Effects of active immunization against LHRH and melatonin on growth and plasma hormone concentrations in red deer stags during their second year

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SUMMARY

The effects of immunization against luteinizing hormone releasing hormone (LHRH) and melatonin were studied during autumn and winter 1989 in yearling red deer stags initially aged 14 months and weighing 90–96 kg. Four out of five stags immunized against LHRH and six out of eight stags immunized against melatonin developed detectable levels of antibody titre. Immunization against LHRH reduced plasma LH concentration and both delayed and reduced weight loss associated with the rut (autumn): however, it had no effect upon carcass weight and dressing-out percentage was slightly lowered, showing that non-carcass components had been affected. Immunization against melatonin had no effect either on weight loss during the rut or on the rate of liveweight gain during winter and spring. Plasma concentrations of LH and testosterone were not affected by melatonin immunization: however, plasma prolactin concentration was consistently, but non-significantly, higher in immunized than control animals.

INTRODUCTION

Seasonal changes in fertility in both male and female red deer are mediated by changes in the secretion of the gonadotrophic hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the anterior pituitary gland. These changes are themselves regulated through the secretion of luteinizing hormone releasing hormone (LHRH) from the hypothalamus. The increase in LHRH pulse frequency and amplitude constitutes the 'drive' to the reproductive system causing the resurgence of gonadal activity, leading to mating and conception occurring during the rut. Factors governing the release of LHRH from the hypothalamus are poorly understood, although there is evidence that the pineal gland is involved in the photoperiodic control of reproduction through its secretion of melatonin (Lincoln 1985).

In New Zealand, red deer have their rutting season in the autumn, lasting 65 days from March to May (Fennessy & Milligan 1987). In temperate deer species the rut marks the change from the growth phase in summer to growth stasis in winter. When LHRH activity is blocked by active immunization, thus in effect creating temporary castrates, there is no breeding season (Bolt 1971; Lincoln 1985). Following castration there is no seasonal rutting behaviour in red deer stags (Lincoln 1971). Ryg & Jacobsen (1982*a*) reported loss of weight in intact male reindeer during the rutting season, whereas the weight of castrates was stable during this period.

In red deer, the secretion of melatonin from the pineal gland during the hours of darkness entrains seasonal reproductive rhythms with annual photoperiod. The effect of melatonin is mediated by LHRH secretion from the hypothalamus (Lincoln *et al.* 1984). Since melatonin appears to influence the secretion of LHRH, active immunization against melatonin may modify the secretion of LHRH and thus reproductive activities.

The effect of active immunization against LHRH and melatonin upon the liveweight gain (LWG) of 15-month-old red deer (*Cervus elaphus*) stags during the breeding season and the following winter was investigated. Animals of this age normally undergo a growth stasis for the following 5 months. Active immunization against LHRH can be regarded as a

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temporary immuno-castration; immunization against LHRH and melatonin were examined as potential means of reducing this growth stasis.

MATERIALS AND METHODS

Vaccination, weighing and blood sampling procedures

Two groups of 13-month-old red deer (*Cervus elaphus*) stags (25 in total) were grazed together on perennial ryegrass/white clover pasture at Massey University, New Zealand from January 1989 onwards. For the anti-LHRH experiment, five of the 13-month-old stags (96·4 ± 3·04 kg initial liveweight; LW ± S.E.) received a primary subcutaneous injection (1 ml on either side of the neck) of LHRH antigen conjugated to ovalbumin in DEAE-dextran adjuvant in oil base on 17 January. One ml of the vaccine was composed of 0·5 mg antigen in 0·5 ml adjuvant and 0·5 ml physiological saline. Booster injections of the same volume (2 ml) were given on 28 February. Another five red deer stags of the same age (90·9 ± 1·16 kg initial LW) did not receive any injections and served as a control group.

For the anti-melatonin experiment, eight 13-month-old red deer stags (89·9 ± 1·58 kg initial LW) that had previously received subcutaneous injections (1 ml on either side of the neck) of a 5-methoxy-tryptamine hemi-succinamide:human serum albumin conjugate in Freund's complete/incomplete adjuvant in March, June and July 1988 were vaccinated on 17 January. Each ml of vaccine comprised 0·5 mg antigen, 0·5 ml Freund's incomplete adjuvant and 0·5 ml physiological saline. Further booster injections of the same volume (2 ml) were given on 27 January and 25 May, 1989. Another seven stags of the same age (94·9 ± 1·99 kg initial LW), that had not been previously immunized, did not receive any injections and were used as controls.

Every 3 weeks, starting on 28 February 1989 until slaughter, the stags were injected i.m. with 20–30 mg xylazine (Rompun; Bayer, NZ) to cause mild sedation, and were weighed. Jugular blood samples were collected in 10 ml vacutainers using Na heparin as anticoagulant and were kept on ice. They were then centrifuged at 4 °C, 1850 *g* for 20 min to obtain plasma for measuring anti-LHRH and anti-melatonin antibody titres and for hormone assays. Each plasma sample was stored at −20 °C in five 1 ml lots.

The anti-LHRH group was sent for slaughter at the Feilding Deer Slaughter Premises (DSP) on 13 July 1989 and the anti-melatonin group was slaughtered on 15 December 1989. At the DSP, the carcass weights (kg), carcass GR tissue depth (mm) and rump fat width (mm) were recorded and testes were weighed. GR was measured as the depth of soft tissue over the 12th rib at a distance of 16 cm from the carcass midline as an index of carcass fatness (Kirton 1989).

Laboratory analyses

Antibody titre determination

Anti-LHRH antibody titre was measured by the procedure outlined by Abraham (1974) using [^{125}I]LHRH as ligand, which was prepared by the method of Djura & Hoskinson (1986). The antibody titre is defined as the dilution of antiserum which bound 50% of the [^{125}I]LHRH available and is expressed as a reciprocal.

Anti-melatonin antibody titres were determined as the dilution of plasma necessary to bind 10 pg of [^{3}H]melatonin/ml when 20 pg of [^{3}H]melatonin/ml was available. The results were expressed as titres as reported by Abraham (1974).

Hormone assays

LH concentrations were determined using a heterologous radioimmunoassay procedure described for sheep plasma by Scaramuzzi *et al.* (1970) and validated for deer plasma (Asher *et al.* 1986). The intra-assay coefficients of variation for multiple determinations, calculated from determinations of red deer control plasma samples, was 11·4%. All samples were included within a single assay. The smallest detectable amount was 0·48 ng/ml.

Plasma testosterone concentrations were determined using an extraction radioimmunoassay similar to that described by Peterson *et al.* (1978), but omitting the chromatographic step used to separate androgens. The inter-assay coefficients of variation, calculated from determinations of low (mean concentrations = 1·28 ng/ml) and high (9·30 ng/ml) red deer control plasma samples in each assay ($n = 5$) were 21·7 and 13·1% respectively. The intra-assay coefficients of variation for multiple determinations of the same control samples were 13·1 and 8·8% respectively. The smallest detectable amount was 0·1 ng/ml.

Prolactin was determined using the method of van Landeghem & van der Weil (1978) as modified by S. W. Peterson *et al.* (pers. comm.) and validated for red deer plasma (S. N. McCutcheon, pers. comm.). The first antibody against ovine prolactin was raised in rabbits. The antiserum was supplied by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, Bethesda, USA, in association with the National Hormone and Pituitary Programme, University of Maryland School of Medicine, Baltimore, USA. It was stored frozen at a 1:100 dilution in assay buffer and was further diluted to 1:40000 for the assay. Rabbit gamma globulin was added to the first antibody mix to provide 1 μg per assay tube to facilitate formation of the antibody pellet. The second antibody was a donkey anti-rabbit precipitating serum (IDS Gamma-B precipitating antiserum for radioimmunoassay, Washington, Tyne and Wear, UK).

The dilution range for the ovine reference standard was 1–1200 ng/ml. The inter-assay coefficient of variation was 14·0% and the intra-assay coefficient of variation was 9·1%, with the smallest detectable amount being 0·2 ng/ml.

Statistical analyses and calculation of data

The experimental data were analysed using General Linear Models (GLM). LWG (g/day) during the rut season was calculated for the 65-day period from 22 March to 25 May. Winter LWG (g/day) in the anti-melatonin experiment was calculated for the 85-day period from 25 May to 18 August and spring LWG as the 118-day period from 18 August to 14 December. In all cases, liveweight at the start of the rut (22 March) was used as a covariate. Least squares means were used to test the differences between treatments. Carcass weight (kg) was analysed using initial liveweight (22 March) as a covariate, whilst rump fat width and carcass GR tissue depth (mm; Kirton 1989) were analysed using carcass weight as a covariate.

RESULTS

Anti-LHRH experiment

Eighty percent (four out of five) of the stags vaccinated with the LHRH antigen gave a significant anti-LHRH antibody response (> 1:140; designated responders). The antibody titres peaked in April at 1:694 ± 231 (mean ± S.E.), declined slightly by early May and remained at an average of 1:520 ± 52 throughout the remainder of the experiment (Fig. 1).

Mean plasma LH concentrations for immunized and control stags are shown in Fig. 2*a*. LH concentrations for the immunized group were generally lower than those for the control group, with the difference attaining significance in late May ($P < 0·05$). Plasma testosterone concentrations (Fig. 2*b*) declined from March to May, with the immunized group having lower concentrations in the earlier stages, although the differences did not attain significance ($P > 0·05$).

Mean liveweights at the start of the rut (22 March) were respectively 98·4 kg (S.E. 3·45) and 94·7 kg (S.E. 1·30) for immunized and control stags. The anti-LHRH vaccinated group gained weight slightly (11 g/day) during the rut period (Table 1), whilst the control group lost weight (−55 g/day; $P < 0·05$). Both groups continued to lose weight in the post-rut period, with weight loss appearing to be greater for the vaccinated group ($P > 0·05$). However, overall weight loss was less for the anti-LHRH vaccinated stags ($P = 0·13$). Carcass weight was similar for the two groups, and similar values for rump fat width, testes weight and GR were recorded for both groups. Dressing-out % was slightly lower for the vaccinated group than for the control group. Values presented in Tables 1 and 2 refer to all immunized deer; removing stags that had no detectable antibodies did not affect liveweight change in either experiment.

Anti-melatonin experiment

Seventy-five percent (six out of eight) of stags vaccinated with the anti-melatonin antigen showed

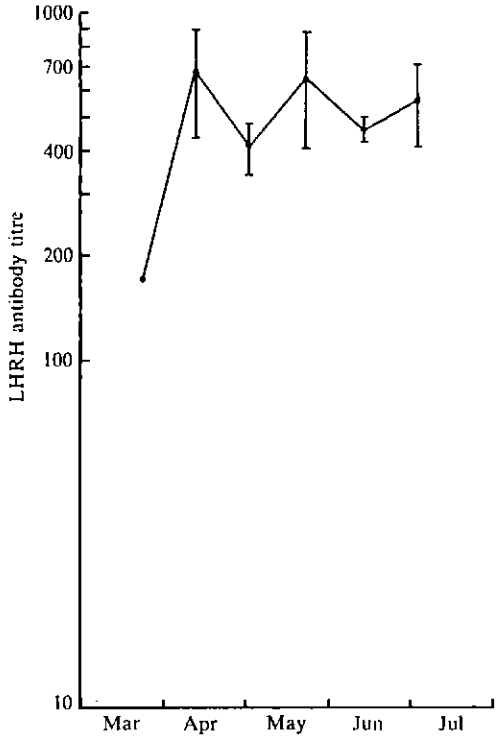

Fig. 1. LHRH antibody response in 13-month-old red deer stags given the primary immunization on 17 January 1989 and a booster immunization on 28 February. Mean values for the four animals giving anti-LHRH titre, out of five animals treated. Bars represent S.E.

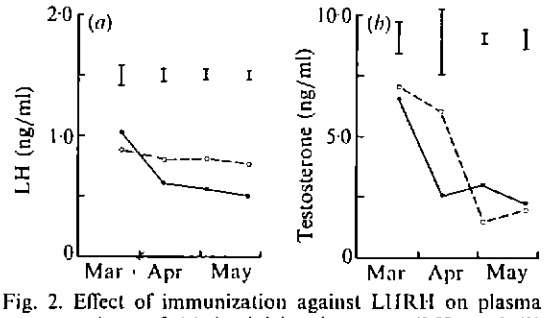

Fig. 2. Effect of immunization against LHRH on plasma concentrations of (*a*) luteinizing hormone (LH) and (*b*) testosterone. Control deer (○); immunized deer (●). Mean values for all five deer per group. Bars represent S.E.

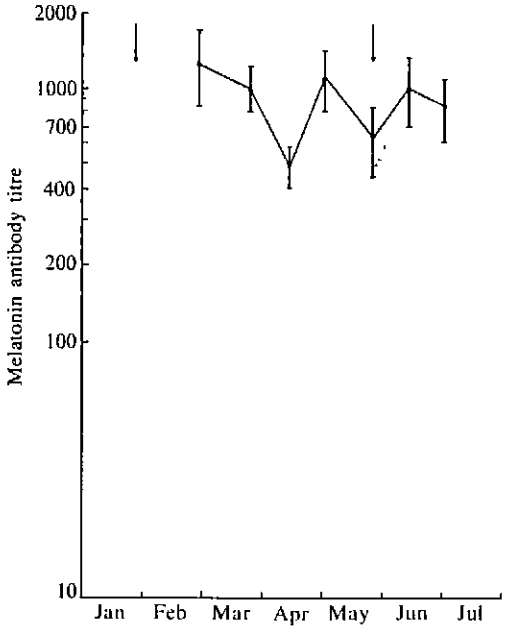

Fig. 3. Melatonin antibody response in red deer stags given booster immunizations on 27 January 1989, aged 13 months, and on 25 May 1989, aged 17 months. All had previously received immunizations against melatonin during March, June and July of 1988. Mean values for the six animals giving anti-melatonin titre, out of eight animals treated. Arrows (↓) indicate first and second booster immunizations. Bars represent S.E.

Table 1. *Effect of immunization against LHRH upon liveweight gain (g/day) of yearling red deer stags during the rut (22 March–25 May) and post-rut (26 May–4 July) seasons 1989, and on carcass components (13 July) in New Zealand*

	Control	LHRH immunized	S.E.
Number of stags	5	5	—
LWG (g/day)			
Rut	−55	11	18·7
Post-rut	−4	−46	21·9
Overall	−35	−11	9·7
Carcass data			
Carcass weight (kg)	53·5	53·0	0·44
Dressing-out (%)	57·3	55·9	0·38
Rump fat cover (mm)	105·2	102·8	5·39
Testes weight (g)	78·5	74·0	5·12
GR tissue depth (mm)	3·4	3·0	0·45

detectable antibody titres. The mean antibody titres of the responders peaked in February at 1:1272±411 (mean±S.E.) and declined to 1:486±97 in April (Fig. 3). It rose again to 1:917±98 in May and stabilized close to that value for the remainder of the experiment.

Plasma concentrations of LH and testosterone declined during the March to May period (Fig. 4), there being no significant effect due to immunization against melatonin ($P > 0{\cdot}05$). Plasma prolactin concentrations were consistently higher for immunized than for control animals (Fig. 5), but due to the variation encountered, the difference did not attain significance.

Mean liveweights at the start of the rut (22 March) were respectively 93·3 kg (S.E. 1·48) and 97·9 kg (S.E. 2·30) for immunized and control animals. There was no significant difference in the growth rates of anti-melatonin vaccinated and control groups during the rut, during winter and during spring (Table 2). Carcass weight, dressing-out %, testes weight and GR for both the immunized and the control group were similar (Table 2; $P > 0{\cdot}05$). Rump fat width for immunized deer was lower ($P < 0{\cdot}05$) than for control deer.

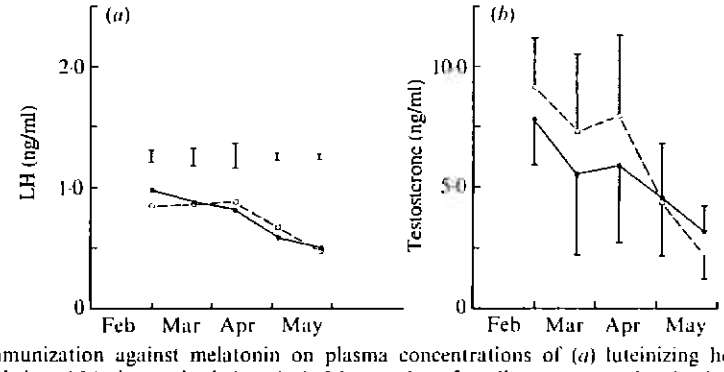

Fig. 4. Effect of immunization against melatonin on plasma concentrations of (*a*) luteinizing hormone (LH) and (*b*) testosterone. Control deer (○); immunized deer (●). Mean values for all seven control animals and all eight animals receiving anti-melatonin immunization. Bars represent S.E.

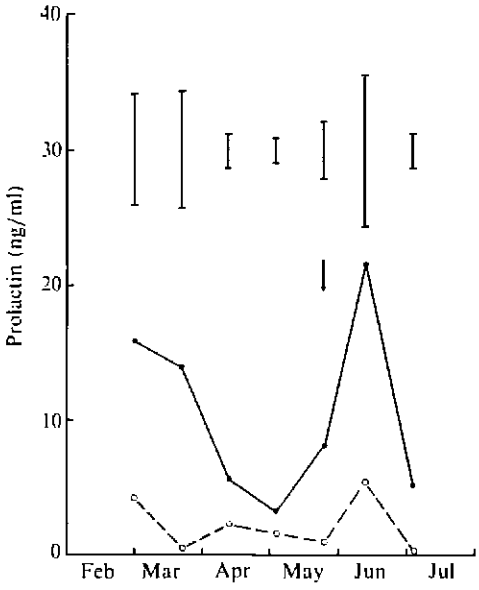

Fig. 5. Effect of immunization against melatonin on plasma concentration of prolactin. Control deer (○); immunized deer (●). Mean values for all seven control animals and all eight animals receiving anti-melatonin immunization. Arrow (↓) indicates second booster immunization. Bars represent S.E.

Table 2. *Effect of immunization against melatonin upon liveweight gain (g/day) of yearling red deer stags during the rut (22 March–25 May), during winter (26 May–18 August) and during spring (19 August–14 December) 1989, and upon carcass components (15 December) in New Zealand*

	Control	Melatonin immunized	S.E.
Number of stags	7	8	—
LWG (g/day)			
Rut season	−34	−32	27·8
Winter	57	60	19·0
Spring	141	151	15·3
Overall	72	76	10·9
Carcass data			
Carcass weight (kg)	61·0	64·1	1·39
Dressing-out (%)	55·1	55·8	0·31
Rump fat width (mm)	121·3	114·1	1·87
Testes weight (g)	99·0	98·1	0·78
GR tissue depth (mm)	6·2	6·6	0·59

DISCUSSION

Anti-LHRH experiment

Antibody titre values were lower (1:173 to 1:1349) than those measured on fallow deer in Australia using a similar antigen (1:2580±452; R. M. Hoskinson, pers. comm.). They were also lower than the mean value of 1:10867±7224 recorded for three red deer stags by Lincoln *et al.* (1984), which was associated with a total block of the seasonal reproductive development induced by treatment with melatonin implants. Antibody titre values for individual stags in the present experiment were not correlated with their individual rates of body growth.

The weight loss shown by control stags during the rut season was consistent with other reports (Lincoln 1971; Pollock 1975; Suttie & Simpson 1985) and occurred despite the stags being too young to exhibit adult rutting behaviour (Lincoln & Guinness 1973) and not being in contact with hinds. Immunization resulted in a small gain in liveweight over the rut, in agreement with the findings of Ryg & Jacobsen (1982*a*) with reindeer, who castrated six yearling male reindeer in spring and compared the weight gain and food intake with those of intact males. The intact reindeer lost weight during late September and early October, coinciding with the seasonal testosterone peaks, whereas the weights of the castrates were stable. Weight losses in the post-rut period suggest that anti-LHRH immunization in this experiment resulted in a delayed rut, with the overall weight loss being slightly reduced.

The main response to immunization would appear to be in non-carcass components, as the treatment had no effect upon carcass weight but lowered dressing out %. Drew *et al.* (1978) reported that castration at 5 months of age reduced growth and increased fatness in red deer stags slaughtered at 16 and 27 months of age. However, no effects upon fatness were found from anti-LHRH immunization, possibly due to the short term nature of the immunocastration, which was aimed at the rut period only.

The primary vaccination was given on 17 January (mid-summer), at a time when the seasonal plasma concentration of LH in red deer stags was highest (7·5 ng/ml; Fennessy *et al.* 1985). Freudenberger *et al.* (1991) commenced their vaccination sequence in September (early spring), *c.* 6 months before the onset of the rut, and reported heavier liveweight and carcass weight in yearling stags immunized against LHRH. Hence, the vaccination sequence may have been commenced too late in the present experiment.

Anti-melatonin experiment

Seventy-five percent of the stags vaccinated with the melatonin antigen gave an anti-melatonin antibody response, with antibody titre values ranging between 1:210 and 1:3167. The mean titre value at its peak in February (1:1272±411; mean±S.E.) was lower than the peak value of 1:3479±1200 recorded by McConnell *et al.* (1987) in the tammar wallaby (*Macropus eugenii*) using a similar antigen. Antibody titres recorded for individual stags within the

vaccinated group were not correlated with growth rates. The vaccinated animals did not grow faster than the control animals. However, Duckworth & Barrell (1989) reported that red deer stags immunized against melatonin were 7–10% heavier than their controls between 9 and 11 months of age and at 16 and 20 months of age (ie during late winter/spring). Duckworth & Barrell (1989) commenced the vaccination sequence at birth, whereas, in the present trial, the vaccination sequence commenced at weaning (3 months of age). Elsewhere, it has been shown that commencing the vaccination sequence at birth in red deer leads to much higher anti-melatonin titres than those reported here (Ataja *et al.* 1992).

Mean plasma prolactin concentrations of the control group showed typical autumn/winter low levels after a decline from peak concentrations during mid-summer, as reported by Barrell *et al.* (1985). The mean levels in the immunized group tended to be higher than those of the control group, although the difference did not attain significance. As prolactin injections during winter have been shown to increase voluntary feed intake (VFI) and LWG in young male reindeer (Ryg & Jacobsen 1982*b*) and in young male red deer (Suttie & Corson 1991), this would have been expected to increase the VFI and LWG of the immunized stags. Further experimentation is required in this area.

The anti-melatonin vaccinated and control groups showed similar growth patterns, losing an average of 33 g/day during the rut season and gaining an average of 59 and 146 g/day, during winter and spring respectively. This slow growth rate results in very inefficient venison production. Ataja *et al.* (1992) achieved growth rates of 145 g/day in winter and 225 g/day in spring, which enabled weaner red deer stags to attain the desired liveweight of 92 kg (> 50 kg carcass), so that they could be slaughtered at 12 months of age.

The lack of effect of the anti-melatonin treatment on dressing-out % and GR may be explained by its failure to change LWG in the present study. However, as the vaccinated stags had lower rump fat width ($P < 0.05$) than the control group and had heavier testes weight in another study (Ataja *et al.* 1992), it may be that immunization had altered the pattern of seasonal cycles. This should be investigated in future studies.

Milne *et al.* (1990) showed that oral melatonin administration advanced peak VFI in non-lactating red deer hinds by 14 days, but the seasonal pattern remained the same as for control animals. Thus it is possible that the VFI and bodyweight rhythms are endogenous in red deer, although they may be modified by immunizations of the type used by Duckworth & Barrell (1989).

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