Effects of immunization against GnRH upon body growth, voluntary food intake and plasma hormone concentration in yearling red deer stags (*Cervus elaphus*)

D. O. FREUDENBERGER[1]*, P. R. WILSON[1], T. N. BARRY[1]‡, Y. X. SUN[1]†
R. W. PURCHAS[1] AND T. E. TRIGG[2]

[1] *Massey University, Palmerston North, New Zealand*
[2] *Peptide Technology Ltd, Dee Why, NSW 2099, Australia*

(*Revised MS received 26 January 1993*)

SUMMARY

Red deer stags in New Zealand were given a series of immunizations against GnRH at 9–12 months of age (spring/early summer) in 1989 and 1990 and the effects upon plasma concentrations of luteinizing hormone (LH) and testosterone during the rut (15–17 months; autumn) and upon body growth to slaughter at 22 months (spring) were studied in two experiments. Control animals showed a sharp peak in plasma LH and testosterone concentration during late summer/early autumn, just preceding the rut, with scrotal circumference increasing to a maximum during the rut; body growth stopped during the rut in Expt 1 but not in Expt 2. Immunization caused the development of significant antibodies against GnRH during late spring and summer, and reduced but did not eliminate the increase in plasma LH and testosterone and scrotal enlargement leading up to the rut. Immunization did not affect body growth or voluntary feed intake during the rut in either experiment, but in Expt 1 early immunization significantly increased growth during both the pre-rut and post-rut periods. Immunization did not effect dressing out percentage, slightly increased carcass fatness in Expt 1 but not Expt 2, and reduced velvet antler growth by 12 months of age.

INTRODUCTION

Voluntary feed intake (VFI) and growth in red deer stags aged 15–20 months is depressed during the rut (breeding season), even when a a high quality pelleted feed is provided *ad libitum* (Fennessy *et al.* 1981). This reduction in VFI limits the productivity of farmed deer during a period when quality pasture is often abundant during the autumn flush. VFI also declines during the autumn and throughout the winter in hinds, but the decline is much more rapid and precipitous in stags (Suttie *et al.* 1987), possibly due to the sharp and sustained rise in plasma testosterone concentration during the rut. High levels of circulating testosterone are necessary for rutting behaviour (Lincoln *et al.* 1972), which has been blocked by active immunization against GnRH in red deer (Lincoln *et al.* 1982).

Permanent surgical castration is known to eliminate weight loss during the rut in reindeer (Ryg & Jacobsen 1982), but increases body fatness in red deer when performed at weaning (Drew *et al.* 1978). Objectives of the present study were to evaluate active immunization against GnRH as a temporary immuno-castration for reducing weight stasis associated with the rut, without having the effect of permanent castration in increasing fatness.

MATERIALS AND METHODS

Experimental design

Experiment 1

Thirty-three red deer stags of *c.* 10 months of age were brought to the Massey University Deer Unit, New Zealand, in early spring 1989, blocked by weight, and randomly allocated to the following three

* Present address: CSIRO Division of Wildlife and Ecology. PO Box 84, Lyneham. ACT 2602, Australia.
† Present address: Department of Biochemistry and Microbiology. PO Box 84. Lincoln University, Canterbury, New Zealand.
‡ To whom correspondence should be addressed.

treatments: early booster, late booster and control. The early booster group received the primary anti-GnRH vaccine on 29 September (early spring), and boosters on 26 October, 13 November, 12 December (early summer) of 1989, and 8 March and 29 March (autumn; rut) of 1990. The late booster group received the same sequence except that the first booster on 26 October was omitted. The control group received a placebo vaccine (adjuvant and vehicle only) on the same dates as the early booster group. Each animal received 5 ml of the vaccine (2·5 ml subcutaneous on each side of the neck) on each occasion, containing 1 mg antigen. The vaccine (Peptide Technology Ltd, Australia) comprised GnRH antigen, with the adjuvant being DEAE dextran in an oil-based vehicle.

Experiment 2

During late winter 1990, thirty-three 10-month-old red deer stags were brought to the University Deer Unit, blocked for weight, and randomly allocated to three treatments: treble booster, single booster and control. The treble booster treatment group received 2·5 ml of priming anti-GnRH vaccine on 29 August, and 2·5 ml booster vaccines on 27 September, 24 October and 20 December 1990, containing 1·5 mg antigen/dose. The single booster group received a 5 ml priming vaccine on 29 August 1990 and a 5 ml booster 6 weeks after the primer, containing 3 mg antigen/dose. The control group received 5 ml of the adjuvant and vehicle placebo at the same times as the single booster group. The formulation of the vaccine had twice as much anti-GnRH antigen per ml as in the first experiment. Each dose of vaccine was given in a single location subcutaneously in the neck. The injection site was clipped, and a sterile needle was used for each animal. Subsequent boosters were given on the other side of the neck.

Animal health

All animals were drenched with anthelminthic prior to the primer vaccine (Ivomec; Merk, Sharpe & Dohme NZ). Faecal egg counts were monitored, and during Expt 2 the stags were again drenched in May and June. Low concentrations of plasma copper were detected during the second month of Expt 2 and the stags received a single oral dose of copper oxide needles (Pitman Moore, NZ). Velvet antler was removed following the administration of xylazine (Rompun; Bayer, NZ) and xylocaine (Willotox; Willows Francis Veterinary, UK) as a local anaesthetic.

Pastures

All stags were maintained at a high plane of nutrition. All the stags grazed perennial ryegrass/white clover pastures that had been top-dressed with single superphosphate (220 kg/ha) and urea (50 kg/ha) prior to winter. Pasture allowance was between 7–12 kg DM/head per day, and during autumn the mean residual DM of pastures after grazing was > 1200 kg/ha. Grazing to no less than this level of pasture residual DM has been shown to maximize the growth of young stags (Ataja *et al.* 1992*a*). Pasture hay and pelleted concentrate supplements were offered during winter (July and August).

Measurements

Each month, animals were weighed and blood was sampled by venipuncture from the jugular vein, while the animals were restrained in a pneumatic crush. The heparinized blood samples were immediately chilled on ice, centrifuged (1850 g; 15 mins) and frozen in 5×1 ml quantities for subsequent analysis of anti-GnRH titre, LH and testosterone. Scrotal circumference was measured with a flexible tape on unrestrained stags held in a dark room.

Voluntary feed intake (VFI) was measured during the rut (April) in Expts 1 and 2. For the first experiment, controlled release capsules (CRC) containing chromic oxide that were designed for sheep (Nufarm NZ Ltd) were orally administered to stags (2/animal) restrained in a pneumatic crush. It was found that the sheep CRC were too small for some of the larger stags, and experimental calf CRCs were used in Expt 2. The concentration of chromium in the gut was allowed to equilibrate for 10 days, and then rectal samples were taken from each stag every 3 days on five occasions. During this period, pasture samples were collected for determination of DM and *in vitro* organic matter digestibility (OMD). The rate of chromium release from the sheep capsules was 121 mg per day, as determined in rumen fistulated red deer (W. J. Parker & A. M. Ataja, unpublished). The rate of chromium release from the calf CRC was 344 mg Cr/day as determined in rumen fistulated red deer by the method of Parker *et al.* (1989). VFI was calculated as described by Parker *et al.* (1990).

In Expts 1 and 2, all animals were slaughtered at *c.* 22 months of age in October at a licensed Deer Slaughter Premises (DSP). The animals were weighed off pasture 24 h prior to slaughter. At the DSP, carcass weight and carcass GR were determined, as was the width of the rump fat pad from the carcass mid-line to the lateral extremity of the pad. GR is measured as tissue depth over the 12th rib, and is an indirect measure of fatness (Kirton 1989). For Expt 1 only, the right forequarter was retained and boned, the humerus weighed and its length measured. The soft tissue of the forequarter was minced and subsampled for Soxhlet fat extraction. During Expt 2, the length of growing antlers (velvet) was measured with a flexible tape.

Laboratory analyses

Antibody titre to GnRH was determined by a direct binding, double antibody radioimmunoassay similar to that described by Aston *et al.* (1985) and Bomford & Aston (1990). This procedure allows a rapid qualitative screening for anti-GnRH sera. A titre was considered positive at 10% or greater of the positive control titre at both 1/50 and 1/500 dilutions.

Plasma LH concentrations were determined using a radioimmunoassay procedure described for sheep plasma by Scaramuzzi *et al.* (1970) and validated for red deer plasma according to the procedures outlined by Xu (1991). The inter- and intra-assay coefficients of variation were 14·7 and 9·8% respectively and the lowest amount detectable was 0·089 ng/ml. Plasma testosterone concentrations were assayed using an extraction radioimmunoassay similar to that described by Peterson *et al.* (1978), but omitting the chromatographic step. The inter- and intra-assay coefficients of variation were 16·8 and 11·7% respectively and the least detectable amount was 0·572 ng/ml.

Faeces were assayed for Cr by atomic absorption spectrophotometry as described by Parker *et al.* (1989). Organic matter digestibility (OMD) was measured by the method of Roughan & Holland (1977).

Statistical methods

Treatment differences in liveweight, scrotal circumference, plasma LH and testosterone concentrations, anti GnRH titre, all across time, were tested by univariate analysis of repeated measures (Gill & Hafs 1971). Differences in these parameters and VFI between treatments at single sampling times were examined by one-way analysis of variance (Snedecor & Cochran 1980). LH and testosterone concentrations were log-transformed prior to analysis. Differences in carcass parameters were examined by analysis of covariance (Packard & Boardman 1988), using carcass weight as the covariate. Means are reported with their standard errors. Treatment effects upon velvet antler length (Expt 2) were assessed using the Chi squared test.

RESULTS

GnRH antibody titre

Antibody titre was highest during December and January (early summer) in Expt 1 (Table 1) and during September–January (spring/early summer) in Expt 2 (Table 2). In Expt 1, the anti GnRH titres developed earlier in spring and persisted longer into late summer/autumn in animals given the early booster compared with those given the late booster treatment. In Expt 2, animals given the single booster treatment developed anti GnRH antibodies earlier

than those given the treble booster treatment, but antibody levels during late summer declined at a slower rate in the treble booster group. During March (early autumn) antibodies to GnRH were still present in both experiments, but had declined to lower levels than those recorded during summer.

Table 1. *Experiment 1: Percentage of animals with an anti-GnRH titre > 10% of a positive control at 1/500 dilution*

1989/90	Early Booster	Later Booster
November	63·6	27·3
December	63·6	63·6
January	88·9	63·6
February	11·1	10
March	22·2	10

Table 2. *Experiment 2: Percentage of animals with an anti-GnRH titre > 10% of a positive control at 1/50 and 1/500 dilutions*

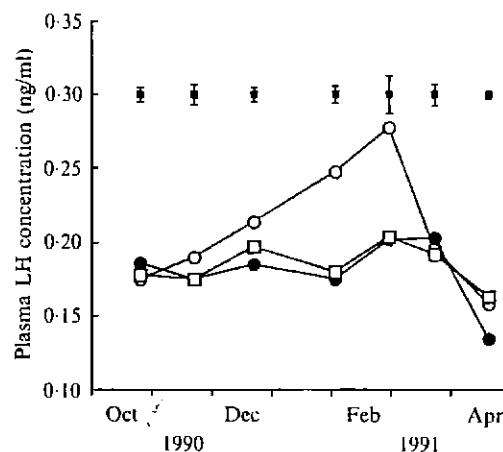| Date 1990/91	Treble booster		Single booster	
	1/50	1/500	1/50	1/500
27 September	82	0	100	27
24 October	100	27	100	82
23 November	100	9	100	18
20 December	82	0	100	0
30 January	91	0	44	0
26 February	36	0	22	0
21 March	27	0	22	0

Fig. 1. Experiment 2: Mean concentration of plasma LH (ng/ml) in red deer stags immunized against GnRH. Single booster, ●; treble booster, □; control (adjuvant only), ○. Vertical bars represent pooled s.e.
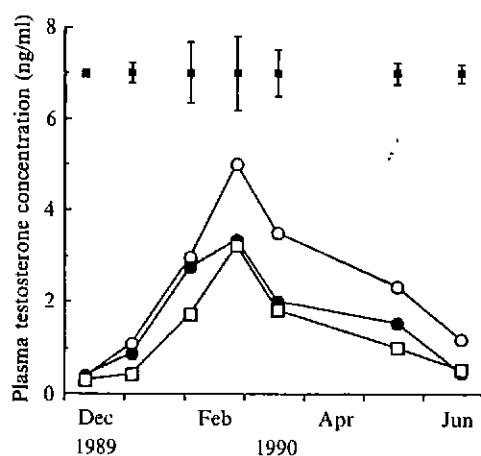
Fig. 2. Experiment 1: Mean concentration of plasma testosterone (ng/ml) in red deer stags immunized against GnRH. Early booster, □; late booster, ●; control (adjuvant only), ○. Vertical bars represent pooled s.e.
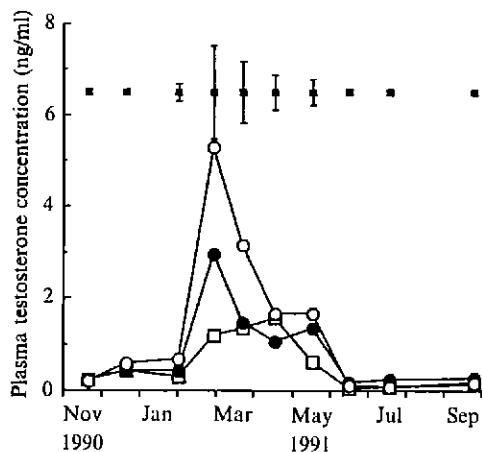

Fig. 3. Experiment 2: Mean concentration of plasma testosterone (ng/ml) in red deer stags immunized against GnRH. Single booster, ●; treble booster, □; control (adjuvant only), ○. Vertical bars represent pooled s.e.

Plasma LH and testosterone concentrations

Plasma LH concentration in control animals peaked in late summer in Expt 2 (Fig. 1). Animals receiving single and treble immunizations had similar plasma LH concentrations, which were lower than those of control animals, with the difference attaining significance in January and February ($P < 0.05$). Plasma testosterone concentration in control animals peaked during late summer/early autumn in both experiments (Figs 2 and 3), just before the onset of the rut, and then declined to very low levels by winter. Plasma testosterone in the immunized groups in Expt 1 was generally lower than for control animals, with
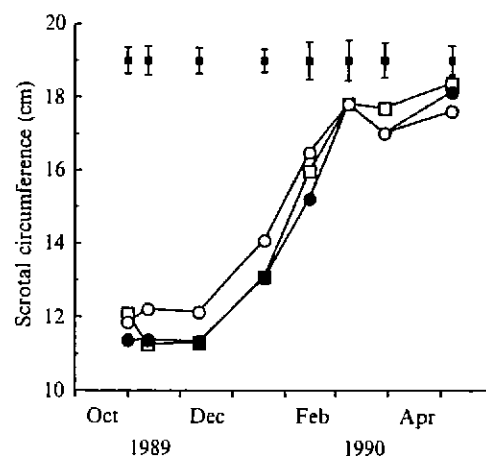

Fig. 4. Experiment 1: Mean scrotal circumference (cm) in red deer stags immunized against GnRH. Early booster, □; late booster, ●; control (adjuvant only), ○. Vertical bars represent pooled s.e.

the difference between the early booster and control animals attaining significance in January, February and March ($P < 0.05$). There was a highly significant ($P < 0.001$) treatment × time interaction for plasma testosterone concentration in Expt 2, explained by both immunization treatments reducing testosterone concentration and in addition by the peak testosterone concentration occurring later in the treble booster group. The treble booster group had lower plasma testosterone concentrations than control animals during February ($P < 0.05$), March ($P < 0.1$), and May ($P < 0.05$).

Scrotal circumference

Scrotal circumference (Figs 4 and 5) increased with time in both experiments ($P < 0.001$), and there were significant treatment × time interactions in both Expt 1 ($P < 0.05$) and in Expt 2 ($P < 0.001$). This was due to the immunization treatments slowing down scrotal enlargement during summer. However, during the rut (autumn) the immunized and control animals had similar maximum scrotal size in Expt 1, and this was also true for the single booster and control animals in Expt 2. The treble booster treatment in Expt 2 appeared both to slightly reduce and to delay the attainment of maximum scrotal size.

Liveweight gain (LWG)

In Expt 1 there was a significant treatment × time interaction ($P < 0.001$), due to animals in the early booster group growing faster than control and late booster animals, which grew at similar rates (Fig. 6). Mean liveweight of the early booster group became greater than that of the control group ($P < 0.05$) in
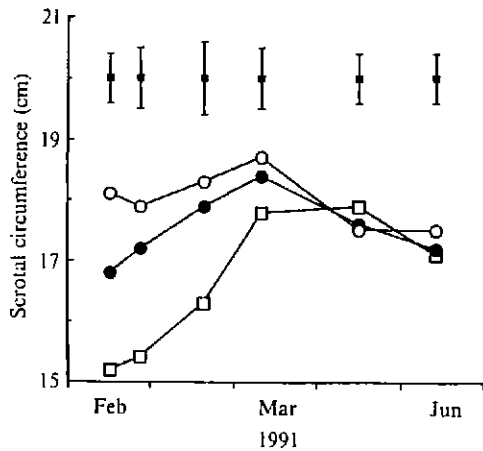
Fig. 5. Experiment 2: Mean scrotal circumference (cm) of red deer stags immunized against GnRH. Single booster, ●; treble booster, □; control (adjuvant only), ○. Vertical bars represent pooled s.e.
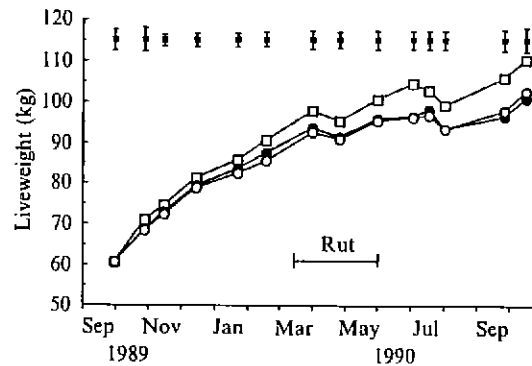

Fig. 6. Experiment 1: Mean liveweight (kg) of red deer stags immunized against GnRH. Early booster, □; late booster, ●; control (adjuvant only), ○. Rut represents the period of sexual activity. Vertical bars represent pooled s.e.
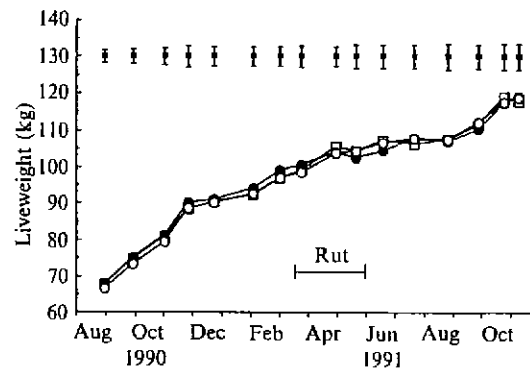

Fig. 7. Experiment 2: Mean liveweight (kg) of red deer stags immunized against GnRH. Single booster, ●; treble booster, □; control (adjuvant only), ○. Rut represents the period of sexual activity. Vertical bars represent pooled s.e.

October 1989 and from February 1990 until the end of the study (October 1990). All three groups showed weight stasis during the rut, but between June 1990 and October 1990 (winter/spring; 19–23 months of age) the early booster group had a greater ($P < 0.05$) daily weight gain (59 ± 9.3 g/day) than the late booster (38 ± 4.8 g/day) or control groups (40 ± 3.7 g/day).

There was no effect of the immunization treatments upon growth in Expt 2 (Fig. 7), and unlike Expt 1 there was an increase in liveweight during most of the rut.

Voluntary feed intake during the rut

Voluntary feed intake (VFI) was not affected by treatments in Expt 1 and mean values were 3.17 ± 0.23 (s.e.), 2.99 ± 0.19 and 3.08 ± 0.47 kg DM per day for control, late booster and early booster groups respectively. These convert to 103 ± 7.2, 97 ± 6.1 and 97 ± 15.1 g DM/kg $W^{0.75}$ per day respectively. Likewise there was no effect of the treatments upon VFI during the rut in Expt 2, with mean values being 2.57 ± 0.111, 2.94 ± 0.339 and 2.64 ± 0.128 kg DM/day for the control, single booster and treble booster groups respectively. These convert to 77 ± 3.4, 91 ± 11.1 and 80 ± 3.8 g DM/kg $W^{0.75}$ per day.

Organic matter digestibility (OMD) of the feed on offer during the rut (autumn) was very high, being 79.9 ± 1.96 (s.e.) % in Expt 1 and 81.1 ± 1.74 % in Expt 2. Feed on offer at all other stages of both experiments was of similar quality.

Carcass characteristics

Mean liveweight 24 h prior to slaughter in Expt 1 was 8–10 kg greater ($P < 0.05$) in the early booster group

than in the control and late booster groups, and this difference was reflected in a 4–5 kg greater ($P < 0.05$) mean carcass weight (Table 3). Dressing out percentages were similar. There was a general tendency ($P < 0.10$) for the immunized groups to be slightly fatter than the control group. In Expt 2 there were no differences in carcass weight, dressing out percentage and carcass fatness between any of the experimental groups (Table 4).

Velvet antler production

In Expt 2, both immunization treatments suppressed velvet antler production by one year of age (Table 5), the normal time of velvet antler removal. A greater proportion of immunized animals had velvet antler < 100 mm long at this time relative to control animals, as indicated by the Chi squared for this comparison (3.17) being greater than the critical value required for significance at the 10% level (2.71).

Table 3. *Experiment 1: Carcass characteristics (mean ± S.E.) of red deer immunized against GnRH or given an adjuvant only (control). Liveweight and carcass weight are given as unadjusted means, all other parameters are adjusted by carcass weight*

	Control	Early Boost	Late Boost
Liveweight (kg)	102·3 ± 1·48	110·2 ± 4·92	100·8 ± 1·67
Carcass weight (kg)	58·9 ± 1·19	63·7 ± 2·90	58·3 ± 1·00
Dressing out (%)	57·7 ± 0·11	57·6 ± 0·12	58·0 ± 0·11
GR† (mm)*	4·0 ± 0·09	4·4 ± 0·10	4·9 ± 0·09
Tissue fat (%)*	3·31 ± 0·076	3·84 ± 0·077	4·03 ± 0·084
Rump fat width (mm)*	76·1 ± 0·91	85·8 ± 1·00	83·4 ± 0·90
Humerus weight (g)*	441 ± 1·7	450 ± 1·9	455 ± 1·7
Humerus length (mm)*	248 ± 0·6	250 ± 0·6	252 ± 0·6

* Adjusted to equal carcass weight.
† Tissue depth over 12th rib.

Table 4. *Experiment 2: Carcass characteristics (mean ± S.E.) of red deer immunized against GnRH or given an adjuvant only (control). Liveweight and carcass weight are given as unadjusted means, all other parameters are adjusted by carcass weight*

	Control	Single boost	Treble boost
Liveweight (kg)	118·8 ± 3·88	119·2 ± 4·51	117·8 ± 2·51
Carcass weight (kg)	67·0 ± 2·07	67·7 ± 2·87	66·8 ± 1·80
Dressing out (%)	56·6 ± 0·12	56·7 ± 0·13	56·7 ± 0·14
GR† (mm)*	6·4 ± 0·21	6·6 ± 0·23	6·1 ± 0·24
Fat depth (mm)*	6·6 ± 0·24	6·1 ± 0·28	7·7 ± 0·29
Rump fat width (mm)*	97·9 ± 1·24	96·4 ± 1·42	97·6 ± 1·50
Testes weight (g)*	55·1 ± 0·94	56·8 ± 1·04	58·1 ± 1·11
Metacarpal weight (g)*	227·7 ± 1·22	230·4 ± 1·34	224·6 ± 1·31
Metacarpal length (mm)*	252 ± 0·9	249 ± 1·0	245 ± 1·1

* Adjusted to equal carcass weight.
† Tissue depth over 12th rib.

Table 5. *The number of stags producing velvet antler less than or greater than 100 mm length on 20 December 1990. (The normal time for 12-month velvet antler removal.) Values in parentheses are percentage of total animals in group*

	Control (n = 12)	Immunized* (n = 22)
Velvet > 100 mm	7	6
Velvet < 100 mm	5 (42)	16 (73)

* Single booster + treble booster groups.

DISCUSSION

In both experiments the control yearling red deer stags showed testes enlargement over summer and a peak in plasma LH and testosterone concentration in late summer/early autumn, just before the onset of the rut. This is in accordance with other work involving similar red deer stags (Suttie et al. 1984a, b; Fennessy et al. 1985). The period of the rut (late March–late May) was associated with reduced growth in Expt 1, as was expected and as found by Ataja et al. (1992b), but no growth reduction was observed in Expt 2. No reason can be given for the difference between experiments.

Whilst active immunization against GnRH depressed plasma concentrations of LH and testosterone in both experiments, the reductions were not as marked as those observed by Lincoln et al. (1982, 1984). The effect of the immunizations upon scrotal circumference can be interpreted as a temporary suppression of testes enlargement in the period leading up to the rut, rather than a reduction in maximum testes size. Collectively, these data indicate that the immunization treatments caused a partial rather than a complete suppression of the rut. Indeed, treatments such as the treble booster in Expt 2 seem to have both reduced the magnitude and delayed the rut, as also found by Ataja et al. (1992b), and this was evident when handling this group in the deer yards.

Perhaps because the rut was only partially suppressed, this explains the similarity in VFI and LWG between the immunized and control groups during the rut. However, in Expt 2 the immunization treatment did suppress velvet antler production during the spring, at a time when GnRH antibody levels were high. Ataja *et al.* (1992*b*) found that immunizations in early and late summer both delayed and reduced the magnitude of weight loss associated with the rut, showing that, like permanent castration (Ryg & Jacobsen 1982), anti GnRH immunization can be used to prevent weight loss during the rut in some circumstances. In the present study, the highest levels of anti GnRH antibodies were present during late spring and summer (October–January), whereas Ataja *et al.* (1992*b*) reported the presence of anti GnRH antibodies during autumn (April and May). Ataja *et al.* (1992*a, b*) showed that antibody production to GnRH and melatonin antigens was very slow in growing red deer stags, which was why immunization was commenced early in the present experiments. To completely suppress the rut effectively with the existing vaccine, it may be that a series of immunizations commencing in late spring and continuing into late summer/early autumn is needed.

The mechanism of the increased growth in the early booster group (Expt 1) during the pre-rut and post-rut periods is difficult to explain. Body growth in growing red deer stags is positively correlated with insulin-like growth factor-I (IGF-I) production (Suttie *et al.* 1989), and this should be measured in future anti GnRH immunization experiments.

Although Drew *et al.* (1978) found that castration at 5 months of age increased carcass fatness in stags slaughtered at 15 and 27 months, anti GnRH immunization in the present study only slightly increased fatness in Expt 1 and did not affect fatness at all in Expt 2. It is concluded that anti GnRH immunization offers possibilities for suppressing the rut in yearling red deer stags without increasing fatness when slaughtered at a later age; however, anti GnRH immunity may need to be kept at high levels from early summer through to late autumn.

REFERENCES

ASTON, R., COOPER, L., HOLDER, A. T., IVANGI, J. & PREERE, M. A. (1985). Monoclonal antibodies to human growth hormone can distinguish between pituitary and genetically engineered forms. *Molecular Immunology* 22, 271–275.

ATAJA, A. M., WILSON, P. R., BARRY, T. N., HODGSON, J., HOSKINSON, R. M., PARKER, W. J. & PURCHAS, R. W. (1992*a*). Early venison production from red deer (*Cervus elaphus*) as affected by grazing perennial or annual ryegrass pastures, pasture surface height and immunization against melatonin. *Journal of Agricultural Science, Cambridge* 118, 353–369.

ATAJA, A. M., BARRY, T. N., HOSKINSON, R. M. & WILSON, P. R. (1992*a*). Effects of active immunization against LHRH and melatonin on growth and plasma hormone concentrations in red deer stags during their second year. *Journal of Agricultural Science, Cambridge* 118, 371–377.

BOMFORD, R. & ASTON, R. (1990). Enhancement of bovine growth hormone activity by antibodies against growth hormone peptides. *Journal of Endocrinology* 125, 31–38.

DREW, K. R., FENNESSY, P. F. & GREER, G. J. (1978). The growth and carcass characteristics of entire and castrate red deer stags. *Proceedings of the New Zealand Society of Animal Production* 38, 142–144.

FENNESSY, P. F., MOORE, G. H. & CORSON, I. D. (1981). Energy requirements of red deer. *Proceedings of the New Zealand Society of Animal Production* 41, 167–173.

FENNESSY, P. F., SUTTIE, J. M. & FISHER, M. W. (1985). Reproductive physiology of male red deer. In *Proceedings of a Deer Course for Veterinarians* 2, 101–106. (Deer Branch, New Zealand Veterinary Association, C/- Department of Veterinary Clinical Sciences, Massey University, New Zealand.)

GILL, J. L. & HAFS, H. D. (1971). Analysis of repeated measurements of animals. *Journal of Animal Science* 33, 331–336.

KIRTON, A. H. (1989). Principles of classification and grading. In *Meat Production and Processing* (Eds R. W. Purchas, B. W. Butler-Hogg & A. S. Davies), New Zealand Society of Animal Production Occasional Publication No. 11, pp. 143–158. Hamilton, New Zealand; New Zealand Society of Animal Production.

LINCOLN, G. A., GUINNESS, F. & SHORT, R. V. (1972). The way in which testosterone controls the social and sexual behavior of the red deer stag (*Cervus elaphus*). *Hormones & Behavior* 3, 375–396.

LINCOLN, G. A., FRASER, H. M. & FLETCHER, T. J. (1982). Antler growth in male red deer (*Cervus elaphus*) after active immunization against LH-RH. *Journal of Reproduction and Fertility* 66, 703–708.

LINCOLN, G. A., FRASER, H. M. & FLETCHER, T. J. (1984). Induction of early rutting in male red deer (*Cervus elaphus*) by melatonin and its dependence on LHRH. *Journal of Reproduction and Fertility* 72, 339–343.

PACKARD, G. C. & BOARDMAN, T. J. (1988). The misuse of ratios, indices and percentages in ecophysiological research. *Physiological Zoology* 61, 1–9.

PARKER, W. J., MCCUTCHEON, S. N. & CARR, D. H. (1989). Effect of herbage type and level of intake on the release of chromic oxide from intraruminal controlled release capsules in sheep. *New Zealand Journal of Agricultural Research* 32, 537–546.

PARKER, W. J., MCCUTCHEON, S. N. & GARRICK, D. J. (1990). The suitability of chromium controlled release capsules for estimating herbage intakes of grazing

ruminants. *Proceedings of the Australian Association of Animal Breeding and Genetics* 8, 151–154.

PETERSEN, A. J., FAIRCLOUGH, R. J. & SMITH, J. F. (1978). Radioimmunoassay of androstenedione and testosterone in cow plasma at the time of luteolysis and oestrus. *Journal of Reproduction and Fertility* 52, 127–129.

ROUGHAN, P. G. & HOLLAND, R. (1977). Predicting in-vivo digestibilities of herbages by exhaustive enzymic hydrolysis of cell walls. *Journal of the Science of Food and Agriculture* 28, 1057–1064.

RYG, M. & JACOBSEN, E. (1982). Effect of castration on growth and food intake cycles in young male reindeer. *Canadian Journal of Zoology* 60, 942–945.

SCARAMUZZI, R. J., CALDWELL, B. V. & MOOR, R. M. (1970). Radioimmunoassay of LH and estrogen during the estrous cycle of the ewe. *Biology of Reproduction* 3, 110–119.

SNEDECOR, G. W. & COCHRAN, W. G. (1980). *Statistical Methods.* 7th Edn. Ames, Iowa: Iowa State University Press.

SUTTIE, J. M., CORSON, I. D. & FENNESSY, P. F. (1984a).

Voluntary intake, testis development and antler growth patterns of male red deer under a manipulated photoperiod. *Proceedings of the New Zealand Society of Animal Production* 44, 167–170.

SUTTIE, J. M., LINCOLN, G. A. & KAY, R. N. B. (1984b). Endocrine control of antler growth in red deer stags. *Journal of Reproduction and Fertility* 71, 7–15.

SUTTIE, J. M., FENNESSY, P. F., VEENVLIET, B. A., LITTLEJOHN, R. P., FISHER, M. W., CORSON, I. D. & LABES, R. E. (1987). Energy nutrition of young red deer. *Proceedings of the New Zealand Society of Animal Production* 47, 111–113.

SUTTIE, J. M., FENNESSY, P. F., CORSON, I. D., LAAS, F. J., CROSBIE, S. F., BUTLER, J. H. & GLUCKMAN, P. D. (1989). Pulsatile growth hormone, insulin-like growth factors and antler development in red deer (*Cervus elaphus scoticus*) stags. *Journal of Endocrinology* 121, 351–360.

XU, Z. Z. (1991). *Endocrine and genetic control of seasonal breeding in sheep.* PhD thesis, Massey University, New Zealand.