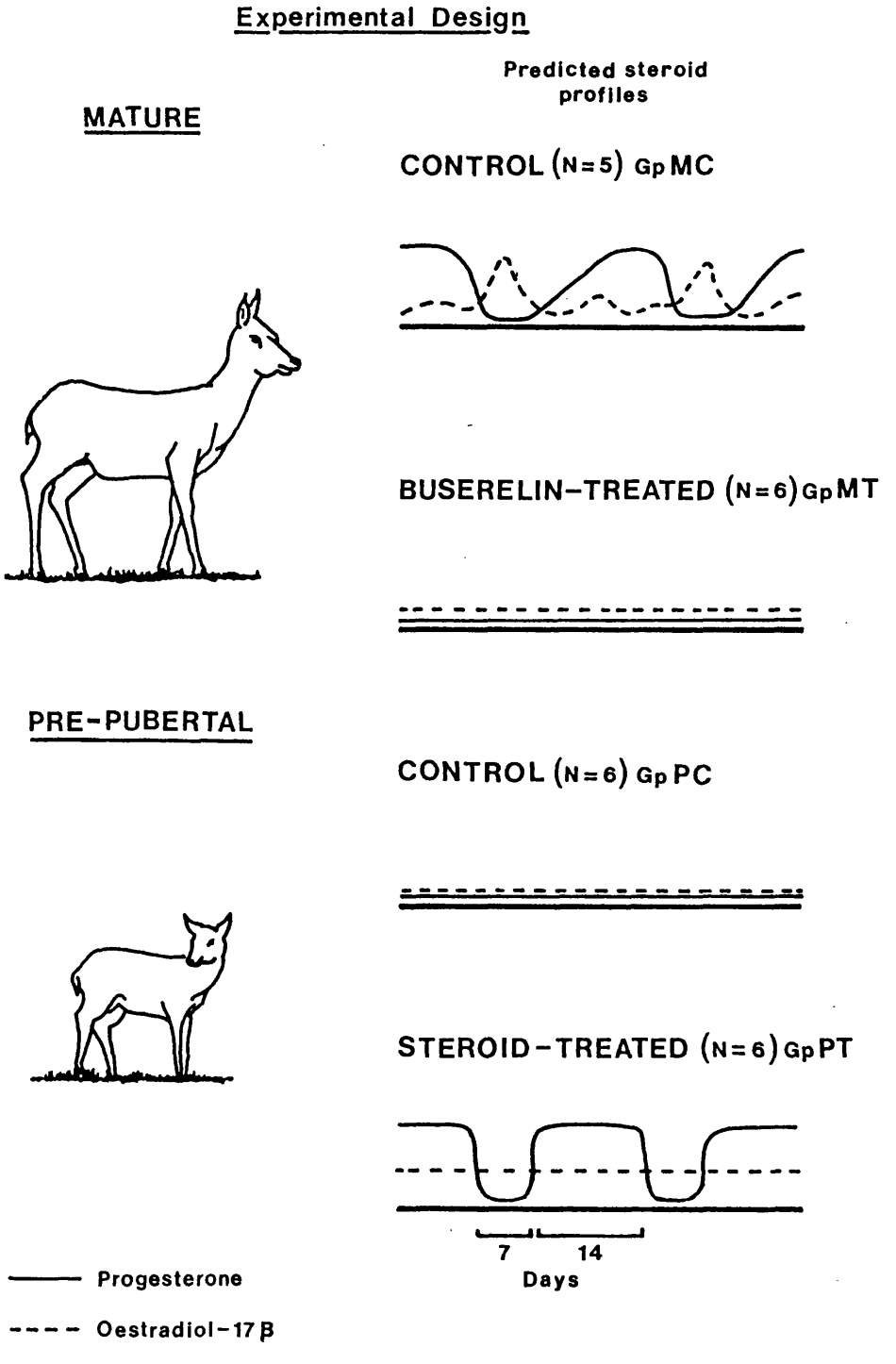
The experiment was performed at the Institute of Zoology field station at Whipsnade Wild Animal Park, Bedfordshire, U.K. (51°30'N) between September 1989 and July 1990.

The role of ovarian steroids in determining the phase of seasonal changes in appetite, live weight, coat growth and plasma prolactin in female red deer was investigated by manipulating steroid profiles of mature and pre-pubertal animals as follows:

Mature animals

One group of 6 mature hinds were treated with the GnRH agonist Buserelin (HOE 766; donated by Dr. J. Sandow, Hoechst

FIGURE 5.1.


Experimental Design
Predicted steroid profiles
MATURE
CONTROL (N=5) Gp MC
BUSERELIN-TREATED (N=6) Gp MT
PRE-PUBERTAL
CONTROL (N=6) Gp PC
STEROID-TREATED (N=6) Gp PT
7
14
Days
Progesterone
Oestradiol-17β

Aktiengesellschaft, Frankfurt, Germany) to remove oestrous cycles over one complete breeding season (group MT). A second group of 5 similar hinds were included as controls (group MC). Groups were balanced for live weight on the 12 September. The mean live weight for groups MT and MC were 98.0 (± 2.7) kg and 98.6 (± 3.6) kg respectively.

Each treated hind was given 6.6 mg of Buserelin in 2 biodegradable implants every 4 weeks from 14 September 1989 until 1 March 1990 (8 doses in total: see section 2.6.2.3. for details including pre-experimental trial of buserelin). Treatment commenced approximately 5 weeks prior to the normal breeding season of the study herd and was intended to suppress ovarian activity until after oestrous cyclicity had ceased in 5 untreated control animals (group MC).

Cloprostenol 0.75ml (Estrumate, Coopers Animal Health, Crewe, Cheshire, U.K.) was given to all mature hinds on the 23 October to cause regression of any persistent corpora lutea present in buserelin-treated hinds.

Pre-pubertal animals

One group of 6 pre-pubertal females had steroid treatment imposed to simulate oestrous cycles over the period of the adult breeding season (group PT). The second group were included as controls (group PC). Groups were balanced for live weight on 12 September. The mean live weight for groups PT and PC were 40.4 (± 1.8) kg and 40.6 (± 2.0) kg respectively.

Implants of the ovarian steroids progesterone and oestradiol-17β were given to animals in group PT (implants are described in section 2.6.2.2.) to simulate 21 day oestrous cycle. Each cycle consisted of 7 days without exogenous progesterone (≃ 'follicular phase') followed by 14 days with a single progesterone implant (≃ 'luteal phase'). The 21 day cycle was repeated throughout the entire period of the normal adult breeding season (from previous data). The first progesterone implant was administered on 17 October (1989) and the final one removed on the 26 March (1990). This represented a total of 8 simulated oestrous cycles. In addition, animals were

given a single implant containing oestradiol-17β on the 17 October which was left in place until 26 March.

The progesterone implant was designed to achieve plasma concentrations of the hormone similar to those present during the luteal phase (3-10 nmol/l). The implant used to deliver oestradiol-17β was designed to maintain constant elevation of the steroid, rather than mimic the changes in concentration believed to occur during the oestrous cycle. The reason for this was two-fold. Firstly, oestradiol concentrations have yet to be determined in red deer. Secondly, to simulate the pattern of changes observed in ewes, would have required implants to have been inserted and removed at least twice during each cycle (Hauger *et al.*, 1977; summarized in Fig. 1.2). This would have resulted in an unacceptable degree of disturbance to feeding behaviour. Thus, the aim was to simply achieve plasma oestradiol-17β concentrations approximating to the average observed during the oestrus cycle of the ewe (Hauger *et al.*, 1977). For this purpose the implant was design to achieve an elevation of 10-15 pmol/l in peripheral plasma. For experimental purposes it was assumed that endogenous secretion of oestradiol-17β in the pre-pubertal deer is similar to that of sheep (i.e. 3-10 pmol/l, Foster and Ryan, 1981; Foster *et al.*, 1986).

A schematic representation of the hormonal profiles of animals in each group is shown in Figure 5.1.

Animals and husbandry

All mature hinds and 4 of the pre-pubertal animals were selected from the Institute herd (primarily Scottish in origin). The remaining 8 calves came from Rahoy Deer Farm (Strontian, Morvern, Scotland). The calves, all born in the first week of June were separated from their mothers within 24 hours of birth and artifically reared at Whipsnade. Weaning occured at the beginning of October 1989. Prior to the experiment adult hinds were maintained on a common pasture. Both adults and calves were accustomed to extensive handling.

From the 12 September until 18 December animals were kept in their respective groups. From 18 December until the end of the

experiment, animals were individually housed in a single building. The floor area of the pens used was 2.3 x 3 m. Each animal had visual contact with those in neighbouring pens. Approximately twice a week, all deer were allowed a period of several hours exercise in outside yards, but without access to food. All mature hinds, and pre-pubertal animals after weaning, were offered the same an *ad libitum* pelleted lucerne diet, plus mineral supplements, throughout the experiment.

MEASUREMENTS

Details of some of the materials and methods outlined below are described in chapter 2.

The effects of treatment on the reproductive axis were determined as follows:

(a) Plasma progesterone concentrations

Plasma progesterone concentrations were measured in twice weekly blood plasma samples from September 1988 until April 1990 in all animals. For mature control hinds progesterone values were used to determine the onset and termination of the breeding season (as described in section 2.6.4.).

(b) Oestrous behaviour

All incidences of observed oestrus were recorded. Characteristic behaviour associated with oestrus is outlined in section 2.3.1.

(c) Plasma luteinizing hormone concentations

The pituitary LH response to a challenge of 100 µg GnRH (via 2 ml saline) was determined on 4 occassions during the latter half of the normal breeding season (5, 12, 19 and 26 Feb). A large dose was chosen to minimise relative dose effects due to variation in animal liveweight between groups, and was intended to produce a maximal LH response. The challenge procedure is outlined in section 2.6.2.4.

(d) Plasma oestradiol-17β concentrations

Plasma concentrations were determined in a limited number of samples from representative individuals in each group. These were

kindly assayed by Dr R. Webb (A.F.R.C., Institute of Animal Physiology and Genetics, Roslin, Midlothian, EH25 9PS).

The effects of ovarian steroids on seasonal changes in VFI, live weight, coat growth and plasma prolactin concentrations were assessed as follows:

(a) Voluntary food intake

VFI was determined by the method described in section 2.1.1.1. Daily intake values were used to produce a weekly mean estimate for each treatment group from 30 November, and for individual animals from the 18 December until the end of the study.

(b) Live weight

Live weight was recorded at weekly intervals.

(c) Coat growth

In situ measurements of pelage type and primary fibre length were made at weekly intervals (as described in section 2.4).

(d) Plasma prolactin concentrations

Plasma prolactin concentrations were determined from once weekly blood samples.

5.3. STATISTICAL ANALYSIS

Oneway analysis of variance were applied to the results (unless stated otherwise) and the the differences between means were examined by the method of Least Significant Difference.

The onset and termination of oestrous cyclicity were determined by the method described in section 2.6.4.

Delta LH values were log transformed so that an assumption of equal variances could be made. A value of 0.01 was added to all LH data to permit log-transformation of zero responses. Data were compared with analysis of variance for repeated measures.

Mean weekly estimates of daily VFI were expressed as actual dry matter intake/day and also corrected for metabolic live weight to remove the effects of body size (Kleiber, 1961). An initial analysis of variance for repeated measures was performed to assess the effects of time on VFI (using uncorrected data). Data (corrected for metabolic live weight) were subsequently analysed by a process of quadratic smoothing in order to determine the timing of peak and

nadir values, as well as mean and maximal values. The method adopted was the same as that used by Loudon *et al.* (1989). In order to avoid the problems associated with a marked between-weeks variation in VFI, seasonal changes were analysed separately for each animal, the value at each observation time being estimated by quadratic smoothing. Smoothing took place over ten values either side of the one to be estimated. This window width was chosen following a process of cross-validation (Stone, 1974). Mean values for each group were determined, and from this the mean time of peak and nadir values calculated. The computed peak and nadir values and the timing of these events were subsequently compared by oneway analysis of variance.

To determine the effect oestrus on daily VFI in mature control hinds (group MC), the intake on the day of observed oestrus was compared to the average of the previous and subsequent 10 days. This method was chosen to overcome the effect of progressive seasonal reductions or increases in VFI. The 10 day window width was chosen as the oestrous cycle in this species is approximately 21 days in duration (see 5.1. for references). Due to the shorter interval between observations of oestrus in group MT, VFI on the day of oestrus was compared to the mean for the days between each occurance. Student's t-test was used to compare the mean intake on the day of oestrus, with mean average intake for the periods between each oestrus, for individual animals in group MT.

Live weight differences were compared by analysis of variance with repeated measurements.

An initial analysis of variance with repeated measures was performed to assess the effects of time on winter and summer coat growth. Data were subsequently analysed to determine treatment effects on: (i) the onset of winter and summer primary fibre growth (see below), (ii) the maximum length recorded for each coat, and (iii) the duration of each moult (time taken to change from 100% existing to 100% new pelage on coat scoring). For the summer coat the onset of growth was defined as the date of first primary length measurement $>$ 0.5cm. This method could not be used for the winter coat as some pre-pubertal animals had commenced coat growth prior to the start of measurements. To estimate the time growth began linear

regression analysis was carried out on the first 10 primary fibre length measurements for individual animals. The onset of growth was estimated from the regression equation for each animal.

The onset of the spring rise in plasma prolactin concentrations was defined as the date of the first of 5 consecutive plasma samples with concentrations greater than twice the mean concentration, for each animal between mid-October and mid-January (inclusive).

All data are expressed as mean ± s.e.m. (unless stated otherwise).

5.4.1. EFFECTS OF TREATMENT ON REPRODUCTIVE STATUS

5.4.1.1. PLASMA PROGESTERONE CONCENTRATIONS

The plasma progesterone profiles of the 4 groups are showed in Figure 5.2.

The effects of sexual maturity

All 5 mature control animals (group MC) underwent regular cycles from, at least, the onset of the experiment in early September until April the following year. The last luteal phase commenced 2 April (± 10.1 days).

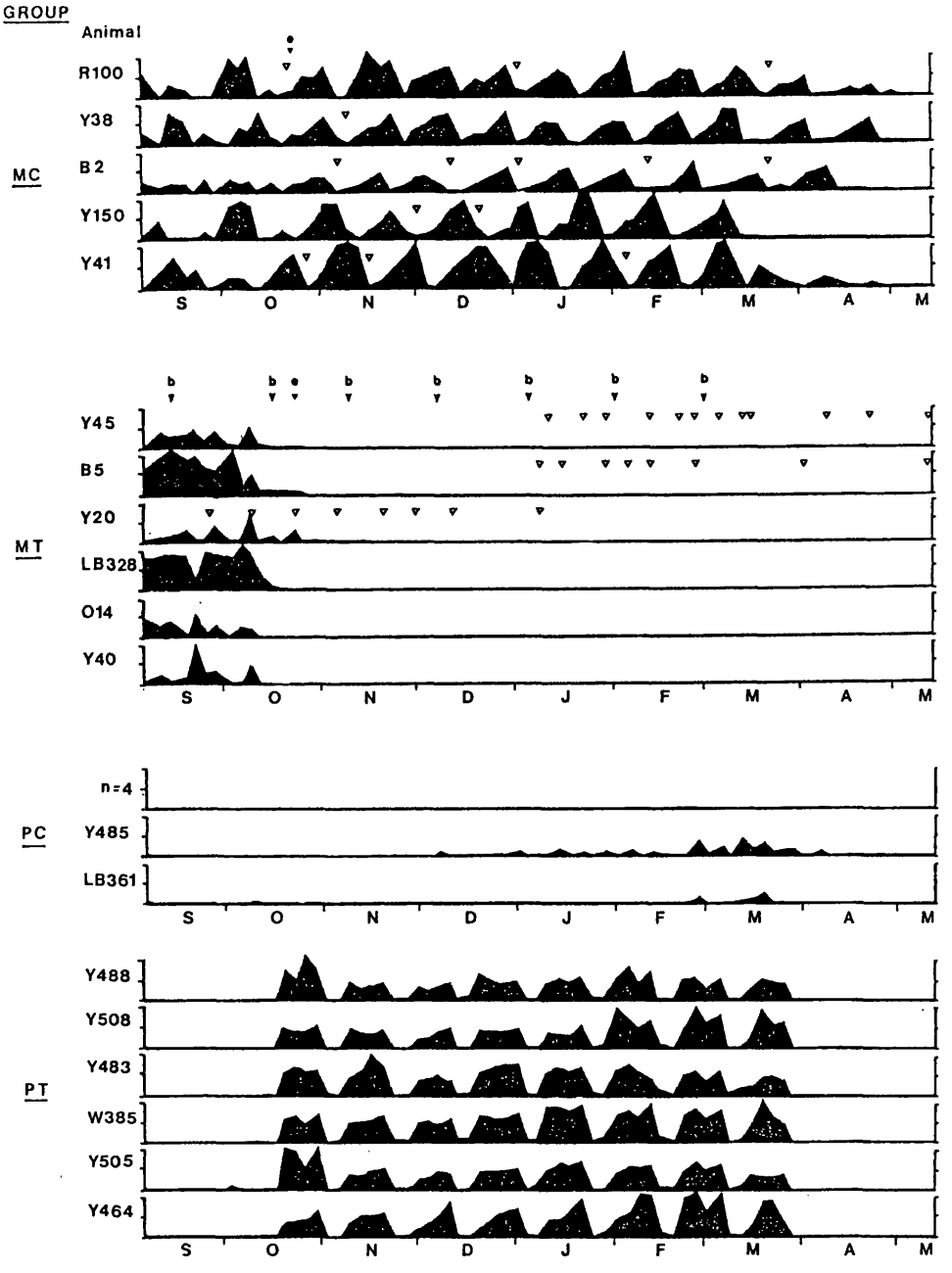
The plasma progesterone concentrations of four of the pre-pubertal control group (group PC) remained low (< 0.6 nmol/l) throughout the study. In two, however, raised concentrations were detected towards the end of the adult breeding season (see Figure 5.2). Plasma concentrations were highest in y485. In this animal concentrations satisfied the criteria used to determine oestrous cyclicity atthe onset of the breeding season (described in section 2.6.4.).

The effects of steroid manipulation

Elevated plasma progesterone concentrations were observed in all buserelin-treated mature animals (group MT) prior to the onset of buserelin treatment (13 September). In 3 of the 6 animals in this group plasma progesterone concentrations remained elevated for more than a month. However, 29.7 (± 0.72) days after the onset of treatment plasma progesterone concentrations declined to less than 1 nmol/l in all hinds. These remained at < 1nmol/l for the remainder of the study.

Steroid-implanted pre-pubertal animals (group PT) underwent clear progesterone 'cycles' of similar duration and amplitude to those of the mature cycling hinds (see Figure 5.2). The mean concentration of plasma progesterone during the simulated 'luteal' phase was 5.0 (± 0.34) nmol/l and during the 'follicular' phase 0.56 (± 0.041) nmol/l. Thus, implants successfully mimicked the changes in plamsa progesterone observed in mature cycling females.

FIGURE 5.2: The plasma progesterone profiles (nmol/l) of mature control (MC), mature buserelin treated (MT), pre-pubertal control (PC), and pre-pubertal steroid-treated (PT) groups. Each division on figure = 5 nmol/l. 'b' denotes date of buserelin administration; 'e' date of estrumate administration to mature animals, and '▽' incidences of observed oestrus.



4.5.1.2. OESTROUS BEHAVIOUR

Observations of oestrus are detailed in Figure 5.2.

Effects of sexual maturity

In group MC oestrous activity (indicated by triangle symbols), coincided with periods of low plasma progesterone concentrations. The average oestrous interval observed in consecutive cycles was 20.3 (± 0.33) days (n=3).

Oestrus was not observed in any of the animals of group PC (including y485, which exhibited high plasma progesterone concentrations).

Effects of steroid manipulation

Three animals in group MT (LB328, 014 and Y40) exhibited no evidence of oestrous activity. The remaining three hinds (Y20, B5, and Y45) exhibited oestrus on repeated occasions. The intervals between observations of oestrus were in multiples of approximately 7 days (see Table 5.1).

TABLE 5.1.
Oestrous activity in buserelin-treated hinds (group MT).

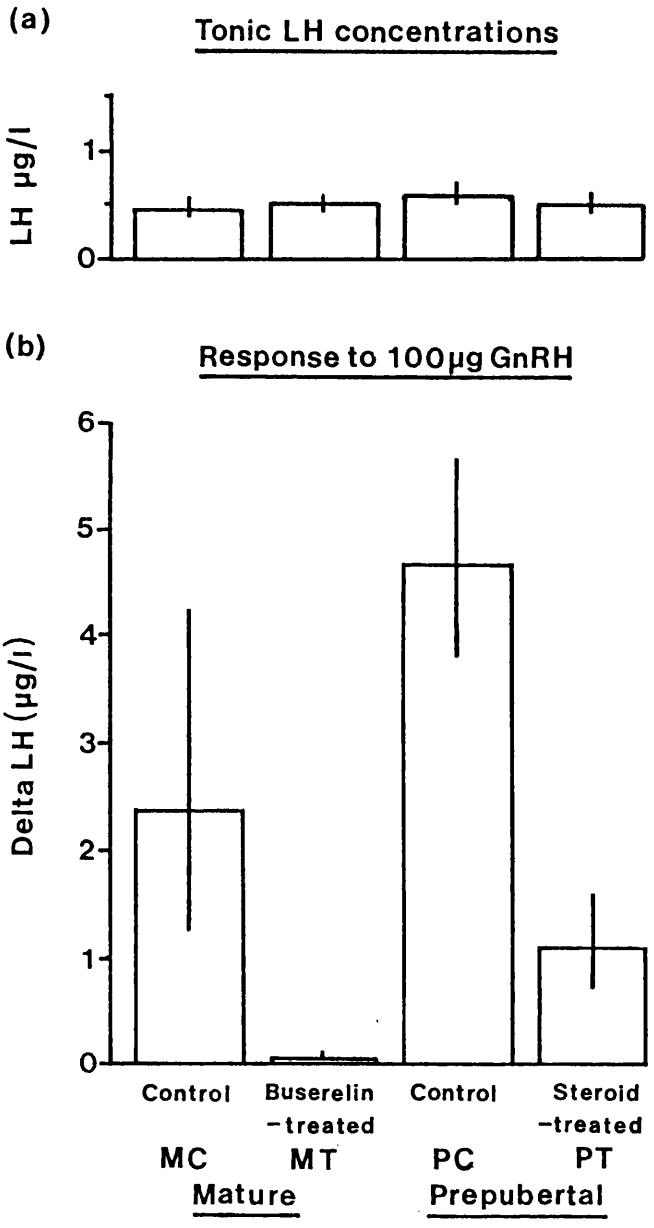
Y20		Y45		B5	
Date	Interval (days)	Date	Interval (days)	Date	Interval (days)
26.09	-	11.01	-	08.01	-
09.10	13	22.	11	15.	7
23.	14	29.	7	29.	14
06.11	14	12.02	14	05.02	7
20.	14	21.	9	12.	7
30.	10	26.	5	26.	14
12.12	12	06.03	8	02.04	35
08.01	27	13. *	8	14.	12
		15. *	2	04.06	23
		09.04	25		
		23.	14		
		14.05	21		

* : same oestrus ?

One animal in group PT showed oestrous behaviour on 16 February. This animal's progesterone implant had been removed on the

FIGURE 5.3: (a) Mean plasma LH concentrations (µg/l) for each group prior to GnRH challenges (at t0). (b) The response of hinds to 100 µg exogenous GnRH after 20 minutes. Defined as the LH concentration at t20 - t0 (delta LH). Values for (a) and (b) are the mean value (+ 95 % confidence limits) for each group for the 4 GnRH challenges, carried out at weekly intervals during February.

All groups, except group MT, exhibited a significant elevation in plasma LH at t20 ($P < 0.001$). For delta LH values, all groups were significantly different from each other ($P < 0.01$).



13 February, at which time the oestradiol implant was found to be missing. The latter was replaced on the 15 February. Thus, oestrus occured in the absence of exogenous progesterone, 24 hours after the replacement of the oestradiol implant.

5.4.1.3. PLASMA LUTEINIZING HORMONE CONCENTRATIONS

The mean average t0 plasma LH concentration for the 4 GnRH challenges was unaffected by experimental treatment (see Fig. 5.3). In contrast, the mean average delta LH values were significantly different between the 4 groups (see Fig. 5.3).

Effects of sexual maturity

The mean average delta LH value was significantly higher in pre-pubertal than mature control groups (4.69 ± 0.518 *vs* 2.42 ± 0.577 µg/l, $P < 0.001$).

Effects of steroid manipulation

Plasma LH concentrations for group MT were not significantly elevated 20 minutes after the GnRH challenge (delta LH = 0.02 ± 0.515 µg/l).

The delta LH values of group PT animals (1.10 ± 0.518 µg/l) were significantly lower than those of the pre-pubertal (PC) and mature (MC) control groups.

5.4.1.4. PLASMA OESTRADIOL-17β CONCENTRATIONS

Effects of sexual maturity

The plasma oestradiol-17β concentrations of 4 animals from group MC were measured twice weekly over the period of one oestrus cycle per animal. The results, including plasma progesterone concentrations and oestrus observations, are described in Figure 5.4. Although oestrus and periods of low plasma progesterone concentrations coincided, these were not consistently associated with either low or high oestradiol-17β concentrations. The overall mean concentration throughout the cycle for these animals was 15.6 (± 2.45) pmol/l.

FIGURE 5.4: Peripheral plasma concentrations of oestradiol-17β ('E2', pmol/l: ●——●) and progesterone ('Po', nmol/l: O----O) concentrations in 4 mature control animals (group MC) during a single oestrous cycle. 'O' denotes observed oestrus.

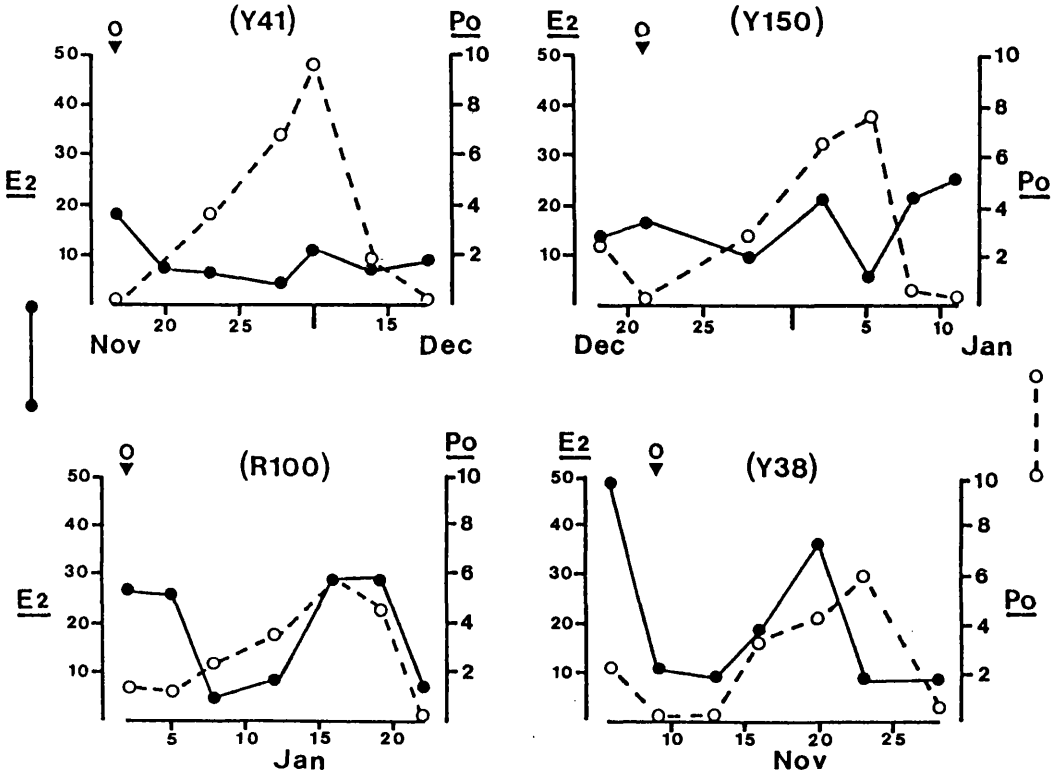


FIGURE 5.5: Mean average (± s.e.m.) plasma oestradiol-17β concentrations (pmol/l) for groups during the adult breeding season in experiment 3. Results for the buserelin-treated animals (group MT) have been separated into hinds that did (A, n=3) and did not (B, n=3) exhibit oestrus. Values for groups MC, PC, and PT were significantly different from each other ($P < 0.05$).

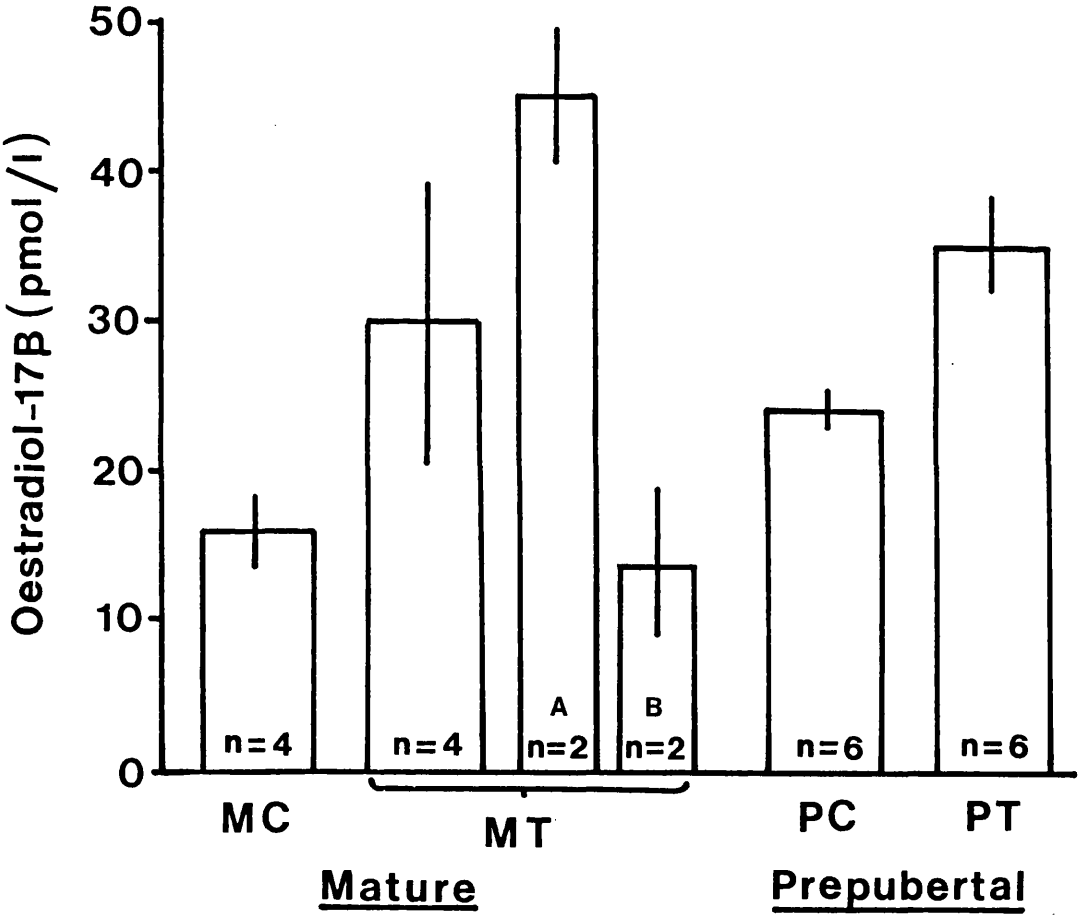
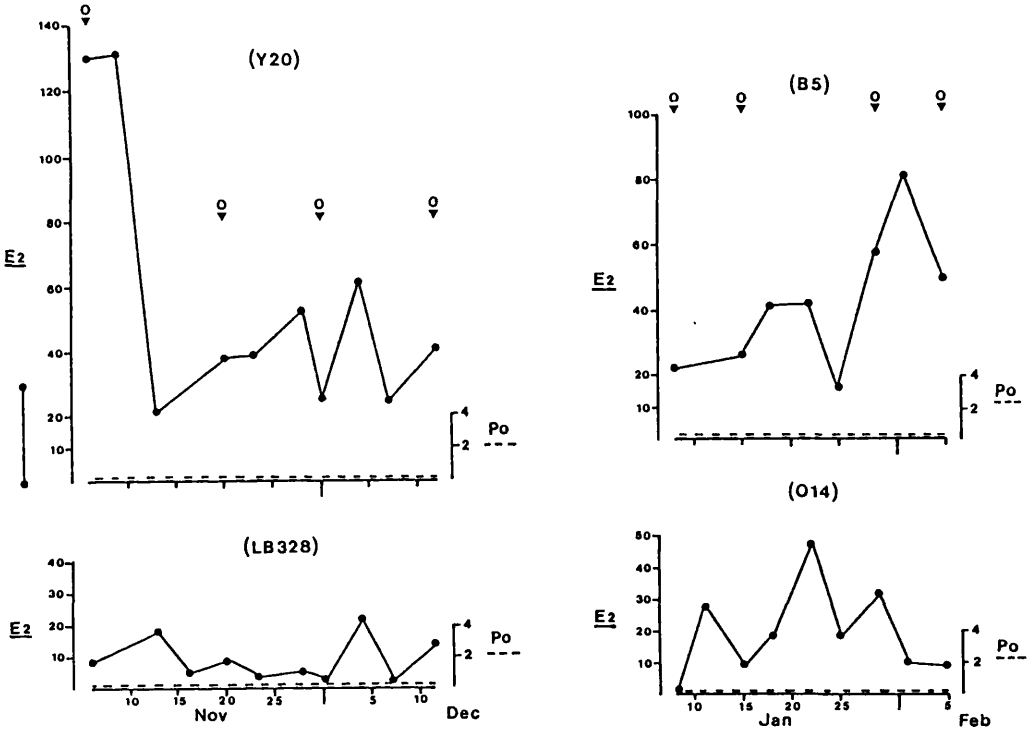


FIGURE 5.6: Comparison of oestradiol-17β ('E2', pmol/l:●——●) and progesterone ('Po', nmol/l: -----) concentrations in buserelin-treated adult hinds exhibiting and not exhibiting oestrus (Nov - Dec: 2 animals, Jan - Feb: 2 animals). 'O' denotes observed oestrus behaviour.



The mean plasma oestradiol-17β concentration for group PC (2 samples per animal) was 24.2 (± 1.62) pmol/l. This was significantly higher than the average for group MC ($P < 0.05$) (see Fig. 5.5).

Effects of steroid manipulation

Plasma oestradiol-17β concentrations were measured in 4 of animals in group MT (2 which had exhibited oestrus and 2 in which it was not observed). The average plasma oestradiol-17β concentration for the 4 animals was 29.9 ± 9.75 pmol/l (see Fig. 5.5). There were substantial differences within the group (see Fig. 5.6). Values were greater in those animals exhibiting repeated oestrus (means values: Y20: 51.0 ± 12.27 pmol/l; B5: 41.1 ± 7.22 pmol/l) than in those that did not (LB328: 8.74 ± 2.13 pmol/l; 014: 18.9 ± 4.66 pmol/l).

Oestradiol implants raised circulating concentrations of the steriod in pre-pubertal (group PT) females by approximately 11 pmol/l ($P < 0.05$). The mean plasma concentration for group PT (2 samples per animal) was 35.4 (± 3.35) pmol/l. This was significantly higher than the average concentration for group MC ($P < 0.01$; see Fig. 5.5).

5.4.2. THE EFFECT OF OVARIAN STEROIDS ON THE TIMING OF SEASONAL CHANGES

5.4.2.1. VOLUNTARY FOOD INTAKE

Seasonal changes in VFI are shown in Figures 5.7, 5.8, and 5.9, and in Table 5.2. All groups exhibited a significant increase in VFI between mid-winter and the end of the study.

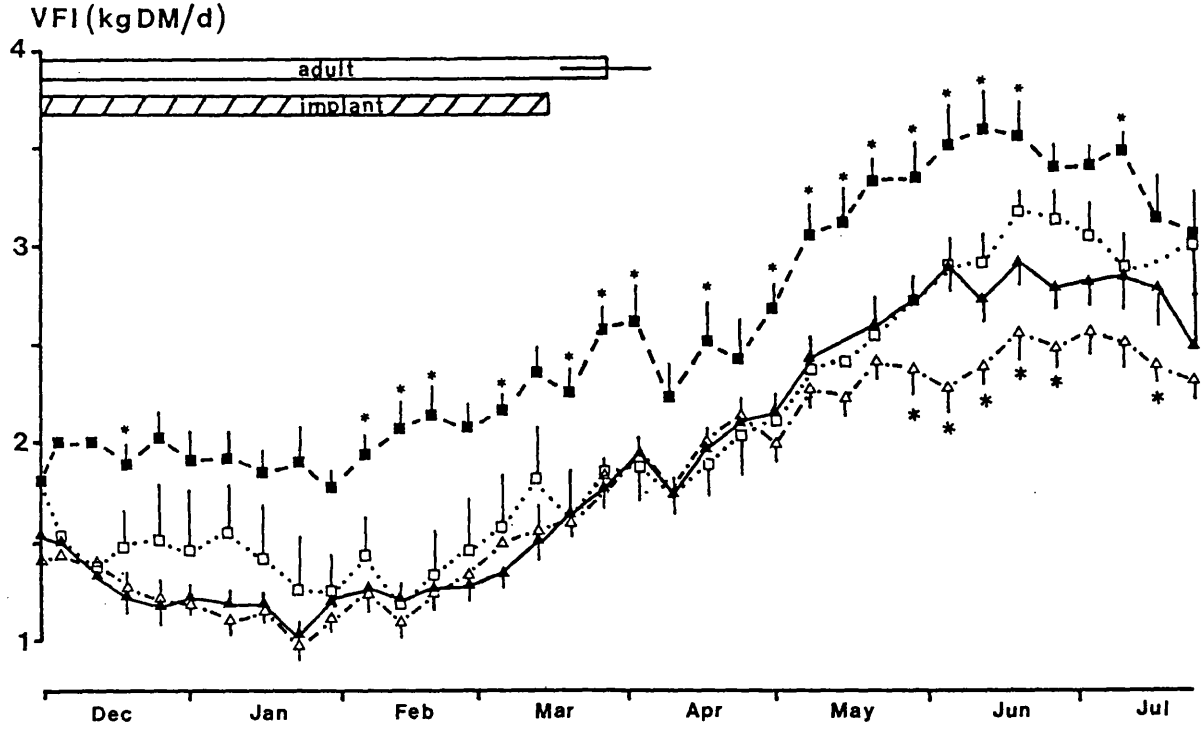
(i) Absolute food intake:

Effects of sexual maturity

The absolute VFI (kg DM/day) of the mature control group (group MC) was significantly greater than that of the pre-pubertal control animals (group PC) throughout the period of individual measurements (see Fig. 5.7).

The daily VFI of group MC hinds exhibited an average 27.0% reduction (range 10.0 - 46.3%) on the day of observed oestrus, compared to the mean intake for the rest of the oestrous cycle (based on data from 3 animals and 6 observations of oestrus).

FIGURE 5.7: Mean absolute food intake (± s.e.m. kg DM/day) of mature control (MC, ■), pre-pubertal control (PC, ▲), mature buserelin treated (MT, □) and pre-pubertal steroid-treated (PT, △) groups. Bars represent the duration of the adult breeding season (clear) and pre-pubertal steroid treatment (hatched). MC *vs* PC ($P < 0.05$, all time points except 23 Apr, 23 Jul); MT *vs* MC ('*' = $P < 0.05$); MT *vs* PC not significantly different. PT *vs* PC ('*' = $P < 0.05$); PT *vs* MC ($P < 0.05$, all time points except 16 and 23 Apr).



Effects of steroid manipulation

The absolute VFI of buserelin-treated mature hinds (group MT) was significantly lower than that of the mature control hinds (group MC) from early February until early June (see Fig. 5.7). The VFI of group MT was similar to that of group PC throughout the study.

The daily VFI of group MT hinds displaying oestrus activity exhibited an average 32.9 (± 1.69)% reduction on the day of observed oestrus (compared to the mean intake for the period between each oestrus). The decline in intake associated with oestrus was significant for both Y45 and B5 ($P < 0.05$). There was insufficient VFI data to compare the effect in Y20.

The VFI of the steroid-treated pre-pubertal animals (group PT) was similar to that of group PC until 28 May (see Fig. 5.7). Throughout June VFI was significantly lower in group PT ($P < 0.05$).

(ii) Food intake relative to metabolic liveweight

Effects of sexual maturity

The daily VFI corrected for metabolic live weight (g DM/$kg^{0.75}$/day) was similar between groups MC and PC at the time of the winter nadir (see Table 5.2 and Fig. 5.8). The peak VFI during summer was significantly lower in group MC ($P < 0.05$). There were, however, no differences in the timing of the nadir and peak in food intake related to sexual maturity.

Effects of steroid manipulation

There was no significant effect of buserelin treatment on the timing of either the winter nadir or summer peak in VFI. The level of VFI in group MT, however, tended to be lower than that of either groups MC or PC at both the seasonal VFI nadir and peak (see Table 5.2 and Fig. 5.8). Within group MT there was significant variation in VFI accompanying the occurence of oestrus (see Table 5.2. and Fig. 5.9). At the time of the winter nadir, the food intake of buserelin-treated animals displaying oestrus was significantly lower than the VFI of animals which did not (Table 5.2). The VFI of group MT animals exhibiting oestrus was also less than that of either groups MC or PC. At the time of the summer peak in VFI, however, the relationship was reversed. The food intake of animals in group MT

FIGURE 5.8: Mean (± s.e.m.) food intake relative to metabolic liveweight (g DM/kg$^{0.75}$/day) for mature control (MC, ■), pre-pubertal control (PC, ▲), mature buserelin treated (MT, □) and pre-pubertal steroid-treated (PT, △) groups. Bars represent the duration of the adult breeding season (clear) and pre-pubertal steroid treatment (hatched). Voluntary food intake exhited a significant ($P < 0.05$) elevation above mid-winter values from: 15 Mar.in group MC; 6 Mar. in group PC; 13 Mar. in group MT, and 11 Mar. in group PT. See Table 5.2 for remaining statistical analysis of treatment effects.

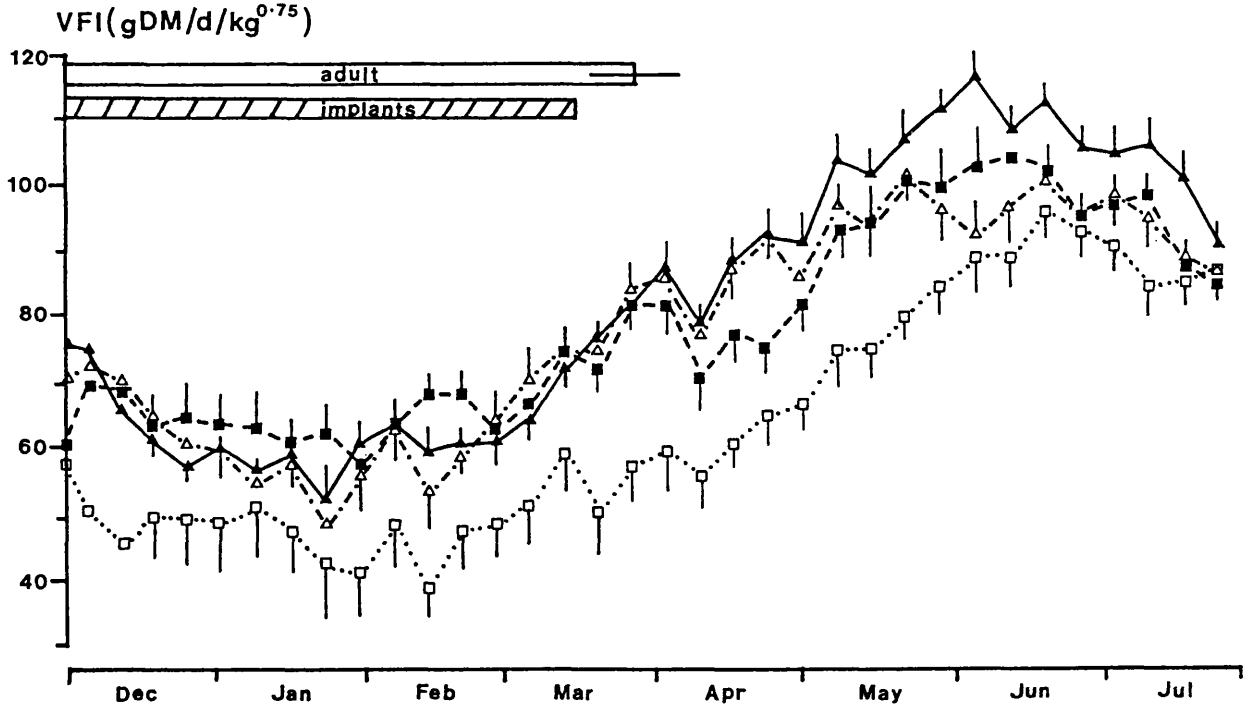
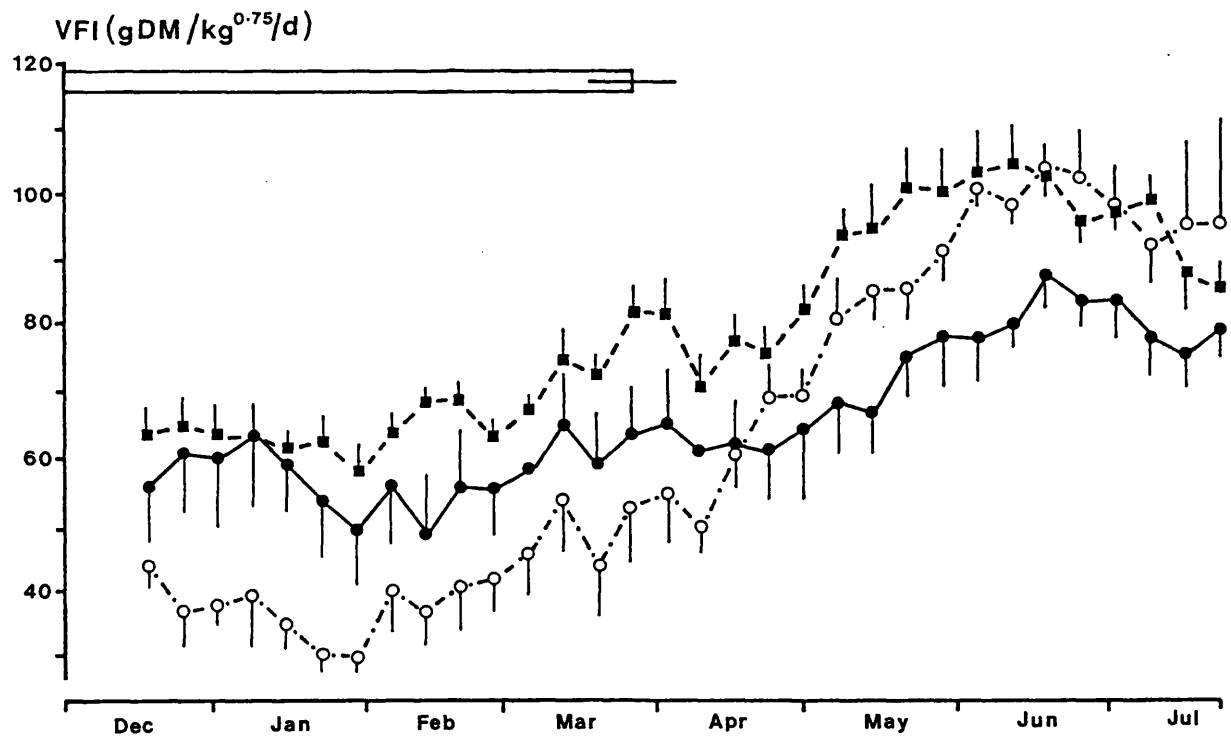


FIGURE 5.9: Mean (± s.e.m.) food intake relative to metabolic liveweight (g DM/kg$^{0.75}$/day) for mature control (MC, ■) and for mature buserelin-treated animals (MT) exhibiting (A, O) and not exhibiting oestrus (B, ●). The duration of the adult breeding season is indicated by the bar. Voluntary food intake exhited a significant ($P < 0.05$) elevation above mid-winter values from: 15 Mar. in group MC; 12 Apr. in sub-group MT(A), and 10 May in sub-group MT(B). See Table 5.2 for remaining statistical analysis of treatment effects.



not exhibiting oestrus was less than the intake of those in the same group which did. Their VFI was also less than that of animals in group MC and group PC.

Steroid treatment of pre-pubertal animals reduced the level of VFI at the summer peak compared to untreated animals (see Table 5.2 and Fig. 5.8). There were no differences in the timing of either nadir or peak intakes accompanying steroid treatment.

TABLE 5.2

The effect of treatment on timing and amplitude of the winter nadir and summer peak in VFI (g DM/kg$^{0.75}$/day). Values are $\bar{x}$ ± s.e.m. Results for group MT are presented as group mean, and as means for animals that did (A) and did not exhibit oestrus (B) (n=3 for both). Values with different superscripts (within each parameter) are significanty different ($P < 0.05$).

Group	(1) Winter nadir date	(1) Winter nadir VFI	(2) Summer peak date	(2) Summer peak VFI	(3) Change in VFI (1 - 2)
MATURE					
Group MC	14 Jan (± 9.2)	58.3^{c} (± 2.85)	29 Jun (± 4.7)	103.0^{e} (± 5.26)	44.7^{h} (± 3.47)
Group MT (all hinds)	29 Jan (± 7.0)	40.2 (± 7.02)	23 Jun (± 6.4)	96.6 (± 7.58)	56.5 (± 12.8)
Oestrus (A)	22 Jan (± 14.0)	29.0^{d} (± 1.27)	25 Jun (± 14.0)	109.9^{e,f} (± 9.66)	81.0^{i} (± 10.8)
No oestrus (B)	5 Feb (± 0.0)	51.3^{c} (± 10.9)	23 Jun (± 1.6)	83.4^{g} (± 3.97)	32.0^{j} (± 10.6)
PRE-PUBERTAL					
Group PC	16 Jan (± 5.8)	52.9^{c} (± 2.32)	30 Jun (± 2.4)	115.0^{f} (± 2.90)	62.1^{k} (± 3.32)
Group PT	20 Jan (± 5.3)	53.0^{c} (± 4.33)	25 Jun (± 10.5)	101.8^{e} (± 3.57)	48.8^{h} (± 2.93)

5.4.2.2. LIVE WEIGHT

Seasonal changes in live weight are shown in Figures 5.10, 5.11 and 5.12. All groups exhibited a significant increase in live weight during the study.

Effects of sexual maturity

The spring increase in live weight largely paralleled that of

VFI in mature (MC) and pre-pubertal (PC) control groups (see Fig. 5.10). Animals in group MC were heavier than those in group PC throughout the study ($P < 0.01$).

Effects of steroid manipulation

There was a trend for smaller liveweight gains in buserelin-treated animals (see Fig. 5.11). As a result the overall mean live weight of group MT (98.6 ± 0.71 kg) was significantly less than that of group MC (101.1 ± 0.75 kg; $P < 0.05$). The live weight of buserelin-treated animals exhibiting oestrus was significantly lower than that of animals which did not at the start of the study (92.3 ± 1.9 *vs* 103.7 ± 1.2, respectively, $P < 0.001$). The difference in live weight between these sub-groups was significant throughout the study (see Fig. 5.12).

There was no significant effect of steroid-implant treatment on the live weight of pre-pubertal animals (see Fig. 5.10). From late June, however, there was a trend for lower gains in group PT.

5.4.2.3. COAT GROWTH

Two pelage changes were observed during this study, the growth of winter coat during autumn followed by the growth of summer coat the next spring. Observations are summarised in Figure 5.13.

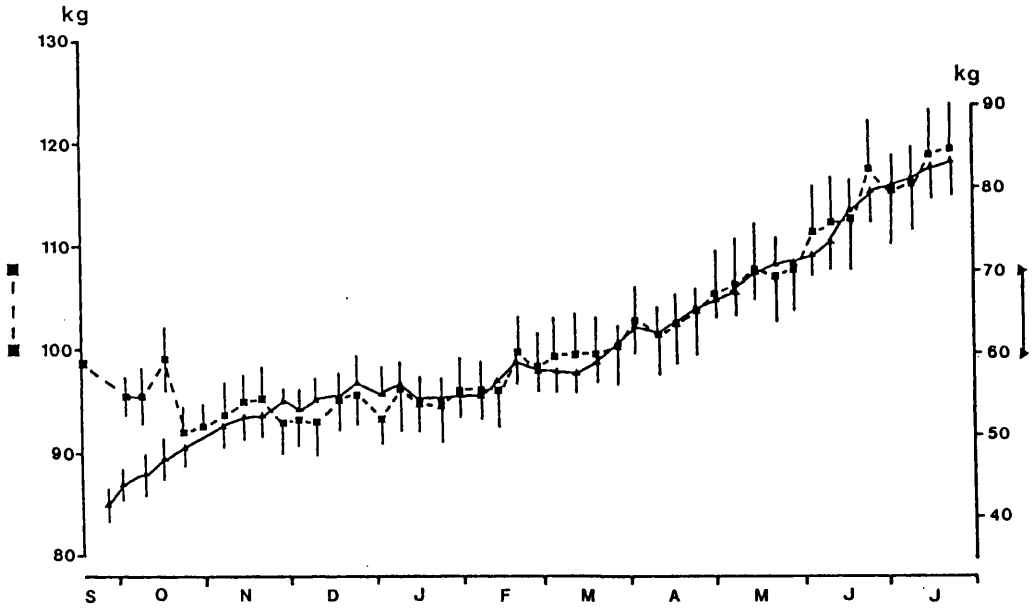
Growth of winter coat

The effects of treatments on the growth of the winter coat are described in Table 5.3. Winter coat growth commenced in some animals prior to the start of measurements on 19 September. To estimate the time growth began regression analysis was carried out on the first 10 primary fibre length values for each animal.

Effects of sexual maturity

The growth of primary fibres commenced approximately 14.5 days earlier in pre-pubertal than mature animals. The duration of the moult was significantly longer in the pre-pubertal animals (see Table 5.3)

FIGURE 5.10: (a) Changes in live weight ($\bar{x}$ + s.e.m.) of mature (MC, ■) and pre-pubertal (PC, ▲) control groups. MC *vs* PC, $P < 0.01$ at all time points. NOTE: different scale for groups MC and PC. Live weight was significantly elevated above mid-winter values from 23 Apr. in group MC and 2 Apr. in group PC, ($P < 0.05$).



(b) Changes in live weight ($\bar{x}$ + s.e.m.) of pre-pubertal control (PC, ▲) and steroid-treated (PT, △) groups. Differences are not significant. Live weight was significantly elevated above mid-winter values from 2 Apr. in group PC and 9 Apr. in group PT, ($P < 0.05$.)

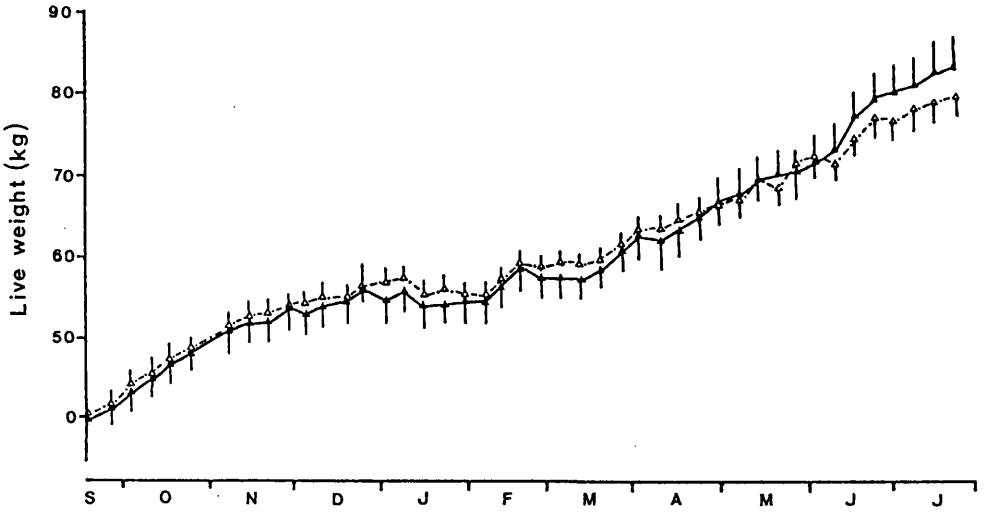
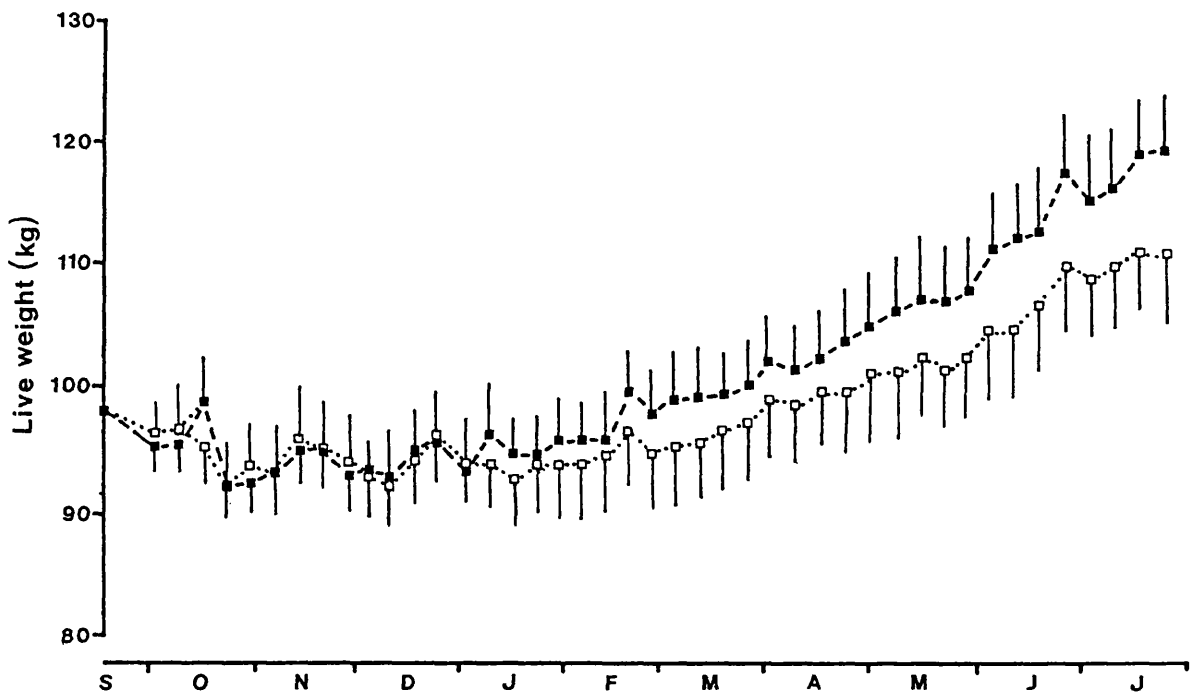


FIGURE 5.11: Changes in live weight ($\bar{x}$ + s.e.m.) of mature control (MC, ■) and mature buserelin-treated (MT, □) groups. The overall average weight for group MC was significantly greater than that of group MT, ($P < 0.05$).



Effects of steroid manipulation

There were no significant effects of buserelin or steroid implants on the timing of winter primary fibre growth, or the duration of the moult. The maximum primary fibre length for pre-pubertal animals was significantly shorter in the steroid-implanted group (group PT) than untreated animals (group PC).

There were no differences in the pattern of winter primary fibre growth between hinds in group MT associated with variation in observed oestrus and plasma oestradiol-17β concentrations (data not shown).

TABLE 5.3
The growth of the winter coat
Values in parentheses are days after 1 Jan. Values with different superscripts are significantly different ($P < 0.05$) ($\bar{x}$ ± s.e.m.).

Group	Onset of growth	Max. length (cm)	Duration of moult (days)
MATURE			
Total	26 Sep (268.6 ± 1.1)	5.82 ± 0.122	18.5 ± 2.23
Group MC	26 Sep[a] (269.0 ± 1.4)	5.90[cf] ± 0.091	18.2[g] ± 2.80
Group MT	25 Sep[a] (268.3 ± 1.8)	5.75[c] ± 0.214	18.7[g] ± 3.52
PRE-PUBERTAL			
Total	11 Sep (254.2 ± 2.8)	6.42 ± 0.120	32.3 ± 3.31
Group PC	8 Sep[b] (251.3 ± 4.6)	6.67[d] ± 0.167	31.1[h] ± 6.28
Group PT	14 Sep[b] (257.0 ± 3.3)	6.17[ef] ± 0.105	33.3[h] ± 3.02

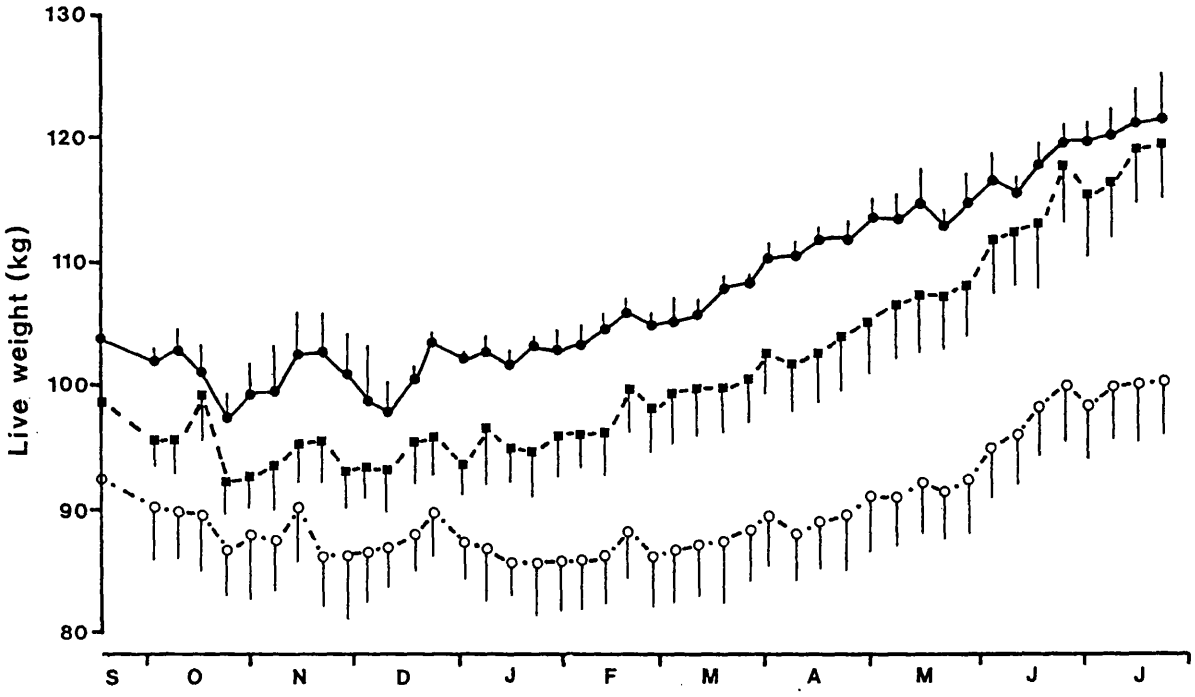
Growth of summer coat

The effects of treatments on the growth of the summer coat are described in Table 5.4.

Effects of sexual maturity

The growth of the summer primary fibres commenced approximately

FIGURE 5.12: Changes in live weight ($\bar{x}$ + s.e.m.) of mature control (MC, ■) and mature buserelin-treated hinds (group MT) exhibiting (A, O) and not exhibiting oestrus (B, ●). A *vs* B, $P < 0.01$ (for all time points). Live weight was significantly elevated above mid-winter values from 23 Apr. in group MC; 18 Jun. in sub-group MT(A), and 29 Jan. in sub-group MT(B), $P < 0.05$. Sub-group MT(B) exhibited significant fluctuations in weight between Oct. and Jan. ($P < 0.05$).



15 days earlier in pre-pubertal than mature animals. By the 23 July, however, the length of the coat was similar irrespective of sexual maturity. The duration of the moult was significantly longer in the pre-pubertal animals.

TABLE 5.4.
Growth of the summer coat.
Values in parentheses are days after 1 Jan. Values with different superscripts are significantly different ($P < 0.05$). ($\bar{x} \pm$ s.e.m.)

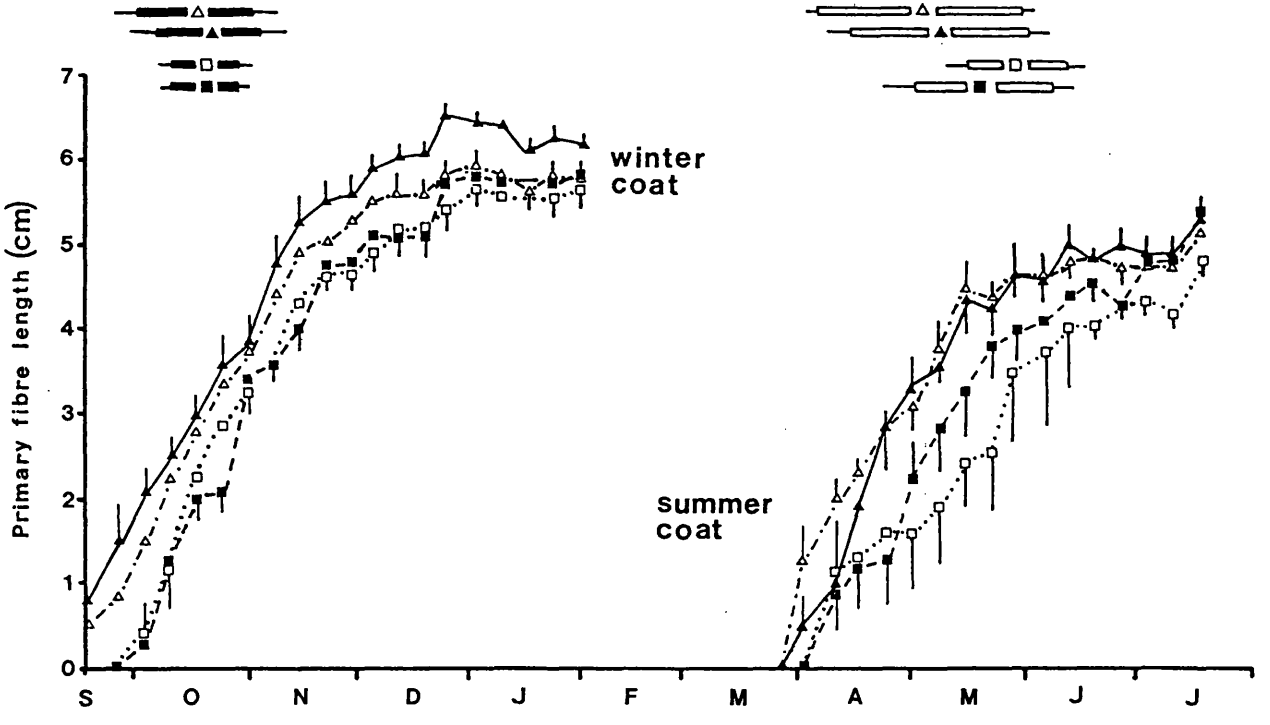
Group	Onset of growth	Max. length (cm)	Duration of moult (days)
MATURE			
Total	24 Apr[a] (114.4 ± 6.4)	5.09 ± 0.132	37.7 ± 5.83
Group MC	19 Apr (109.0 ± 6.3)	5.40[c] ± 0.171	42.2[e] ± 9.34
Group MT	29 Apr (119.0 ± 10.7)	4.83[d] ± 0.105	34.0[e] ± 7.78
PRE-PUBERTAL			
Total	9 Apr[b] (99.2 ± 2.5)	5.25 ± 0.097	57.5 ± 3.27
Group PC	13 Apr (102.7 ± 4.0)	5.33[c] ± 0.167	52.5[e,f] ± 5.92
Group PT	6 Apr (95.7 ± 2.5)	5.17[c] ± 0.105	61.0[f] ± 3.04

Effects of steroid manipulation

There was no significant effect of buserelin or steroid implants on the timing of summer coat growth. The maximum primary fibre length on the 23 July was, however, significantly less in buserelin-treated animals than in remaining groups.

There were no differences in the pattern of summer coat growth between hinds in group MT associated with variation in observed oestrus and plasma oestradiol-17β concentrations (data not shown).

FIGURE 5.13: The growth of winter and summer coats in mature control (MC, ■), pre-pubertal control (PC, ▲), mature buserelin treated (MT, □) and pre-pubertal steroid-treated (PT, △) groups. The duration of each moult is indicated by the group coded bars. Values are $\bar{x}$ ± s.e.m. There was no significant interaction between date and treatment for the growth of winter or summer coats. See Tables 5.3. and 5.4. for remaining statistical analysis of treatment affects.



5.4.2.4. PLASMA PROLACTIN CONCENTRATIONS

The plasma prolactin concentrations of the 4 groups are summarized in Figure 5.14 and 5.15. All groups exhibited a substantial rise in plasma concentrations during spring. The timing of this change was not significantly different between the treatment groups. The mean dates were: for animals in group MC, 15 March (± 11.5 days); for group PC, 9 March (± 8.0 days); for group MT, 16 March (± 9.4 days); for PT, 7 March (± 4.6 days).

There were no differences in the onset of the spring rise between hinds in group MT, associated with variation in observed oestrus and plasma oestradiol-17β concentration (data not shown).

FIGURE 5.14: Mean (± pooled s.e.m.; n=6) plasma prolactin concentrations (µg/l) for (a) mature (group MC, - - - -) and pre-pubertal (group PC, ———) control animals; (b) pre-pubertal control and steroid-treated females (group PT, - - -).

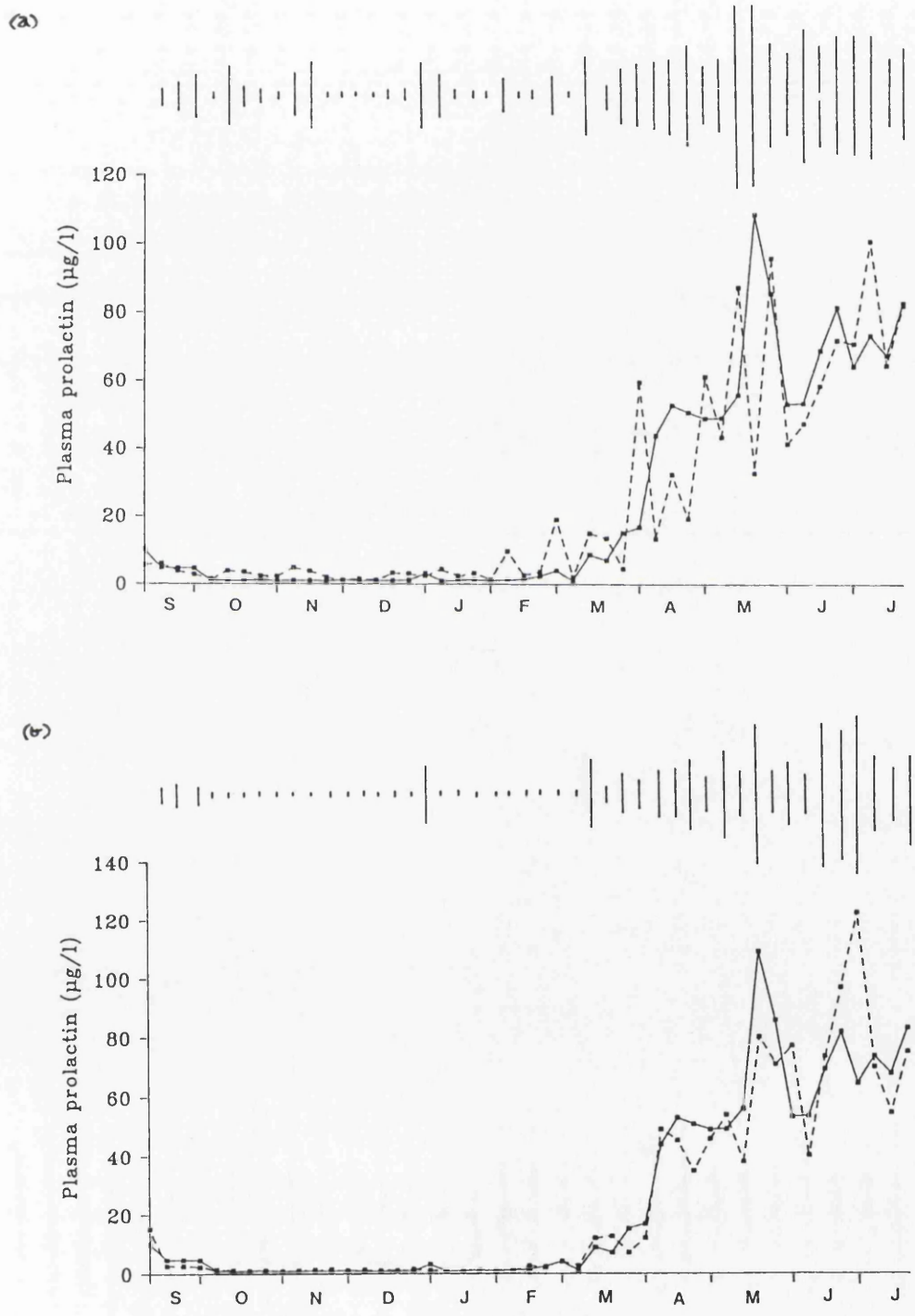
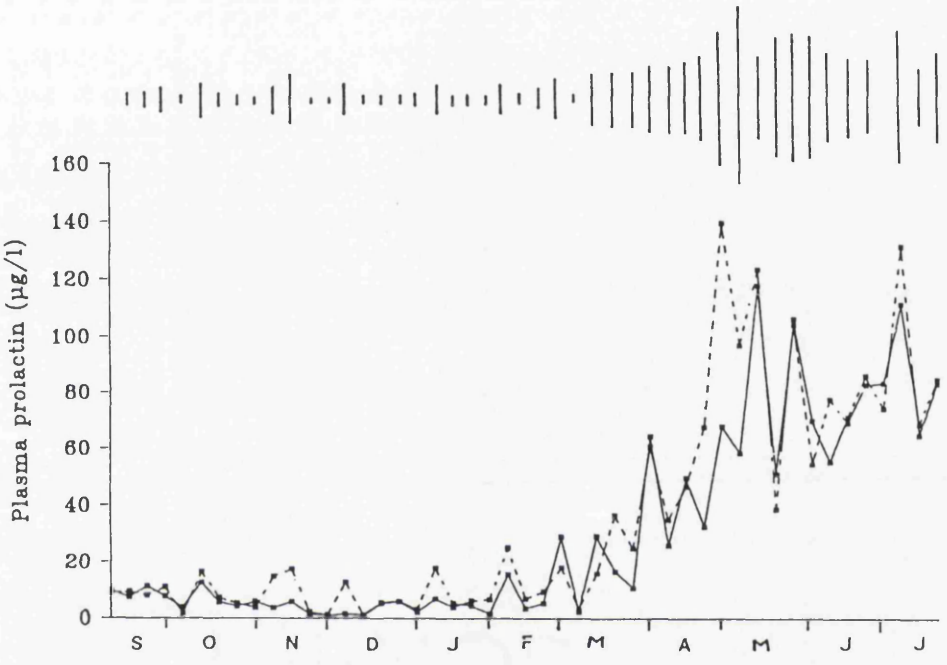


FIGURE 5.15: Mean (± pooled s.e.m.; n=6) plasma prolactin concentrations (µg/l) for mature control (group MC, ———) and buserelin-treated (group MT, - - -) females.



5.5.1. EFFECTS OF TREATMENT ON REPRODUCTIVE STATUS

5.5.1.1. Untreated mature animals

Mature untreated animals underwent cycles of plasma progesterone of similar length and amplitude to those observed during experiment 1, and described previously by Adam *et al.* (1989a). In the latter two studies, and that of Guinness *et al.* (1971), an average of 7-8 cycles were reported. This contrasts with the hinds in the present study, which underwent on average, at least, 10 cycles. Due to the earlier than expected start, the onset of measurements commenced after the beginning of the breeding season (5 Sept) resulting in a duration of > 209 days. This is substantially longer than the 1986/87 breeding season reported for the same herd (159.8 ± 6.9 days; Loudon *et al.*, 1989). There is no explanation for this difference.

Incidences of observed oestrus coincided with periods of low progesterone concentrations as previously reported in deer (Adam *et al.*, 1989a; white-tailed deer: Plotka *et al.*, 1980). The average interval between observations of oestrus in consecutive cycles (20.3 days, range 20-21 days, n=3) was similar to that observed in experiment 2 (18.8 days, range 18-20, n=4) and previous reports of between 18-21 days (Guinness *et al.*, 1971; Kelly *et al.*, 1985; Adam *et al.*, 1985).

There was no consistent correlation between plasma oestradiol-17β concentrations and stage of oestrous cycle in this study with the sampling frequency used. This contrasts with a clear peak in oestradiol-17β concentrations observed on the day of oestrus in other ruminant species (sheep, Hauger *et al.*, 1977; white-tailed deer, Plotka *et al.*, 1980). The concentration on the day of oestrus in group MC animals was 18.0 (± 3.4) pmol/l. This was substantially lower than the values reported for red deer by Kelly *et al.* (1985) which ranged between 154-450 pmol/l. The average value in this study, however, was comparable with concentrations detected on the day of oestrus of 33 pmol/l in sheep (Hauger *et al.*, 1977) and 33.4 (± 2.6) pmol/l in white-tailed deer (Plotka *et al.*, 1980). Kelly *et*

al. (1985) did not describe full assay methodology, and it may be tentatively concluded that red deer exhibit similar concentrations of oestradiol-17β during the oestous cycle to other ruminants.

5.5.1.2. Untreated pre-pubertal animals

Plasma progesterone concentrations in 4 of the pre-pubertal control animals (group PC) were low (< 0.06 nmol/l) throughout the experiment as reported by Loudon *et al.* (1989). Two animals, however, exhibited raised concentrations during the latter part of the adult breeding season (Feb - Mar). In one, y485, these were sufficiently elevated to satisfy the criteria used to define the onset of oestrous cyclicity (see section 2.6.4. for details). This indicates the existence of luteal tissue and by implication an ovulation. There was even evidence of a brief elevation in plasma progesterone concentrations, characteristic of 'silent oestrus', shortly before the sustained rise. This should have provided the necessary priming to enable oestrogens to elicit oestrus behaviour (Short, 1972), although none was observed.

Typically, red deer do not achieve puberty until at least their second potential breeding season. Even at two years old fertility in wild populations is highly variable (0 - 41 %: Hamilton and Blaxter, 1980; Clutton-Brock *et al.*, 1982a). The delayed puberty observed in in large deer species like red deer (Sadleir, 1987) may reflect the time required to achieve a high minimum live weight for reproduction (c.52 kg in female red deer in Scotland, Hamilton and Blaxter, 1980). Recent evidence, however, suggests that even if calves attain live weights during their first potential breeding season, sufficient, in adults, to permit ovulation, puberty does not occur (Loudon and Brinkow, 1990). This could be due to an inability to respond to photoperiodic signals, however, evidence (discussed in section 5.1) suggests this is unlikely. Alternatively, delayed puberty may be a consequence of immaturity of the reproductive system. Some components of the ovulatory mechanism are, however, mature well in advance of the normal age of puberty. The LH surge mechanism is able to respond to an oestradiol positive feedback (A.S.I. Loudon, B.R. Brinklow and B.J. McLeod, *unpublished data*), and the pituitary response to exogenous GnRH with LH release (same

study, and this experiment - see section 5.4.3.). Thus, the growth-related cues determining age of puberty appear to be more complex than a simple critical body weight. Loudon (1987) hypothesised that in sheep puberty occurs when the "proportion of their total energy intake allocated to growth falls below a critical threshold." Such a proposal could explain why the slower maturing red deer, still growing rapidly in its first autumn (Milne *et al.*, 1987) fails to breed despite reaching a theoretically adequate body weight at an appropriate time of year.

The ovarian activity observed in two animals from group PC suggests that a minority of individuals may in fact achieve a sufficiently developed state to display puberty during their first potential breeding season. The close proximity of cycling adults may have provided an important stimulatory cue for the early ovarian activity. As discussed in chapter 1 (section 1.2.11.2) social contact is known to have a profound influence on expression of reproductive activity. This includes oestrus synchronization (Iason and Guinness, 1985) and the ability to influence the onset of reproductive activity in other individuals (M.W. Fisher, *unpublished data*). Isolation from cycling females (and mature stags) may explain the absence of ovarian activity reported by Loudon and Brinklow (1990) in female deer during their first potential breeding season. Although some animals achieved live weights sufficient, in adults, to permit oestrous cyclicity, behavioural and/or pheromonal cues, via social contact, may have been required for the expression of puberty. There is further indirect evidence to support this hypothesis from the same study. Maintained in isolation from mature male and female deer, the timing of puberty (3 Dec ± 18 days) during the second potential breeding season occurred significantly later than the onset of breeding season in mature females from the same herd (< 5 Sept, adult data from this thesis, section 5.4.1).

The presence of elevated plasma progesterone concentrations does not necessarily reflect a fully competent normal *corpus luteum*. Thus, puberty may not have been attained by either animal exhibiting high plasma progesterone concentrations in group PC. Recent evidence from red deer populations in nutritionally abundant habitats, however, indicates that limited precocious puberty may occur in wild

deer (P.R. Ratcliffe, Forestry Commission, *unpublished data*). From data on culls, it was found that a small percentage of calves do conceive during their first potential breeding season. Large sample sizes indicated that 0.6 % of calves possessed a viable foetus, and that 1.5 % of yearlings were lactating.

Although there is evidence to suggest some red deer do conceive during their first autumn, the occurrence of puberty at this time is very limited. This is probably a consequence of both the large mature body size of this species, and the limited period during which puberty can be expressed. In sheep, late born and poorly nourished ewes fail to attain sexual maturity in their first autumn (Foster and Ryan, 1981; Foster *et al.*, 1986, 1988). It has been proposed that these animals have achieved insufficient growth to permit pre-ovulatory events before the onset of seasonal anoestrous in spring (Foster and Ryan, 1981; Foster *et al.*, 1985; Foster *et al.*, 1988). Thus, their transition to sexual maturity is 'masked' by the imposition of a seasonal increase in oestradiol inhibition preventing reproductive activity until the following autumn. The mature body size of red deer may mean that, like late born ewes, the majority fail to achieve sufficient growth before the onset of seasonal anoestrous. There is some limited evidence to support this hypothesis. In British populations of the closely related but smaller sika deer (*Cervus nippon*), it has been estimated, from cull data, that there is a much higher conception rate among females during their first potential breeding season (16.7% *vs* 0.6 - 1.5%, P.R. Ratcliffe, Forestry Commission, *unpublished data*).

In addition to the above, the single progesterone cycle observed in animal Y485 occured at the end of the adult breeding season. This is about three months after the main rut period has finished. In a natural situation there would be few, if any, fertile males in attendance of female groups at this time, reducing the chance of conception. In addition, as oestrus was not observed in Y485, it is unclear whether the behavioural mechanisms were functioning.

Oestradiol-17β concentrations in the pre-pubertal control group (24.2 ± 1.62 pmol/l) were substantially higher than the reported concentrations in pre-pubertal ewes (3 - 10 pmol/l; Foster and Ryan,

1981; Foster *et al.*, 1986). In fact, based on the limited number of samples measured, it appears that concentrations in pre-pubertal females are higher than the average for mature cycling hinds (15.6 ± 2.5 pmol/l; $P < 0.05$). Whether pre-pubertal values differ from those of anoestrus mature females, however, has yet to be determined.

5.5.1.3. Buserelin-treated mature animals

The aim of administering the GnRH-agonist, buserelin, was to suppress oestrous cycles throughout the entire breeding season, and to simulate the steroid hormone concentrations of pre-pubertal animals. Buserelin was successful in preventing oestrous cyclicity as determined by plasma progesterone. Since the treatment began after the unusually early onset of the breeding season, however, all animals experienced at least one oestrous cycle before the onset of treatment.

In three of the treated hinds (Y45, B5 and LB328) there was evidence that buserelin extended existing luteal phases beyond their period of normal duration. This may have been due to the buserelin induced LH surge (cattle, D'Occhio *et al.*, 1989) in some way extending *corpus luteum* (CL) life, or alternatively, with additional FSH secretion, resulting in ovulation and production of a second CL prior to luteolysis of the original. The brief decline in plasma progesterone concentrations observed during the middle of the elevated profile for animal LB328 is consistent with the second explanation (see Fig. 5.2). For a second ovulation to occur during an existing luteal phase would require the presence of a sufficiently developed follicle to ovulate. Evidence from the ewe (Hauger *et al.*, 1977) indicates this to be possible as low amplitude waves of oestradiol secretion and follicular growth occur during this period.

By mid October plasma progesterone concentrations in all buserelin-treated animals had fallen to levels typical of anoestrus or pre-pubertal animals. These concentrations persisted throughout the remainder of the breeding season. Notably, the time between the onset of buserelin treatment and fall of progesterone concentrations to anoestrus levels, showed little variation between animals (29.7, SD ± 1.7 days). After inducing an initial surge of LH (cattle,

D'Occhio *et al.*, 1989) this GnRH agonist induces pituitary insensitivity to GnRH induced gonadotrophin secretion. In the ewe, at least, this results in the suppression of FSH secretion and inhibition of pulsatile release of LH (McNeilly and Fraser, 1987; Picton *et al.*, 1990). Although neither was measured in this study, the failure of a high (100 µg) dose of exogenous GnRH to provoke a detectable elevation in plasma LH concentrations, implies a desensitization of the pituitary in the buserelin-treated deer.

The repeated oestrous behaviour in 3/6 of the buserelin-treated hinds was unexpected as plasma progesterone data indicated that these animals did not exhibit oestrous cyclicity. This observation has not previously been reported in any species. This was accompanied by higher plasma oestradiol-17β concentrations in those animals exhibiting oestrus (46.0 ± 4.9 *vs* 13.8 ± 5.1 pmol/l). Oestradiol-17β is important in eliciting oestrous behaviour (Short, 1972). The reason for the differences in oestradiol concentration in response to a fixed dosed of buserelin may be related to live weight. All animals exhibiting oestrus were lighter throughout the period of treatment (mean at start of study: 92.3 ± 1.9 *vs* 103.7 ± 1.2 kg; $P < 0.001$). This would suggest some dose-related difference in the effect of the agonist on the pituitary and thence the ovaries. The plasma oestradiol concentrations measured in the heavier buserelin-treated animals, not exhibiting oestrus, were similar to the average measured in the cycling hinds (15.6 ± 2.5 pmol/l). The substantially higher concentrations detected in the lighter buserelin-treated animals may have been related to the effect of this agonist on FSH secretion. At present there is no available antisum to measure deer FSH. In the ewe, secretion of this gonadotrophin is inhibited by continuously administered buserelin (McNeilly and Fraser, 1987; Picton *et al.*, 1990). In cattle, however, no effect of treatment on FSH secretion was observed (D'Occhio *et al.*, 1989). If the response in red deer to buserelin is similar to that reported in cattle then this could provide the necessary stimulation to drive follicle development perhaps explaining the elevated oestradiol-17β secretion. Evidence from sheep given buserelin indicates that exogenous FSH can stimulate growth of apparently normal large oestrogenic follicles in the

absence of pulsatile LH secretion (Picton *et al.*, 1990). A proper explanation of this phenomenon awaits further studies detailing the effects of long term buserelin treatment on plasma LH, FSH and oestradiol-17β secretion in deer.

A distinctive feature of the repeated oestrous activity displayed by buserelin-treated animals, was the short interval between observations of oestrus. Where as 18-21 day intervals are usually reported in cycling hinds (Guinness *et al.*, 1971; Adam *et al.*, 1985; this thesis), the buserelin-treated animals exhibited intervals that appeared to be in multiples of approximately 7 days. Based on evidence in the ewe it is believed that luteal phase progesterone secretion normally determines the 21 day interval via its suppressive action on the GnRH pulse generator (Goodman and Karsch, 1981 - see section 1.2.5.3 for details). In buserelin-treated hinds, there was no significant progesterone present to determine the timing of events. Due to this the short interval may have been determined by the periodicity of waves of follicular development in these animals. The approximately 7 day periodicity suggested by the present study is comparable with observations in the ewe. During the 16 day oestrous cycle in this species, waves of follicular development persist during the luteal phase resulting in a large peak in plasma oestradiol 4-6 days after the pre-ovulatory LH peak (Hauger *et al.*, 1977).

During the present study most observations of oestrus in buserelin-treated animals did not occur following periods of elevated plasma progesterone concentrations. This is inconsistent with the claim that, in sheep at least, females require recent exposure to progestrone to exhibit oestrus (Short, 1972). The low plasma progesterone concentrations during anoestrus are believed to explain the occurrence of an ovulation without overt oestrus ('silent' oestrus) at the start of the breeding season (red deer, Kelly and Challies, 1978; Webster and Barrell, 1985). The reason buserelin-treated hinds exhibited oestrus without progesterone 'priming' may be related to the high concentrations of plasma oestradiol-17β detected in these animals. In pre-pubertal red deer (i.e. with low progesterone concentrations), administration of high non-physiological doses of oestradiol-17β have been shown to elicit

oestrus in some animals (A.S.I. Loudon, B.R. Brinklow and B.J. McLeod, *unpublished data*).

5.5.1.4. Steroid-treated pre-pubertal animals

The objective was to simulate aspects of the ovarian steroid profiles of a cycling adult in pre-pubertal animals. The duration and amplitude of elevated plasma progesterone concentrations during the artifical 'luteal' and 'follicular' phases, were similar to those observed in the cycling adults. Due to the early start of the breeding season during the year of the study (see section 5.5.1.1.) the period of steroid treatment commenced at least 5-6 weeks after the onset of oestrous cyclicity in mature animals. As a consequence of this, and the long duration of the breeding season, the pre-pubertal treatment group experienced approximately 3 progesterone 'cycles' less than the mature hinds.

The oestradiol implants, as dicussed in section 5.2, were intended to achieve a plasma concentration of the steroid similar to the average present during the oestrous of the ewe (i.e. about 20 pmol/l, Hauger *et al.*, 1977). The implant design assumed an endogenous concentration of about 3-10 pmol/l in pre-pubertal animals (based on evidence in ewes, Foster and Ryan, 1981; Foster *et al.*, 1986). The actual endogenous plasma concentration in the pre-pubertal deer, however, was considerably higher at 24.2 (± 1.6) pmol/l. Due to this the steroid concentrations in the treated animals were more than twice the average observed in mature hinds (35.4 ± 3.4 *vs* 15.5 ± 2.5 pmol/l; $P < 0.05$).

The pituitary LH response to exogenous GnRH was substantially lower in the steroid-treated pre-pubertal animals than in the control group. This might have been a consequence of the elevated oestradiol-17β concentrations in steroid-treated animals. In the ewe, comparison of the LH secretory pattern of ovariectomized animals with, and without oestradiol implants, has demonstrated that the presence of this steroid reduces LH pulse amplitude (Goodman and Karsch, 1981). It is proposed that oestradiol acts, at least in part, upon the anterior pituitary gland by decreasing its response to GnRH. Therefore, the lower LH response observed in animals treated with exogenous oestradiol in the present study may have

resulted from elevated concentrations decreasing pituitary sensitivity to GnRH.

5.5.2. THE EFFECT OF OVARIAN STEROIDS ON THE TIMING OF SEASONAL CHANGES

The main objective of this study was to test the hypothesis that 'ovarian steroids secreted during the breeding season modulate the expression of an underlying seasonal rhythm, and thereby delay the onset of spring changes in VFI, live weight, coat growth and plasma prolactin'.

5.5.2.1. The effects on voluntary food intake and live weight

Prior to this study available evidence suggested that the increase in VFI may commence and peak later in cycling than pre-pubertal red deer possibly as a result of reproductive activity (Loudon *et al.*, 1989). In this experiment, however, sexual maturity did not influence the timing of either the winter nadir or summer peak in food intake. The level of VFI (relative to metabolic live weight) in pre-pubertal and mature females was similar at the time of the winter nadir, but significantly lower in mature animals at the summer peak. This difference occurred nearly three months after the breeding season suggesting it was not a direct effect of reproductive hormones. Instead, the difference may reflect a greater food requirement for growth in pre-pubertal deer.

Consistent with observations in sheep, a significant reduction in the VFI of cycling hinds was exhibited on the day of oestrus (Tartellin, 1968; Argo, 1986). This inappetance corresponds with the period of elevated oestradiol-17β concentrations reported during the follicular phase in the ewe (Hauger *et al.*, 1977). The failure to detect a clear peak in oestradiol at the time of oestrus in the present study, may reflect the low frequency of sampling. In addition to reducing feeding activity, in rats, oestradiol has been shown to reduce adiposity (Wade and Gray, 1979). It is suggested by these authors that in cycling mammals (particularly at oestrus) elevated oestradiol concentrations act to direct nutrients away from

adipose tissue and the liver, so that they can be used as a fuel for the increased activity observed in cycling animals.

To further investigate the influence of sexual maturity on seasonal changes in VFI and growth the ovarian steroid environment of female deer was experimentally manipulated.

In mature animals oestrous cyclicity was suppressed using buserelin. Although treatment resulted in substantial changes to circulating steroid concentrations (see section 5.5.1.3.) there were no significant effects on the timing of the seasonal nadir or peak in VFI. The amplitude of intake changes, however, was modified. The food intake of buserelin-treated animals was lower than that of the untreated deer throughout most of the study. Within the treatment group were significant differences in food intake correlating to the observed variation in oestradiol-17β concentrations and oestrous behaviour. In the three lightest animals which exhibited repeated oestrus and higher oestradiol concentrations, the level of VFI at the winter nadir was only about 50% of that in the remaining buserelin-treated hinds. In these heavier animals the level of food intake and oestradiol concentrations were similar to the control group. This suggests that the high levels of oestradiol in some buserelin-treated hinds may have resulted in an increased suppression of food intake during winter. This is consistent with evidence in both female sheep (Forbes, 1972) and rodents (Czaja, 1984) demonstrating that exogenous oestradiol depresses food intake. It is important, however, to note that the seasonal increase in VFI in these deer began during the buserelin treatment period.

The suppression of plasma progesterone concentrations by buserelin did not appear to significantly influence VFI. This conclusion is suggested by the similar mid-winter food intake of mature control animals and those which were treated, but did not exhibit oestrus. In these animals oestradiol concentrations were similar.

Within the buserelin-treated group, peak VFI during summer was greater in animals which exhibited higher concentrations of oestradiol during the treatment period. Their change in VFI between nadir and peak represented a 3.8 fold increase in food consumption.

This suggests that the depressed appetite state of these deer during winter was followed by a period of rapidly increased food intake to compensate. The increase in intake commenced before the end of buserelin treatment, at about the same time as increases in food intake in the remaining groups. Similar compensatory responses have been reported in juvenile red deer hinds and stags following experimental depression of winter food intake (Suttie and Hamilton, 1983; Adam and Moir, 1985; Milne *et al.*, 1987). The smaller 1.6 fold increase in food intake between winter and summer observed in the remaining buserelin-treated animals may reflect their significantly higher live weight. If associated with high body condition, then these animals may have required less food for fat deposition.

Although buserelin did not alter the phase of seasonal VFI changes, it did influence the onset of the spring rise in live weight. In the 3 hinds exhibiting highest oestradiol-17β concentrations, live weight increases were delayed compared to the remaining mature animals. It is possble that this was a result of high oestradiol concentrations suppressing fat deposition. In female rats suppression of adiposity is an important metabolic effect of this steroid (Wade and Gray, 1979).

The mechanisms by which oestradiol influences VFI and growth in deer have not been determined. Injection of oestradiol into the brain of ewes results in a suppression of intake suggesting this steroid effects appetite centres in the brain (Forbes, 1972). In the rat, oestradiol receptors have been located in both the hypothalamus and the pituitary (Eisenfeld, 1970). Oestradiol has a stimulatory effect on the pituitary-adrenal axis which results in elevated cortisol secretion (sheep, Sillence *et al.*, 1987). Glucocorticoids are catabolic in muscle, causing increased muscle degradation (Thomas *et al.*, 1979) and reduced growth rates when administered to young rats (Odedra *et al.*, 1980). The higher endogenous levels of cortisol observed in female compared with male sheep (Sillence *et al.*, 1987) and cattle (Henricks *et al.*, 1984) are believed to explain the slower growth rates of females. This evidence suggests that suppression of growth in buserelin-treated animals experiencing high oestradiol concentrations may, at least partly, have been a consequence of elevated cortisol secretion.

Imposing simulated oestrous cycles on pre-pubertal animals failed to influence the timing of VFI or live weight changes providing further evidence that steroids released during the breeding season do not modulate the timing of seasonal changes in VFI. Increasing plasma progesterone and oestradiol-17β concentrations also failed to influence the level of VFI or growth during the treatment period. A similar lack of effect on growth during a comparable long-term elevation of oestradiol-17β has been reported in ewe lambs (Foster *et al.*, 1986).

Whilst there were no differences in VFI and growth during the steroid treatment period, the peak summer intake was lower in treated pre-pubertal than the control deer . This was associated with a trend of lower live weight gains from June. Thus it appears that exposure to oestrous cycle concentrations of oestradiol and/or progestrone resulted in a reduction in future growth by the treated pre-pubertal females. This suggests that the reduced growth observed in following puberty in deer (Fennessy *et al.*, 1981) may be a consequence of this initial exposure to ovarian steroids.

Evidence from this experiment indicates that oestrous cyclicity does not delay the onset of spring increases in VFI and live weight as suggested by data from an early study (Loudon *et al.*, 1989, see section 5.1). The difference between observations in this study and those reported by Loudon *et al.* (1989) may be related to experimental design. In the earlier study, mature and pre-pubertal animals were kept at different latitudes (51°30'N and 56°54'N respectively). Due to this the two age groups were exposed to different environmental conditions. The lower spring temperatures and/or the larger daylength changes experienced by pre-pubertal deer at the higher latitude, may explain the difference in timing of VFI changes. Both these environmental factors, but particularily photoperiod, influence the timing of seasonal changes (see chapter 1). The synchrony of changes in mature and pre-pubertal animals during the present study may also reflect the influence of social factors as all of the animals were maintained in the same building and were exercised together (see section 1.2.11.2).

5.5.2.2. Effect on coat growth and plasma prolactin concentrations

Two pelage changes were observed during this study. Both of these were delayed by two weeks in the mature control females compared to the control pre-pubertal females. It is perhaps significant that each moult coincided with a change in the reproductive state of the mature deer. The onset of winter primary fibre growth commenced at the beginning of the breeding season, whereas, summer primary fibre growth began at the end of the breeding season. These observations are consistent with the hypothesis examined by this study that 'ovarian steroids secreted during the breeding season modulate expression of an underlying seasonal rhythm, and thereby delay changes in coat growth'. Manipulations of ovarian steroid concentrations were used to determine if the phase delay observed in mature animals could have been due to the action of these steroids.

Experimental treatments commenced too late to provide evidence of the involvement of steroids in the delayed onset of winter coat growth. Suppression of oestrous cyclicity in adults and simulation of cycles in pre-pubertal females, however, failed to influence the rate of primary fibre growth. This suggests that ovarian steroids do not influence the rate of growth once commenced.

In addition to a difference in the onset of coat growth, there was variation in the maximum primary fibre length and the duration of the moult associated with sexual maturity. The average pre-pubertal primary fibre length was 13% (0.75cm) greater than that of mature animals. This may reflect an adaptation to reduce the effect of smaller body size (and hence larger surface area to volume ratio) on heat loss. Exposure of pre-pubertal deer to simulated oestrous cycles was associated with a reduction in the the maximum winter coat length. This suggests that ovarian steroids may be responsible for the lower primary fibre length of mature animals. Suppression of oestrous cycles in adults using buserelin, however, did not result in an increased coat length. It may be significant that buserelin reduced only plasma progesterone and not oestradiol-17β concentrations. If it were oestradiol that were responsible for the shorter primary fibre length seen in steroid-treated pre-pubertal

deer, then this could explain the absence of variation between mature groups. A further difference accompanying sexual maturity was the longer duration of the moult in pre-pubertal animals (defined as the interval between 100% existing and 100% new pelage). Possibly pre-pubertal animals are more sensitive to the increased heat loss associated with moulting (white-tailed deer, Silver *et al.*, 1969) and attempt to reduce this by retaining their summer coat longer.

Whilst the onset of summer coat growth coincided with the onset of seasonal anoestrus in control mature animals, experimental treatments failed to provide evidence that ovarian steroids could have been responsible for the delay seen in mature animals. Suppression of oestrous cyclicity using buserelin resulted in a trend towards delayed winter primary fibre growth, and a lower maximum length at the end of the study in mature animals. Although this may have been a direct effect of steroid manipulation it could also have been a consequence of the lower VFI and live weight of buserelin treated animals. Experiment 2 (chapter 4) demonstrated that poor nutrition can delay the onset of coat growth in lactating deer.

Simulating oestrous cycles in pre-pubertal animals did not delay the onset of summer primary fibre growth. While this suggests ovarian steroids do not influence the onset of growth it is important to note that the last simulated cycle commenced 20 days earlier than the final oestrous cycle in the mature animals. Thus, it remains impossible to assess whether differences between pre-pubertal and mature animals were related to an effect of oestrous cyclicity.

Despite the coincidence of reproductive and pelage changes there is little evidence from steroid manipulations to indicate that delayed coat growth in the mature compared to the pre-pubertal females was a consequence of oestrous cyclicity. This is consistent with reports that gonadectomy in male mink (Allain and Martinet, 1984) and in hamsters of both sexes (Duncan and Goldman, 1984a) does not significantly influence pelage changes.

The onset of summer coat growth also coincided with the seasonal increase in prolactin secretion. This is consistent with evidence that prolactin is involved in mediating photoperiodic effects on coat growth (Martinet *et al.*, 1984 - see section 1.2.7.). Plasma prolactin concentrations, however, were unaffected by sexual maturity despite differences in coat growth. Possibly variation in the timing of pelage changes resulted from a maturity related difference in the sensitivity of hair follicles to the same photoperiod or prolactin signal.

5.5.3. SUMMARY

The main objective of this study was to test the hypothesis that 'ovarian steroids secreted during the breeding season modulate the expression of an underlying seasonal rhythm, and thereby delay the onset of spring changes in VFI, live weight, coat growth and plasma prolactin'.

This was investigated by suppressing the breeding season in a group of mature females, and by simulating the ovarian steroid concentrations of cycling hinds in a group of pre-pubertal animals.

Administration of continuous release buserelin successfully suppressed oestrous cyclicity in mature females. Treatment reduced plasma progestrone concentrations to a level comparable with anoestrous or pre-pubertal animals. The overall effect on plasma oestradiol-17β was to elevate concentrations to a similar level to that observed in pre-pubertal deer. Live weight, however, appeared to influence the response to buserelin treatment. In the lighter animals, plasma oestradiol concentrations were higher than untreated mature or pre-pubertal deer. These treated deer also exhibited repeated oestrus at intervals considerably less than the normal 21 days. In the heavier buserelin-treated animals, plasma oestradiol concentrations were similar to those in untreated mature females.

Progesterone implants were successful at simulating oestrous cycle concentrations of this steroid in pre-pubertal animals. Oestradiol-17β implants elevated plasma concentrations of the hormone as intended. The endogenous concentrations present in pre-pubertal animals, however, were higher than expected. As a result

plasma oestradiol concentrations following treatment were significantly higher than those present in mature cycling hinds.

Comparison of untreated mature and pre-pubertal females revealed that the timing of spring changes in VFI and live weight were similar. There was also no evidence that *normal* elevations of ovarian steroids during the breeding season significantly modify the average daily intake of female deer. This is demonstrated by the similarity of VFI between cycling adults and pre-pubertal animals with, and without the simulated oestrous cycle steroid profiles. There was, however, a transient reduction of intake associated with oestrus. Although not demonstrated in this study, evidence in sheep suggests this may be a related to high pre-ovulatory oestradiol concentrations (Hauger *et al.*, 1977). Appetite suppression by oestradiol may also explain the lower VFI in 3 of the buserelin-treated animals. In the pre-pubertal animals, VFI and growth were lower during mid-summer in the animals which had previously experienced simulated oestrous cycles. This suggests that exposure to ovarian steroids at puberty may reduce the potential for further growth.

The onset of growth of both the winter and summer coats were delayed in mature compared to pre-pubertal females. Although these pelage changes coincided with the onset and termination of the breeding season in the adults, manipulation of steroid concentrations failed to influence the timing of coat growth. This might suggests that oestrous cyclicity in mature females was not responsible for the difference. Steroid implants were, however, accompanied by a reduction in winter coat length in the pre-pubertal deer. Despite differences in the timing of summer coat growth, the onset of the seasonal rise in plasma prolactin concentrations occured at the same time in both mature and pre-pubertal deer.

CHAPTER 6
GENERAL DISCUSSION

6.1. The control of seasonality in red deer with reference to British populations.

Red deer exhibit seasonal rhythms of metabolism, reproduction and pelage which have evolved as a response to the variation in climate and food resources characterising temperate zone habitats. Studies of enhoused deer maintained on an *ad libitum* diet suggest that seasonal changes in photoperiod are utilised by deer to entrain these rhythms to environmental periodicity (see chapter 1). Few studies, however, have examined the influence that variation in food resources exerts on expression of seasonal rhythms. The aim of this thesis was to investigate the interaction between herbage availability and endogenous rhythms to provide a better understanding of the control of seasonal changes in wild red deer.

The first experiment (chapter 3) demonstrated that the seasonal appetite changes exhibited by red deer fed to appetite (Kay, 1979; Loudon *et al.*, 1989) can influence the herbage intake of grazing deer. The experiment revealed, however, that expression of appetite changes are dependent on the availability of herbage resources. When resources are too scarce to satisfy the appetite level deer appear to maximise herbage intake under the prevailing conditions. An important factor limiting the ability of deer to compensate for low herbage resources is an apparent ceiling on the duration of daily grazing activity, which is probably imposed by the need to ruminate. The maximum grazing time observed in this study was estimated at about 12.6 h/day (section 4.5.1.1.). This is similar to the maximum times previously reported in a variety of ruminant species (see section 1.2.12.1).

The majority of red deer in the British Isles occupy the open moorland areas of the Scottish Highlands. These are an atypical habitat for this species. Throughout most of their geographical range (see section 1.1) red deer are associated with open woodland or forest edge habitats (Kay and Staines, 1981). Extensive

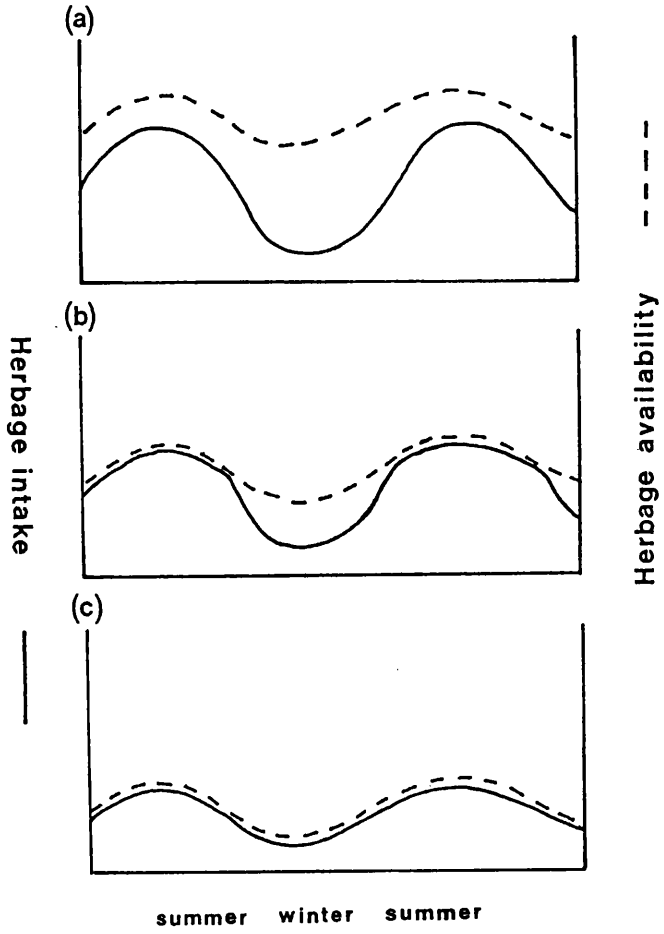
deforestation forced the Scottish populations onto moorland areas. These are characterised by impoverished acidic soils with a relatively low annual production of plant biomass (Kay and Staines, 1981). The nutritional constraints imposed by the moorland environment are illustrated by the relatively small size of Scottish red deer. In general, these deer are half as heavy as their counterparts found in the forested areas of central Europe (Mitchell *et al.*, 1977). The potential for Scottish red deer to achieve greater live weights has been demonstrated by removing male calves from the wild and rearing them on high quality diets. Under these conditions stags have been reported to achieve live weights of 150-180 kg by two years of age (Blaxter *et al.*, 1974) compared to maximum mature weights of 120 kg in wild populations (Mitchell *et al.*, 1976). In view of the results of the first experiment it is probable that the food intake of deer in these impoverished habitats is primarily determined by herbage availability during much of the year.

The first experiment showed that adequate herbage availability is required for expression of appetite changes. During the second experiment non-lactating hinds consumed significantly less herbage than lactating hinds grazing the same low availability pasture. This suggests that for a given availability of herbage, differences in appetite between individuals means that for some deer herbage resources may be adequate but not for others. Thus, herbage intake in grazing mammals, especially in marginal habitats, is controlled by a complex interplay of appetite and food availability. A simple model outlining the interaction between these factors is described in Figure 6.1.

Administering exogenous melatonin between mid-summer and autumn advanced the timing of the onset of the breeding season in both non-lactating (15 days, experiment 1) and lactating (18 days, experiment 2) hinds. This response is consistent with the involvement of photoperiod in the control of the timing of the seasonal reproductive cycle (see chapter 1).

The principal influence of herbage availability on the breeding season was related to the ability to exhibit oestrous cycles, and

FIGURE 6.1: A model for the interaction between herbage availability (-----) and appetite in the control of food intake (———) in grazing deer:
(a) If herbage resources are abundant then the level of food intake is primarily determined by the appetite state of the individual.
(b) Under certain conditions herbage resources may be sufficient for for deer to achieve their appetite requirements at certain times of the year but not at others.
(c) If herbage resources are scarce then hinds may be unable to meet their appetite requirements, in which case food intake is primarily determined by herbage availability.
The food intake cycle illustrated is based on the the voluntary food intake (VFI) of enhoused red deer hinds maintained on an *ad libitum* diet (Loudon *et al.* 1989). Under these conditions the VFI cycle reflects seasonal changes in appetite.



not on the timing of the onset of the breeding season. It appears that hinds commence oestrous cycles at particular time irrespective of the effects of herbage abundance on food intake, unless they are in poor body condition in which case oestrous cyclicity is suppressed. Avoiding conception when in poor body condition may be important for the hind's survival as the additional energetic demands of pregnancy could prove fatal to an animal with low energy reserves during winter. The insensitivity of the timing of the breeding season to nutritional status may be related to the importance of conceiving (and therefore calving) during a specific period of the year. Failure to synchronize parturition with optimum environmental conditions in early summer has been shown to exert a heavy cost on reproductive success (see section 4.1). Thus, while fluctuations in food resources have had an important role in the evolution of seasonal reproduction, the acute control of the timing of the breeding season appears to be independent of prevailing herbage abundance.

Considering the apparent importance of achieving conception during a brief period in autumn it is unclear why red deer should have such a long potential breeding season. In the absence of a successful mating, hinds exhibit continual oestrous cyclicity for up to 5 months (see experiment 1 and 3). In fact, in southern England some hinds continue to cycle to within 1½ months of the normal calving period (see experiment 3). There is no clear explanation for this phenomenon. The reason could be partly historical. The ancestral Artiodactyla from which deer subsequently evolved first appeared in tropical forests (Rose, 1982). In a largely aseasonal environment polyestry is common. When seasonality evolved there may have been relatively little selection pressure on the time of termination of the breeding season since almost all females conceive at their first or second oestrus (red deer, Guinness *et al.*, 1971; Adam *et al.*, 1985). In addition, greater precision is conferred by the seasonality of male potency and sexual behaviour which occurs over a briefer period than the potential female breeding season (Lincoln, 1985). It may also be significant that if hinds fail to achieve sufficient body condition to exhibit oestrus cycles at the beginning of the breeding season, then it is unlikely that condition

will improve before the onset of seasonal anoestrus. This is due both to declining seasonal appetite state and herbage availability at this time.

If as suggested there has been little selection pressure on the time of termination of the breeding season, there is no obvious reason why seasonal anoestrus occurs when it does in spring. One possiblity relates to the seasonal rise in food resources that occurs at about the same time as anoestrus commences. The function of anoestrus may be to prevent non-pregnant hinds in too poor body condition to exhibit oestrous cycles at the beginning of the breeding season, from conceiving when food availability (and body condition) increases in spring.

Thus, the prolonged potential breeding season and the relatively invariant time of its onset, suggests that the primary role of seasonal anoestrus is to prevent conception occurring too early, rather than too late.

The timing of seasonal changes in coat growth exhibited a greater sensitivity to environmental influences than reproduction. Melatonin treatment resulted in a 35 day advance in the onset of winter primary fibre growth. In addition, there was a delay in fibre growth associated with lactation and poor nutrition. Not only was the timing of coat growth delayed, but the density of hair fibres in winter coat during January was reduced following experience of limited herbage resources the previous summer. These effects on the winter coat may increase heat loss. For deer living in exposed moorland habitats with limited food resources and low fat reserves this may have significant effect on survival during harsh winters.

Seasonal changes in coat growth were closely associated with changes in plasma prolactin concentrations. The growth of summer coat primary fibres coincided with the spring increase in plasma prolactin concentrations (experiments 3), whereas, the onset of growth of winter coat fibres commenced as plasma concentrations declined after mid-summer (experiment 2). The advance in the onset of winter coat growth following melatonin treatment was associated with an early and precipious decline in plasma prolactin concentrations. The delayed winter coat growth in poorly nourished lactating hinds

was accompanied by elevated plasma prolactin concentrations, possibly due to a change in suckling activity arising out of lower milk production. These observations are consistent with a hypothesis that prolactin is involved in the control of pelage changes (see section 1.2.7.).

This thesis also examined influences of the breeding season on the timing of seasonal changes in VFI, live weight, coat growth and prolactin secretion. Comparison of the timing of these changes in mature and pre-pubertal females revealed that the only difference related to sexual maturity was a two week delay in the timing of winter and summer coat growth in mature deer. This, however, did not appear to be related to differences in ovarian steroid secretion. Suppressing the oestrous cyclicity in mature females and stimulating oestrous cycle steroid concentrations in pre-pubertal animals demonstrated that ovarian steroids do not significantly modify the phase of seasonal rhythms in red deer.

The steroid-treated pre-pubertal females showed a reduced level of food intake during the following summer compared to the non-treated pre-pubertal females. This indicates that ovarian steroids are probably the cause of the reduced growth observed following puberty (Fennessy *et al.*, 1981). It is well established that oestrogens influence epiphysial plate closure.

To conclude, it appears that there is a complex interaction between herbage availability and the endogenous rhythms controlling seasonal changes. Expression of seasonal changes in appetite are highly dependent on the availability of food resources. In addition, nutritional status influences not only the timing of coat growth but also the density of hair fibres. The timing of reproduction, however, seems to be relatively insensitive to the effects of herbage availability, although if body condition is too poor hinds can not exhibit oestrous cycles.

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