12

Seasonal Profiles of Plasma Testosterone, Prolactin, and Growth Hormone in Red Deer Stags

G. K. Barrell, P. D. Muir, and A. R. Sykes

Animal Sciences Group, Lincoln College, Canterbury, New Zealand

Abstract

Blood samples were collected from adult red deer stags (*Cervus elaphus*) each week for 2 years to define the seasonal secretion patterns of testosterone, prolactin, and growth hormone and to relate these to different phases of the annual antler growth cycle. Plasma prolactin levels were low in winter, rose in spring near the time when hard antlers were cast, and reached peak values at mid summer. As prolactin levels subsequently declined in late summer, plasma testosterone concentrations increased and reached their peak levels during the rutting period in April. Plasma testosterone levels were lowest at the time of antler casting but elevated levels were associated with shedding of velvet and antler mineralisation.

Taken together with other data on drug-induced suppression of testosterone levels, these results support the view that high levels of testicular steroids are required for antler hardening and that casting of antlers is associated with diminished testicular secretion of steroids. Furthermore this study indicated a possible association between prolactin secretion and the antler casting process. No relationship between plasma GH levels and antler growth was apparent.

Keywords: Cervus elaphus, *antlers, seasonal changes, testosterone, prolactin, growth hormone, testis*

Introduction

In red deer stags (*Cervus elaphus*) the precise nature of the mechanisms which regulate the annual patterns of antler growth, mineralisation, and casting are not fully understood. A number of studies have investigated hormonal secretion patterns, particularly of reproductive hormones. Luteinising hormone and testosterone secretion profiles determined on 6 occasions throughout the year have been linked to different phases of the antler cycle in red deer (Lincoln and Kay 1979), but the temporal association between changes in hormonal secretion and specific events during the antler cycle have not been described.

Long-term studies of plasma levels of testosterone have been carried out in males of other species such as reindeer (Leader-Williams 1979), reindeer and caribou (Whitehead and McEwan 1973), white-tailed deer (McMillin *et al* 1974; Bubenik *et al* 1977; Brown *et al* 1978), Columbian black-tailed deer (West and Nordan, 1976), and roe deer (Sempéré and Boissin 1981; Sempéré and Lacroix 1982). Seasonal changes have also been reported for growth hormone (GH) and cortisol in male white-tailed deer (Bubenik *et al* 1975) and for GH and thyroid hormones in male reindeer (Ryg and Jacobsen 1982). The patterns of plasma prolactin and testosterone in red deer stags through a 2-year period have been described by Suttie (1980).

The present study investigated changes in plasma testosterone and prolactin levels in adult red deer stags in New Zealand and related these to the antler growth cycle. In addition, plasma GH levels were measured in 3 stags throughout 1 year.

Castration of stags has dramatic effects on antler status. For instance, castration during the hard antler phase causes casting within a few weeks (Goss 1963; Lincoln 1971), while castration of stags during velvet antler growth causes antlers to remain in the velvet state and prevents casting indefinitely (Wislocki *et al* 1947; Goss 1963). These results provide strong evidence of testosterone having a major role in the antler growth and mineralisation processes and in preventing premature casting. Testicular function can be suppressed in entire male animals by blocking release of pituitary luteinising hormone with a suitable progestagen (Bolt 1971); thus, treatment of stags with a synthetic progestagen, medroxy progesterone acetate, results in premature casting of the hard antlers (Muir *et al* 1982).

Materials and Methods

Red deer stags over 2 years old were used in this study. Initially they were housed indoors in

Biology of Deer Production © *The Royal Society of New Zealand, Bulletin 22, 1985, pp. 185–190*

individual pens and fed *ad libitum* a pelleted diet; they were exposed to the natural photoperiod. The study started in August 1980 but by the end of June 1981 all stags had been shifted outdoors on to fescue and ryegrass – white clover pastures supplemented with lucerne hay and pelleted concentrate. Sometimes they were near hinds (e.g. in adjacent paddocks), but they never ran with any female deer.

Blood samples were collected by jugular venipuncture every 2 weeks from mid August 1980 until November 1980 and thereafter every week until August 1982. The number of stags bled at each sampling occasion varied (mean $n = 8$) but was never less than 3. Plasma was separated by centrifugation then stored at −20°C until assayed.

Testosterone concentrations in plasma were measured by a direct radioimmunoassay based on those described by Garnier *et al* (1978) and Schanbacher and D'Occhio (1982). Duplicate 100 μl samples of plasma, diluted in phosphate buffer containing 0.1% gelatin, were incubated at 70°C for 30 minutes. After cooling and addition of tritiated tracer and antiserum (#250, rabbit anti-testosterone-11-BSA serum supplied by Dr G. D. Niswender) the reaction mixtures were incubated overnight at 40°C. Nonbound tracer was removed by centrifugation with dextran-coated charcoal. Assay sensitivity was 0.15 ng/ml and within- and between-assay coefficients of variation were 5.7% and 12.0% respectively for a sample containing 3.6 ng/ml of testosterone. As the only significant cross-reaction with other steroids was from 5α-dihydrotestosterone, which is present in ruminant blood in low concentrations only (Garnier *et al* 1978), the assay was considered specific for testosterone in the present case.

Plasma prolactin was determined by the double antibody radioimmunoassay described by Barrell and Lapwood (1978). This assay utilised a radio-iodinated preparation of highly purified ovine prolactin (LER-860-2, provided by Dr L. E. Reichert, Jr) for tracer and rabbit antiserum raised against bovine prolactin (provided by Dr E. Payne). Results were expressed as ng of NIH-P-S12 ovine prolactin equivalents. Serial dilutions of deer plasma produced inhibition curves which were parallel to the standard curve, thus validating the assay for use with cervine plasma.

Growth hormone was determined in plasma samples collected from 3 stags every 2 weeks over a 12-month period (August 1980 to August 1981). This hormone was measured at The Medical Unit, Princess Margaret Hospital, Christchurch, using a radioimmunoassay based on that of Hart *et al* (1975). The assay used a highly purified preparation of ovine GH for tracer and standards (code no. 1-3AFP5285C) and an antiserum (code no. 2 AFP-C0123080) (all provided by Dr A. F. Parlow). Results were calculated as ng of ovine GH, this being justified on the grounds that inhibition curves produced by serial dilutions of deer plasma were parallel to the standard curve. Separation of antibody-bound from free tracer was achieved by use of polyethylene glycol. This assay had a lower detection limit of 1.13 ng/ml and a reproducibility at mid range of 1.7% and 14.2% for intra- and inter-assay coefficients of variation respectively.

Testis diameters were measured every 2 weeks from August 1981 by taking the cranial – caudal distance between 2 lightly spring-loaded plates held on to the intact scrotum of standing stags. Liveweights were recorded weekly until mid June 1981 and every 3 weeks thereafter. Stags were observed daily to record antler casting dates, to determine when velvet antlers reached commercial grade harvest (normally about 60 days after casting), and to note the date when velvet shedding commenced.

Results

Mean plasma testosterone and prolactin levels obtained during the second year of the study are shown in Fig. 1. For testosterone the annual pattern consisted of low values (less than 1 ng) throughout winter and spring followed by an increase during late December which eventually rose to peak levels (up to 48 ng/ml) in April. An equally seasonal pattern of change was recorded for prolactin but elevated concentrations of this hormone occurred between spring and autumn, with peak mean values (up to 55 ng/ml) occurring near the summer solstice. In individual stags (e.g. Fig. 2) it was clearly evident that plasma prolactin levels were reduced almost to baseline values before any major elevation in plasma testosterone levels occurred.

Key events in the antler growth cycle were closely linked temporally with the changes in hormone levels recorded. Casting of hard antler remnants (buttons) occurred near the onset of increase in plasma prolactin concentrations and the bulk of velvet antler growth (up to commercial grade harvest stage) occurred during the period when high prolactin values were recorded, before any appreciable change in testosterone values. Velvet shedding commenced at the time of the increase in plasma testosterone levels, and peak testosterone values were obtained during the rutting period, by which time the antlers were hard and clean. Testicular diameter (Fig. 3) increased almost twofold between October and mid March. Generally the changes in testicular size were in

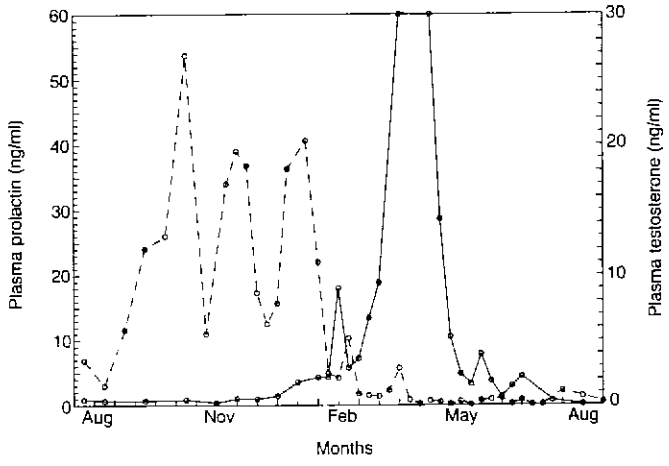

Fig. 1: Seasonal changes in mean plasma levels of prolactin (····) and testosterone (——) recorded from red deer stags (mean $n=8$) between August 1981 and August 1982.

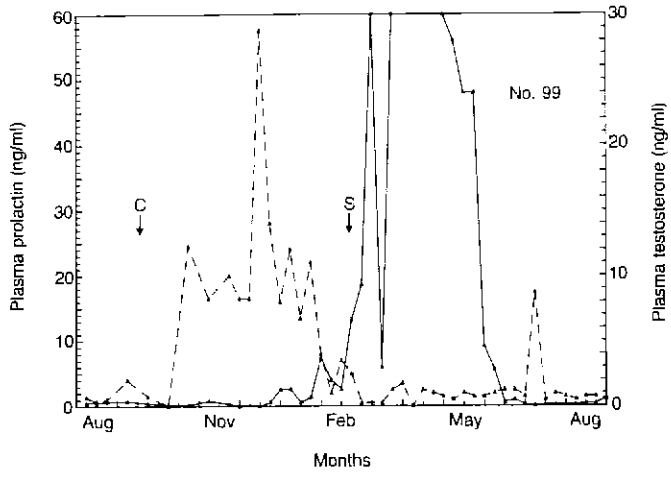

Fig. 2: Seasonal changes in plasma levels of prolactin (····) and testosterone (——) recorded from a red deer stag between August 1980 and August 1981. Dates of antler casting (C) and velvet shedding (S) are indicated by arrows.

phase with the pattern recorded for plasma testosterone concentrations, although substantial increases in testicular size were recorded prior to any major change in plasma testosterone levels.

Seasonal changes in liveweight and plasma GH levels are shown in Fig. 4. Liveweight increased steadily throughout spring and summer reaching maximum values during autumn. Thereafter, from the beginning of the rut period in April, liveweights decreased such that some stags lost up to 50 kg by the end of winter. Changes in plasma GH were generally unremarkable except for a period of consistently elevated levels during May and June, at which time some abrupt losses in body weight occurred.

Discussion

Several important observations arose from the

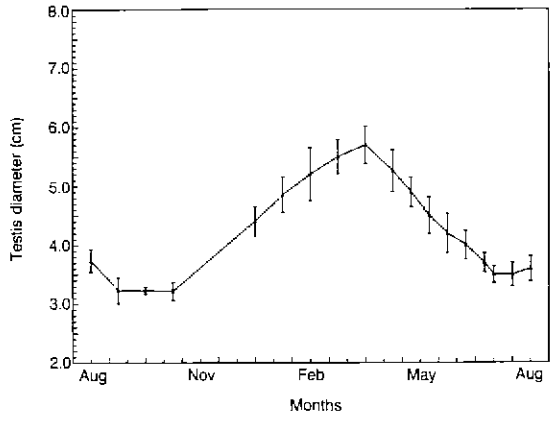

Fig. 3: Seasonal changes in testis diameter (mean ± s.e.m.) of 6 red deer stags recorded between August 1981 and August 1982.

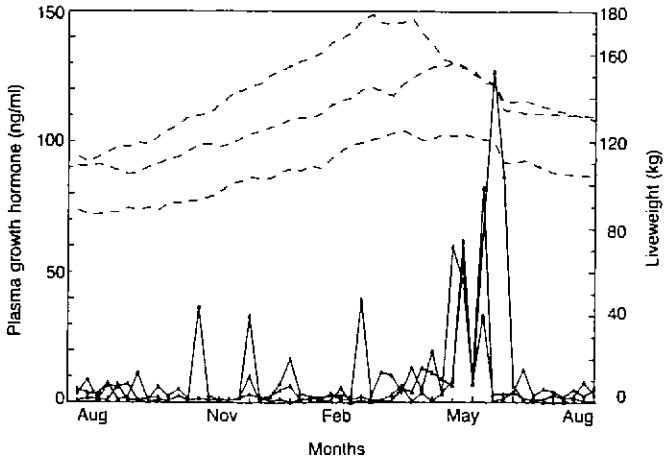

Fig. 4: Seasonal changes in plasma GH levels (——) and liveweight (----) recorded from 3 red deer stags between August 1980 and August 1981.

present study. First, the casting of hard antlers occurred at a time when no change in plasma testosterone levels had been observed for 2 – 3 months but at about the time that the annual rise in plasma prolactin secretion commenced. Secondly, growth of velvet antlers occurred only when plasma testosterone secretion was low, before the seasonal rise in levels. Thirdly, shedding of velvet and maturation of antlers was associated with high plasma levels of testosterone, which did not occur normally until after prolactin secretion had subsided well below the seasonal maximum. Finally, GH secretion did not appear to be related to any phase of the antler cycle but was elevated during the period of major body weight loss.

Clearly, because values for hormone concentrations were based on single plasma samples from each stag on the collection dates, this study cannot provide a detailed investigation of the secretory profiles of each hormone. As a basis for sampling, it was assumed that when secretion of a hormone was elevated and thus the amplitude and frequency of secretory episodes were increased, then there would have been a high probability of obtaining a high value for the amount of hormone in any single plasma sample. Conversely, many samples with small amounts of hormone would have indicated diminished secretory activity. Although there is no substantial evidence that any of the hormones in this study undergo circadian patterns of secretion, this would be of little concern here since blood samples were always collected at similar times of day (between 1000 and 1400 hours).

Casting of hard antlers in this experiment occurred when testosterone levels were barely detectable which is consistent with the testosterone pattern recorded in red deer stags by Lincoln and Kay (1979) at the corresponding time of year. However, in both studies testosterone levels were near the lower limits of measurements of the assays used (0.15 – 0.20 ng/ml) and it is possible that reductions in secretion did occur but were simply not detected. Premature casting of hard antlers can be induced in red deer stags by castration (Lincoln 1971), by immunisation against gonadotrophin releasing hormone (Lincoln *et al* 1982), and by progestagen (MPA) treatment (Muir *et al* 1982). Each of these treatments would suppress testosterone release, but they do not explain how this suppression of testicular secretion occurs in the natural situation. Photoperiodic regulation of antler cycles by use of artificial lighting regimes has been demonstrated in sika (Goss 1969) and red deer (Pollock 1975). This evidence, together with the fact that casting normally occurs around the time of the spring equinox, indicates that a photoperiodically mediated factor could be involved in the casting process. Prolactin is a possible candidate for this role, as the release of prolactin appears to be influenced by changes in the length of the daily photoperiod in red deer stags (Brown *et al* 1979; Suttie 1980), so that the possible close association of prolactin secretion with antler casting indicated by the present results warrants further investigation.

Mineralisation of antler tissue and shedding of velvet from the antlers are clearly dependent on testicular steroids. In a castrated red deer stag antlers did not shed velvet until the stag had been given supplementary testosterone (Lincoln *et al* 1970). It is noteworthy that in the present study, high levels of plasma testosterone (greater than 5 ng/ml) which appear to be necessary for complete mineralisation of antlers (G. K. Barrell unpubl.)

were not recorded until after prolactin secretion was substantially reduced from its summer peak. This point is supported by Suttie (1980) who also worked with red deer stags.

In white-tailed deer in Canada, serum GH levels peaked during April (corresponding to October in New Zealand; Bubenik *et al* 1975). This peak coincided with the initial few weeks of antler growth but otherwise serum GH levels were low (less than 10 ng/ml, bovine equivalents) throughout the remainder of the antler growth period. The authors could not attribute a direct role to GH in the regulation of antler growth, except perhaps at the initiation of growth of the new antler. In Norwegian reindeer, serum GH levels fluctuated throughout the year (Ryg and Jacobsen 1982). However, the levels were generally high in late winter – early spring but were not correlated with either weight gain or dry matter intake. Also samples taken from young red deer stags (4 months old at the start of the trial) did not provide any evidence for an association between food intake and plasma concentrations of GH (Brown *et al* 1979). Together with the present data the information available provides little support for GH having a role in the control of antler growth. In sheep, elevations in blood GH levels have been associated with short-term fasting (Driver and Forbes 1981) and, conversely, with a reduction in free fatty acid levels in blood (Hertelendey and Kipnis 1973). These findings tend to link GH release with lipid and carbohydrate metabolism in ruminants, even though the nature of the link is unclear. It is thus possible that the high levels of plasma GH recorded from deer in the present study were a direct result of altered metabolism during the period of massive body weight loss.

Acknowledgements

The animals used in these studies were lent to Lincoln College by courtesy of members of the New Zealand Game Industry Association. We are indebted to Mrs Carolyn Redekopp for performing GH assays. Miss Jan Wilkinson and Mrs Sue Parratt carried out testosterone and prolactin assays and Messrs A S Familton and M Keely helped with deer handling. Gifts of chemicals or biological preparations were kindly provided by: Dr G D Niswender of Colorado State University; Dr A F Parlow of Harbor General Hospital, California; Dr E Payne of Ruakura Animal Research Station, New Zealand; Dr L E Reichert Jr of Albany Medical College, New York; National Institute of Arthritis, Metabolism and Digestive Diseases, U.S.A.; Upjohn New Zealand.

References

Barrell G K and K R Lapwood 1978 Seasonality of semen production and plasma luteinizing hormone, testosterone and prolactin levels in Romney, Merino and Polled Dorset rams. *Anim. Reprod. Sci. 1:* 213 – 228.

Bolt D J 1971 Changes in the concentration of luteinizing hormone in plasma of rams following administration of oestradiol, progesterone or testosterone. *J. Reprod. Fert. 24:* 435 – 438.

Brown R D, R L Cowan and J F Kavanaugh 1978 Effect of pinealectomy on seasonal androgen titers, antler growth and feed intake in white-tailed deer. *J. Anim. Sci. 47:* 435 – 440.

Brown W B, J M Forbes, E D Goodall, R N B Kay and A M Simpson 1979 Effects of photoperiod on food intake, sexual condition and hormone concentrations in stags and rams. *J. Physiol. 296:* 58 – 59P, abstract.

Bubenik G A, A B Bubenik, G M Brown, A Trenkle and D A Wilson 1975 Growth hormone and cortisol levels in the annual cycle of white-tailed deer *(Odocoileus virginianus). Can. J. Physiol. Pharmacol. 53:* 787 – 792.

Bubenik G A, A B Bubenik, G M Brown and D A Wilson 1977 Sexual stimulation and variations of plasma testosterone in normal, antiandrogen and antiestrogen treated white-tailed deer (*Odocoileus virginianus*) during the annual cycle. *Proc. 13th Int. Cong. Game Biol.* 377 – 386.

Driver P M and J M Forbes 1981 Episodic growth hormone secretion in sheep in relation to time of feeding, spontaneous meals and short term fasting. *J. Physiol. 317:* 413 – 424.

Garnier D-H, Y Cotta and M Terqui 1978 Androgen radioimmunoassay in the ram: results of direct plasma testosterone and dehydroepiandrosterone measurement and physiological evaluation. *Annales de Biologie animale. Biochimie et Biophysique 18:* 265 – 281.

Goss R J 1963 The deciduous nature of deer antlers. *In: Mechanisms of Hard Tissue Destruction.* R F Sognnaes editor, Publication No. 75, American Association for the Advancement of Science, Washington, D.C. pp. 339 – 369.

Goss R J 1969 Photoperiodic control of antler cycles in deer. I. Phase shift and frequency changes. *J. Exp. Zool. 170:* 311 – 364.

Hart I C, D S Flux, P Andrews and A S McNeilly 1975 Radioimmunoassay for ovine and caprine growth hormone: its application to the measurement of basal circulating levels of growth hormone in the goat. *Horm. and Metabolic Res. 7:* 35 – 40.

Hertelendey F and D M Kipnis 1973 Studies on growth hormone secretion: V. Influence of plasma free fatty acid levels. *Endocrinol. 92:* 402 – 410.

Leader-Williams N 1979 Age-related changes in the testicular and antler cycles of reindeer, *Rangifer tarandus. J. Reprod. Fert. 57:* 117 – 126.

Lincoln G A 1971 The seasonal reproductive changes in the red deer stag *(Cervus elaphus). J. Zool. 163:* 105 – 123.

Lincoln G A, H M Fraser and T J Fletcher 1982 Antler growth in male red deer (*Cervus elaphus*) after active immunization against LH-RH *J. Reprod. Fert. 66:* 703 – 708.

Lincoln G A and R N B Kay 1979 Effects of season on the secretion of LH and testosterone in intact and castrated red deer stags *(Cervus elaphus). J. Reprod. Fert. 55:* 75 – 80.

Lincoln G A, R W Youngson and R V Short 1970 The social and sexual behaviour of the red deer stag. *J. Reprod. Fert. Suppl. 11:* 71 – 103.

McMillin J M, U S Seal, K D Keenlyne, A W Erickson and J E Jones 1974 Annual testosterone rhythm in the adult white-tailed deer *(Odocoileus virginianus borealis). Endocrinol. 94:* 1034 – 1040.

Muir P D, G K Barrell and A R Sykes 1982 Modification of antler growth in red deer stags by use of a synthetic progestagen. *Proc. N.Z. Soc. Anim. Prod. 42:* 145 – 147.

Pollock A M 1975 Seasonal changes in appetite and sexual condition in red deer stags maintained on a six-month photoperiod. *J. Physiol. 244:* 95 – 96P, abstract.

Ryg M and E Jacobsen 1982 Seasonal changes in growth rate, feed intake, growth hormone, and thyroid hormones in young male reindeer *(Rangifer tarandus tarandus). Can. J. Zool. 60:* 15 – 23.

Schanbacher B D and M J D'Occhio 1982 Validation of a direct radioimmunoassay for testosterone in unextracted serum for five species: application to study of the hypothalamic-pituitary-gondal axis in males. *J. Andrology 3:* 45 – 51.

Sempéré A J and J Boissin 1981 Relationship between antler development and plasma androgen concentrations in adult roe deer *(Capreolus capreolus). J. Reprod. Fert. 62:* 49 – 53.

Sempéré A J and A Lacroix 1982 Temporal and seasonal relationships between LH, testosterone and antlers in fawn and adult male roe deer (*Capreolus capreolus* L.): a longitudinal study from birth to four years of age. *Acta Endocrinol 99:* 295 – 301.

Suttie J M 1980 Influence of nutrition on growth and sexual maturation of captive red deer stags. *In: Proc. 2nd Int. Reindeer/Caribou Symp.* Røros, Norway. E Reimers, E Gaare and S Skjenneberg editors, Directoratet for vilt og ferskvannsfisk, Trondheim. pp. 341 – 349.

West N O and H C Nordan 1976 Hormonal regulation of reproduction and the antler cycle in the male Columbian black-tailed deer (*Odocoileus hemionus columbianus*). Part I. Seasonal changes in the histology of the reproductive organs, serum testosterone, sperm production, and the antler cycle. *Can. J. Zool. 54:* 1617 – 1636.

Whitehead P E and E H McEwan 1973 Seasonal variation in the plasma testosterone concentration of reindeer and caribou. *Can. J. Zool. 51:* 651 – 658.

Wislocki G B, J C Aub and C M Waldo 1947 The effects of gonadectomy and the administration of testosterone propionate on the growth of antlers in male and female deer. *Endocrinol. 40:* 202 – 224.