RURAL INDUSTRIES
RESEARCH & DEVELOPMENT CORPORATION

Maintaining year-round production of quality venison

The use of Immunocastration vaccines to control "rutting behaviour"

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Foreword

The Victorian Institute of Animal Science (VIAS) has had a major research program running for a number of years aimed at developing viable anti-LHRH vaccines for use in dogs, cats and pigs. In late December 1993 VIAS was approached by deer producers in NE-Victoria, who had learnt of our work with these immunocastration vaccines. The producers were interested in the potential of immunocastration to control rutting behaviour in stags destined for venison production.

In collaboration with producers from the Murray Deer Breeders Incorporated we conducted an initial preliminary experiment on 2 deer farms in NE-Victoria. This experiment clearly demonstrated that testicular function could be inhibited and aggressive behaviour modified by using anti-LHRH vaccines. However, before these vaccines could be considered as being ready for commercial development, some basic research was required to identify the most appropriate formulation for use in deer; to assess the effects of the vaccine on weight loss, meat and carcass quality, and on aggressive and sexual behaviour. These questions formed the basis of this project.

The experiments were all conducted on farms throughout Victoria, One location was a deer/emu farm in Healesville. This presented us with the opportunity to examine the effects of the vaccine in a small group of emus as a preliminary trial. A copy of the report associated with this work has been included at the end of the final report.

Peter Core
Managing Director
Rural Industries Research and Development Corporation

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Summary Report DAV-100A

Introduction

Production of a "year-round" supply of quality venison has been recognised by the Australian deer industry as being vital for expansion in export and domestic markets. Rutting behaviour in stags however has limited the supply of venison during the breeding season. Aggressive behaviour during the rut renders the stags unsuitable for commercial sale as quality animals as weight loss and injury decrease the value of the carcass. The replacement of lost body condition at the end of the rut represents a substantial waste of valuable feed resources. Furthermore, because of the high level of aggressive behaviour during the rut stags are very difficult to control and cannot safely be yarded for even the simplest of procedures. The cause of this aggressive behaviour is the influence of testosterone as the testes become active during the rut.

Immunocastration offers a possible alternative for the production of a year-round supply of venison. Vaccination against LHRH has been shown to temporarily control testicular function and all related sexual behaviour. This process will allow stags to grow as entires produce velvet but behave as castrates in the targeted breeding season. The result is easy yarding and transporting due to the lack of aggressive behaviour whilst avoiding the fat deposits typical of surgical castrates. The temporary nature of the vaccine allows velvet production to be largely unaffected. The main advantage is that the weight loss associated with stags in the breeding season should be significantly reduced as the immunocastrates will not enter a breeding season as such. This should have direct benefits in that farmers will be able to send stags to slaughter either during or immediately after the breeding season without the added costs of restoring body condition.

Objectives

The objectives of this research project were to develop viable procedures, using immunocastration, to prevent rut induced weight loss and to control the sexual and aggressive behaviours associated with the seasonal rut in farmed male deer. The project was defined within four major experiments.

- Experiment 1: To identify optimal vaccine formulation for use in deer
- Experiment 2: To identify optimal vaccine dosage for use in deer
- Experiment 3: To identify the duration of vaccine efficacy, control of weight loss, antler growth and behavioural changes in response to the immunocastration vaccine
- Experiment 4: To provide additional data regarding behaviour and weight loss during the rut.

Major Research Findings

Experiment 1 and 2: Identification of optimal vaccine formulation and dose for use in deer.

Experiment 1 used Fallow and Red deer, to examine a range of vaccine formulations to identify which produced the best immune response by determination of LHRH antibody concentrations. To this end, three LHRH antigens and five adjuvants were examined for their ability to stimulate a strong immune response, after two doses, with minimal site reaction. The best formulation was selected on the basis of producing the highest and most long lasting anti-LHRH titre. This formulation was then tested in fallow deer at twice, normal and half doses to establish optimal dose rate. Again based on antibody titre we identified that most optimal formulation and dose for deer.

Experiment 3: Efficacy Of Vaccine In Controlling Sexual Behaviour And Weight Loss During The Rut

The aim of experiment 3 was to examine the effect of the vaccine when administered during the rut, how long it remained efficacious and whether use of the immunocastration vaccine could improve weight condition, reduce aggressive behaviour, and improve carcass quality when animals were subsequently slaughtered during the rut. In this experiment 40 fallow deer and 21 red deer were given four and three doses respectively of the immunocastration vaccine. Deer were monitored on a fortnightly basis for antibody concentrations, testosterone, weight, testes size and behaviour. In August, 15 fallow were sent for slaughter and carcass weights compared to a group of castrates slaughtered the same day.

Immune response: The results of the experiment clearly demonstrated that the vaccine was effective in producing an antibody response which in turn led to the reduction in testosterone concentrations during the rut. Vaccine efficacy was however shorter than anticipated with titres rapidly decreasing within 4-6 weeks for the fallow deer and 8 weeks for red deer after each boost. As a result, in order to maintain vaccine efficacy for the full duration of the trial, the fallow and red deer were given four doses and three doses respectively.

Testosterone: In the 2 yr old fallow vaccinates 80 - 92% of the herd had testosterone < 2nM two weeks following each boost. In the vaccinated red deer 80 - 84% of the herd had testosterone concentrations < 2nM two weeks after each boost. In comparison the non-vaccinated controls were well within the breeding season with testosterone > 2nM. On each of these occasions, the vaccinated deer were generally quiet in the yards and holding pens with minimal fighting and generally low risk of injury to the animals. However achieving duration of effect proved difficult as testosterone concentrations increased to active levels within 4-6 weeks of each boost in at least 50% of the herds.

Testes function: A second direct biological effect of the vaccine was seen in the reduction of testes size. Vaccinated 2 yr old fallow deer showed a significant reduction in testes size compared to the controls and remained reduced for the duration of the experiment. By the last weeks of the experiment approximately 50% of the herd had regressed testes as would be expected for a non-breeding season. Of all the vaccinates sent to slaughter only one testes appeared normal by histological examination. All others showed varying degrees of testicular atrophy. Similar results were also observed in the red deer with the testes in the vaccinates being significantly reduced in size in comparison to the controls within 2 weeks after the first booster and remained smaller for the duration of the experiment.

Behaviour: In contrast, the control of aggressive behaviour in the herd was difficult to maintain and seemed to reflect the rapidly changing testosterone concentrations. Although a direct correlation between observed fighting and testosterone concentrations could not be established in the fallow deer both farmer and scientific staff observed that the herd was more difficult to safely yard and handle as testosterone concentration increased. Control of aggressive sexual behaviour however remains essential if deer are to be taken to slaughter during the breeding season.

Weight The vaccinated 2yr fallow deer as a group maintained their weight condition throughout the experiment within approximately ± 2 Kg. Comparison between the vaccinated 2yr old deer and controls showed that there was no weight difference between the two groups in the first 8 weeks of the experiment (Bleed 5). The controls were not weighed again until week 18 (Bleed 9, 22 May). At this stage the two groups were significantly

different ($P<0.05$), with the controls being 4.6Kg below the vaccinated deer and 4.9 Kg below their initial weight. The weight data suggests that the immunocastration vaccine was highly successful at improving liveweight gain in comparison to the controls in 2 yr olds. This finding however is based on the single weight comparison at Week 18 between vaccinates and controls and cannot be used as a definitive result. In contrast, the weight data for the red deer showed that there was no difference between the vaccinated and non-vaccinated deer ($P>0.05$) with both groups increasing in weight through the experiment by an average of 6 Kg.

Eighteen vaccinated fallow deer were sent to slaughter on 1st August 1996. The group had an average liveweight, carcass weight and dressing out percentage of 51 Kg, 29 Kg and 57% respectively. This result was within normal expectations and was not significantly different from a group of castrates also sent to slaughter (from the same farm) on the same day.,

Velvet production: Both herds of vaccinated red and fallow deer were successful in producing a growth of velvet (due to decreasing testosterone concentrations) as early as April. Growth was generally a straight spike or of an irregular shape with the absence of defined browtine or baytine. In all cases the velvet was typical castrate growth and graded at "D" class. Velvet growth in the red deer was observed again in December 1997 and was observed to be typical 3yr old velvet as expected in a normal herd.

Mature stags The vaccine proved to have poor efficacy in mature fallow stags aged 3 years or more. The majority of mature stags showed no reduction in testosterone, testes size and many became and stayed aggressive with the onset of the breeding season. Typically the mature stags were noticeable for thickened necks throughout the trial.

Experiment 4: Further investigation of weight loss during the rut.

This experiment aimed to provide additional data regarding weight loss and gave the opportunity to compare a commercial prototype (Vaccine I) against the VIAS deer immunocastration vaccine we had previously identified as being optimal. Due to constraints in supply of deer and time this experiment was conducted in fallow deer only. Twenty fallow deer were given three doses of Vaccine I, starting in January with subsequent boosts given four then 6 weeks after. The deer were monitored for weight, testes size, behaviour and testosterone concentrations and were compared to a group of 10 non-vaccinated controls and a group of 4 deer vaccinated with vaccine formulation from Experiment 3.

Immune response In general the Vaccine I displayed a slight improvement in terms of producing anti-LHRH titres and decreasing testosterone concentrations. The experiment showed that 90-95% of the herd produced a strong immune response to the vaccine. Titres remained above 400 for 6 weeks after vaccination and testosterone concentrations below 2 nM in more than 80% of the herd for up to 10 weeks after the third dose. This compares with Experiment 3 whereby 80% of the herd showed a strong biological response to the vaccine; and efficacy was shorter with titres dropping below 400 with 4 weeks of the boost in 50% of the herd. These differences seen between Experiment 3 & 4 are considered to be slight since such differences may equally be effected from the different farm locations, nutrition, season temperatures and herd.

Behaviour Experiment 4 clearly indicated that the vaccinated group were quiet and easily handled in the 8-10 weeks following the third vaccination. This provides a period of time from April- June whereby stags of good weight condition could be sent to slaughter.

Weight In terms of controlling weight loss during the rut, Vaccine I produced some positive results in comparison to the non-vaccinated controls in the initial part of the trial. Weight loss in the 8-10 weeks following vaccination was significantly less than that observed in the non-vaccinated group which were at that stage actively in rut. During this time the vaccinated group remained 2-3 Kg heavier than the non-vaccinated controls. This advantage was however negated at the end of the trial when the vaccinated group moved into an apparent delayed rut as the vaccine efficacy deteriorated. At this stage the non-vaccinated controls were rapidly increasing in weight with the emergence of the non-breeding season and new spring pasture growth. In contrast the vaccinated group showed deterioration in weight despite equal access to fresh pasture growth. This same period coincided with increasing testosterone concentrations, and decreased anti-LHRH titres to baseline levels. The deer became increasingly difficult to yard or handle. This aggression and deterioration in body condition continued at least through to our final visit in mid-October.

The exact cost benefit to farmers in terms of body condition and weight loss is however difficult to predict from the findings of this research. In experiment 3, the fallow vaccinated deer were 5Kg heavier than the non-vaccinated entires by the end of May. In experiment 4 the fallow deer maintained a 2-3 Kg advantage over the non-vaccinated stags in the period that the vaccine was efficacious (April-May). In experiment 3, the red deer showed no weight advantage over the non-vaccinated controls at any stage of the experiment. It is therefore likely that the cost benefits to farmers using the immunocastration will be highly variable. Previous research into immunocastration in other countries also found a variable response in regards to weight gain/loss (Ataja, 1992; Freudenberger *et al*, 1991, 1993).

Conclusions:

1. An immunocastration vaccine was developed using a formulation seen as being most optimal for use in deer. This vaccine and another vaccine supplied by a commercial company have been shown to be effective in reducing testicular function in stags in up to 95% of the herd. Whilst antibody titre is high, rut induced weight loss and aggressive behaviour can be reduced.

2. The vaccines have been shown to produce no adverse site reactions in fallow deer. The vaccines produced site reactions in the form of burst abscesses or swellings in approximately 26% of the red deer. Given that the red deer also suffered severe site reactions to copper injections, it is suspected that this finding may be a factor of the species rather that being due to a specific component of the immunocastration vaccine.

3. The success of using "immunocastration" as a tool to minimise weight loss and improve productivity was highly variable throughout the trials. No weight benefit was observed for immunocastrated red deer. Immunocastrated fallow deer were 2-5 Kg heavier than non-vaccinated stags but only whilst the vaccine was efficacious. Thus economic gain in terms of liveweight may be highly variable and vaccination may not be of cost benefit to farmers in terms of producing a heavier carcass.

4. The vaccine is most effective in 2 year old stags and not appropriate for fully mature stags.

5. Limitations of the vaccine include a short period of vaccine efficacy of 8-10 weeks. The use of the immunocastration vaccine does not act to prevent the onset of the rut but rather delays it. Once vaccine efficacy deteriorates, aggressive behaviour and weight loss can be

severe at a time when pastures are good. The net result is that if vaccinates are not slaughtered during the rut, the entire non-vaccinated stags will ultimately be of a higher liveweight and better body condition in comparison to immunocastrates.

6. Other work reported in the literature found a similar result and supports the conclusion that the "delayed rut" is not specific to the formulation tested here but is an outcome pertaining to any form of LHRH immunocastration.

7. Immunocastration offers the potential for supplying venison in the earlier months of the breeding season (April-June). The benefits to the farmer is that the opportunity to slaughter is extended until May.

8. In practice however the vaccine is limited in four ways:
    - firstly by its short duration of effect which therefore requires the use of multiple doses,
    - secondly by the fact that 5-10% of the herd do not respond immunologically or biologically to the vaccine and therefore present problems in terms of behaviour and handling;
    - thirdly the cost benefit to farmers in terms of weight and body condition have been shown to be highly variable;
    - fourthly the vaccine acts to delay the rut not prevent it, thus limiting farmers to the necessity of sending animals to slaughter that season so to avoid significant weight loss and body condition.

1. In its current formulation the vaccines tested in this project are not viewed as being suitable for commercialisation for use in deer.

Background

The need to control rutting behaviour in deer.

The Australian deer industry comprises of a population of approximately 230 000 deer as of 1996 (RIRDC, 1996). Production of a "year-round" supply of quality venison is essential if the industry is to expand in export and domestic markets. Rutting behaviour in stags however has limited the supply of venison during the breeding season. Depending on breed and region, the breeding season in the southern states of Australia may extend from February to September. Aggressive behaviour during the rut renders the stags unsuitable for commercial sale as quality animals as weight loss and injury decrease the value of the carcass. During the rut stags are reported to loose up to 30% of their body weight (Drew, 1997). The replacement of lost body condition at the end of the rut represents a substantial waste of valuable feed resources. Furthermore, because of the high level of aggressive behaviour during the rut stags are very difficult to control and cannot safely be yarded for even the simplest of procedures. The cause of this aggressive behaviour is the influence of testosterone as the testes become active during the rut.

Controlling rutting behaviour in deer:

Surgical Castration

Surgical castration before the rut will control the rutting behaviour. However, the costs of castration and the associated stress make this option non-viable. The code of practice by the Standing Committee on Agriculture, Animal Health Committee (1991), for the farming of deer, stipulates that "if animals are over 6 months of age appropriate anaesthesia or analgesia administered by or under the supervision of a veterinary surgeon is required. Because of the late descent of testicles, some deer cannot be castrated at a young age". In addition, earlier castration precludes the production of velvet and limits growth performance by leading to increased fatness (Drew *et al*, 1978).

Farm Management

There has been some suggestions that the year-round supply of venison can be achieved by better farm management. According to Dr Ken Drew, from AgResearch in Invermay, the supply of male carcasses during the rut is also severely limited in New Zealand. This problem is however tackled by trying to sell mainly female deer and yearlings during the rut when the older males can not be handled. This suggestion however cannot readily be applied to the much smaller Australian industry since there is an equally important need to keep all females for breeding. In addition, the sale of smaller and younger deer is unprofitable to the farmer due to high transport and slaughter costs.

Immunocastration

Seasonal changes in fertility, increases in circulating testosterone in males, and progesterone/oestradiol in females, are governed by the secretion of the gonadotrophic hormones, lutenizing hormone (LH) and follicle-stimulating hormone (FSH). These changes are themselves mediated by the release of Lutenizing Hormone Releasing Hormone (LHRH, also called gonadotrophin releasing hormone GnRH) from the hypothalamus.

Active vaccination against LHRH has the result that LH and FSH are not released and, in males, the release of testosterone is subsequently suppressed. Vaccination against LHRH (immunocastration) has been shown to be effective in dogs, cows, pigs, horses and deer in selectively and temporarily controlling testicular function and all related sexual behaviour.

This process has direct application to the control of rutting behaviour in deer with the subsequent reduction in aggression and weight loss in male deer. The temporary nature of the vaccine allows the period of castration to be short with the result that carcass fatness remains unaffected and velvet production can be maintained in the non-breeding season.

The concept of immunocastration is not new. In 1936, Parker and Rowland first demonstrated ovulation inhibition in rabbits treated with anti-bovine gonadotropin. Since then there have been numerous studies outlining the effect on immunocastration on sexual function and behaviour in many species of animals including dogs (Hennessy *et al* 1996), cows/bulls (Johnson *et* al, 1988; Adams & Adams, 1992; Finnerty *et al*, 1994) , pigs (Bonneau *et* al, 1994; Bonneau & Enright 1995; Hennessy *et al*, 1994, 1995a, 1995b; Oonk *et al* 1995), deer (Lincoln *et al*, 1982; Ataja *et al* 1992; Freudenberger et al, 1991, 1993) and horses (Dowsett *et al*, 1992-1993, 1993). However, despite much world-wide research, on both a pure scientific front and on a commercial front, successes in developing effective vaccines of acceptable reactivity have been extremely limited. To date only one commercial "immunocastration" vaccine has been made available but was recently withdrawn from the market. That vaccine, "Vaxstrate", developed by Webster's and Peptide Technology was only approved for use in female cattle in northern Australia.

Immunocastration in deer - A review

Several research groups in either New Zealand or Europe have been working on an immunocastration vaccine in deer (Lincoln *et al*, 1982; Ataja *et al* 1992; Freudenberger et al, 1991, 1993). These experiments were partially successful in stopping testes function and had limited effects on body weight during the rut. Effects on behaviour were largely not reported. No further reports of progress have been noted in more recent years. A brief review is as follows:

In an investigation of how antler growth is governed by testosterone and possibly gonadotrophic hormones, Lincoln *et al* (1982) of Edinburgh immunised four red deer against LHRH to produce physiological castrates. The immunocastration vaccine was based on a LHRH-human serum albumin conjugate prepared by reaction with carbodiimide. The conjugate was presented in Freunds adjuvant. Each animal received a total of four doses administered over 8 months before the next breeding season. In response to immunisation, all deer had circulating levels of LHRH antibodies, the testes were reduced in size compared to the maximum values of the controls. The blood levels of testosterone were reduced in the immunised animals and the immunised stags showed no rutting behaviour. Three of the four animals cast their antlers prematurely during the rut and development of new antlers was initiated after casting. The effects on the antler cycle were variable and correlated with the antibody titre, only the animal with the highest titre developed antlers that resembled those of a castrate and remained in velvet for more than 6 months.

More recently Ataja *et al* (1992) of New Zealand investigated the effect of immunisation against LHRH and melatonin on growth in five red deer stags. The LHRH vaccine contained LHRH antigen conjugated to ovalbumin in DEAE-dextran adjuvant. Each animal received 1 mg antigen/dose. Two doses were administered, the first in January, the second approx four weeks later in February. Four out of the five stags developed detectable levels of antibody titre. Immunisation against LHRH reduced plasma LH concentrations and both delayed and reduced weight loss associated with the rut (March-May). However in the months following the rut (May-July) the liveweight gain of the immunised animals

deteriorated dramatically suggesting that immunisation had not fully suppressed the rut but delayed its onset. Despite this the immunised animals performed slightly better overall than the controls. Liveweight gain for immunised and control animals was -11 g/day and -35g/day respectively. Mean liveweights at the start of the rut were 93.3 kg and 97.9 kg for immunised and control groups respectively. Animals were slaughtered in July and average carcass weight was 53.5 and 53.0 kg respectively. Thus whilst the immunised animals began the experiment with a slightly lower liveweight, their carcass weights were equivalent (or slightly higher) than that of the controls. Ataja *et al* also found that immunisation had no effect on fatness possibly due to the short term nature of the immunocastration.

Freudenberger *et al* (1991, 1993) of New Zealand also investigated the effect of immunocastration on red deer. In this experiment a total of 66 deer separated into a number of groups were immunised with LHRH vaccine, each group receiving a different vaccination regime. The regimes differed in the quantity of antigen (1-3 mg) and number of booster doses (2-5). The LHRH vaccine (Peptide Technology Ltd) comprised LHRH antigen in DEAE-Dextran. Vaccinations were administered over the Spring-Summer and were completed well before the onset of the next breeding season. Freudenberger *et al* found that whilst immunisation against LHRH depressed plasma concentrations of LH and testosterone, the reductions were not as marked as those observed by Lincoln *et al* (1982). Some treatments appeared to reduce the magnitude and delayed the rut and this was especially observed when handling the deer in the yards. However because the rut was only partially suppressed voluntary food intake and live weight gain were very similar between immunised and control groups. The exception was stags that received the early booster. These animals were significantly heavier four hours prior to slaughter (October), mean (±SE) liveweight was 110.2 ± 4.9, 100.8 ±1.7 and 102.3 ±1.5 kg in the early booster, late booster and control groups respectively, with corresponding carcass weights being 63.67±2.92, 58.26±1.01, 58.92 ±1.72 Kg. Immunisation was found to also reduce velvet production with the majority of animals having velvet less than 100 mm at the time of harvest.

In late December 1993 VIAS was approached by deer producers in NE-Victoria, interested in the potential of immunocastration vaccines to control rutting behaviour in stags destined for venison production. In collaboration with producers from the Murray Deer Breeders Incorporated we conducted an initial preliminary experiment on 2 deer farms in NE-Victoria using an immunocastration vaccine formulation that was readily available. A total of 10 red/wapiti cross deer and 7 fallow deer were vaccinated with 3 doses of vaccine. The experiment commenced on 31st March 1994 and subsequent doses were administered three weekly thereafter. The progress was then monitored for a further 10 weeks (until 21/7/97). Both wapiti/red and fallow deer produced strong immune response to the vaccine with anti-LHRH antibody titres ranging from 206->3500. The vaccinated fallow deer showed a significant reduction in testes size in response to vaccination. Testosterone levels also decreased from active levels (above 2 nM) at the start of the experiment to low inactive concentrations (<2 nM) following the 3 doses of vaccine. Observations in regards to behaviour, indicated that the vaccine was also effective in reducing the aggressive rutting-induced behaviour. Following vaccination the deer were observed to be quiet and unwilling to fight whilst in the yarding pens. In contrast most measurements on the non-vaccinated controls were unable to be taken due to the fighting and stress caused as the controls were yarded. The results were less conclusive for the Wapiti/Red deer. Due to handling difficulties, the testes size could not be measured in the red deer. Although the rut was well underway at the onset of the experiment the controls did not display high active testosterone concentrations or aggressive behaviour. Apart from the strong immune response in the

vaccinated deer, we were therefore unable to identify significant biological differences between vaccinated and control groups.

This preliminary experiment demonstrated that a strong antibody titre was produced in response to vaccination, that testicular function in fallow deer could be inhibited and aggressive behaviour modified by using anti-LHRH vaccines. Time restraints at the beginning of the experiment, caused by the necessity to begin vaccination before the onset of the rut, meant that we were restricted at that time to using a formulation vaccine that was readily available for use. Given the positive results, some basic research was now required to identify the most appropriate formulation for use specifically in deer; to assess the effects of the vaccine on weight loss, meat and carcass quality, and on aggressive and sexual behaviour. These questions formed the basis of the project pertaining to this report.

Objectives

The objectives of this research project were to develop viable procedures, using immunocastration, to prevent rut induced weight loss and to control the sexual and aggressive behaviours associated with the seasonal rut in farmed male deer. It was proposed to achieve this by developing an anti-LHRH vaccination regime which would allow substantial opportunities for the year round production of quality venison.

At the outset, the objectives of the project were defined within three major experiments. Firstly, in order to develop an immunocastration regime, the first and second experiments were required to identify the formulation then dose of vaccine which would produce the best immune response in deer. Once identified, the formulation of choice was then to be tested in a experiment conducted during the breeding season. Thus this protocol required that the three experiments be conducted as follows:

Experiment 1: Vaccine formulation	July 1995- October 1995
Experiment 2: Dose response	November 1995 - January 1996
Experiment 3: Vaccine efficacy experiment	January 1996 - March 1997

The third experiment aimed to answer basic questions in relation to the efficacy of the vaccine in deer. Specifically, duration of vaccine efficacy, control of weight loss, carcass quality, antler growth and behavioural changes were to be addressed in the third experiment. The third experiment was completed with mixed results and with some of the meat quality objectives being unable to be met.

At this time, we were given the opportunity to test an immunocastration prototype vaccine developed by a commercial company. As such we sought approval for conducting a fourth experiment in the following breeding season. This experiment aimed specifically at providing additional data regarding behaviour and weight loss during the rut and as a secondary measure compare the performance of a commercial prototype against the VIAS deer immunocastration vaccine we had previously identified as being “optimal” for use in deer.

Thus at the end of the two year duration of the project, four experiments had been completed meeting the following objectives

Experiment 1: To identify optimal vaccine formulation for use in deer
July 1995- October 1995

Experiment 2: To identify optimal vaccine dosage for use in deer
November 1995 - January 1996

Experiment 3: To identify the duration of vaccine efficacy, control of weight loss, antler growth and behavioural changes in response to the immunocastration vaccine
January 1996 - December 1996

Experiment 4: To gain further information about the effect of immunocastration on behaviour and weight loss.
January 1997 - October 1997

Technical review of the Research Need

In 1996 RIRDC released its Deer Research and Development Plan for 1996 - 2000. This plan clearly indicated that one of the industry strengths laid in the expanding domestic and international markets. Australian farmed deer have a proven record for their good health status and are well placed to meet the increasing demand. One major weakness however is the relatively small size of the Australian deer population. Insufficient deer numbers have limited the industry's ability to provide supplies to export markets or lower production costs. Although increasing population size is of the highest priority to the industry, females are often exported or slaughtered to meet orders. This practice obviously is a major obstacle in expanding the industry.

In order to market a continually expanding industry, a consistent year-round supply of quality product is vital (Tume, 1993). Because of the need to maintain the female population for breeding, stags are the choice for venison production. Venison animals must be in prime nutritional condition, suffering minimal stress and bruising before slaughter, preferably young (15 - 27 months old) and of sufficient carcass weight to make overhead costs viable. Red deer, for example, should be 90 - 100 kg live weight to produce a 50 -55 kg carcass. (Watson & Leah, 1993).

The supply of stags for venison production is however limited during the breeding season, due to aggressive behaviour and weight loss associated with the rut. Voluntary feed intake and growth in red deer stags aged 15-20 months is depressed during the rut even when a high quality pelleted feed is provided *ad libitum* (Fennessy *et* al, 1981). Mature stags may cease eating altogether for some weeks. This depressed appetite coincides with intense physical rutting activity. As a result, mature stags suffer significant weight loss. Weight loss of 15 to 20% of bodyweight is usual in dominant red deer stags in New Zealand (Kelly and Moore, 1977). This depressed appetite and weight loss is observed in rutting males of all species of deer. Sub-dominant males also lose weight but not to the extent as dominant stags (Denholm, 1984). This loss of body condition must be replaced before the animal is suitable for slaughter at significant expense to the farmer. In addition, the slaughter of stags during the rut is non-viable since bruising and injury caused by fighting downgrades the quality of the carcass. Furthermore it is not possible to safely yard, handle or transport entire stags during the rut as fighting increases with the animals being forced into small confined areas. The onset of the rut and subsequent aggressive rutting behaviour and weight loss is attributable to the seasonal changes in daylengths and an increase in testosterone in response to LHRH hormonal activity.

The alternatives for the breeding season are to slaughter females, castrates or young immature stags (15 months old). Slaughter of females is not viable for the Australian industry as already discussed.

Whilst surgical castration of stags provides a supply of male deer which are easily handled throughout the rut, the costs of castration and the associated stress make this option non-viable. The code of practice by the Standing Committee on Agriculture, Animal Health Committee (1991), for the farming of deer, stipulates that "if animals are over 6 months of age appropriate anaesthesia or analgesia administered by or under the supervision of a veterinary surgeon is required. Because of the late descent of testicles, some deer cannot be castrated at a young age". In addition, earlier castration precludes the production of velvet and limits growth performance by leading to increased fatness (Drew *et al*, 1978).

The slaughter of young stags offers some advantages. Aggressive behaviour during the rut is not a problem at this age and so deer can be sent for slaughter at any time through the breeding season. Younger animals are more efficient converters of grass to meat. The older animals have a higher maintenance cost and need more feed to product the same kilo of meat. Cash return is 12 months sooner than with a 27 month slaughter. The disadvantage is that the overhead costs for transport and slaughter are high and non-viable given the smaller weight carcasses. Slaughter of the young stags prevents the production of a cut of velvet and therefore prevents the farmer from accurately establishing the ability of the stag to produce a good velvet harvest.

Immunocastration offers an attractive alternative for the production of a year-round supply of venison. Vaccination against LHRH has been shown to temporarily control testicular function and all related sexual behaviour. This process will allow stags to grow as entires produce velvet but behave as castrates in the targeted breeding season. The result is easy yarding and transporting due to the lack of aggressive behaviour whilst avoiding the fat deposits typical of surgical castrates. The temporary nature of the vaccine allows velvet production to be largely unaffected. The main advantage is that the weight loss associated with stags in the breeding season should be significantly reduced as the immunocastrates will not enter a breeding season as such. This will have direct benefits in that farmers will be able to send stags to slaughter either during or immediately after the breeding season without the added costs of restoring body condition.

Research Methodology, Detailed Results and Discussion of Results

These aspects will be covered in four chapters covering the four major experiments of the trial:

Experiment 1: Optimal vaccine formulation for use in deer

Experiment 2: Optimal vaccine dosage for use in deer

Experiment 3: Vaccine efficacy

Experiment 4: Further investigation of weight loss during the rut.

Experiment 1. Effects Of Vaccine Formulation On The Immune Response.

Aim

Experiment 1 was the first part in a series of three experiments to develop and examine the efficacy of a deer immunocastration vaccine. The aim was to use male and female deer, Fallow and Red, to examine a range of vaccine formulations to identify which produces the best immune response by determination of LHRH antibody concentrations. A range of LHRH antigens and adjuvants were examined for their ability to stimulate a strong immune response with minimal site reaction. Because the experiment was conducted in the non-breeding season (August - November) it was not possible to determine how behaviour, weight loss or carcass quality was affected by each formulation. However, since in other studies (Lincoln *et al* 1982) anti-LHRH titres have correlated with the resulting efficacy of the vaccine it was proposed that the measurement of LHRH titre would be a sufficient indicator to determine which formulation should be most efficacious when administered for controlling the rut.

Materials and Methods

Vaccine formulations

1. LHRH antigen A in DEAE-DEXTRAN
Group 1 formulation (2 mL/dose) was an LHRH conjugate (Batch No. 256003) in DEAE-DEXTRAN.

2. LHRH antigen A in QUIL A
Group 2 formulation (2 mL/dose) used LHRH conjugate (Batch No. 256003) in Quil A.

3. LHRH antigen A in PMMA particles.
Group 3 formulation (4 mL/dose) used LHRH conjugate (Batch No. 256003) in PMMA particles.

4. LHRH antigen A in Montanide 50.
Group 4 formulation (2 mL/dose) used LHRH conjugate (Batch No. 256003) in Montanide ISA 50.

5. LHRH antigen A in Montanide 206.
Group 5 formulation (2 mL/dose) used LHRH conjugate (Batch No. 256003 in Montanide ISA 206 adjuvant.

6. LHRH antigen A in Montanide 25
Group 6 was not included in the experiment due to the limited supply of available deer. This adjuvant was chosen to be excluded because it had produced severe site reactions in earlier experiments carried out in dogs.

7. LHRH antigen B in DEAE-DEXTRAN
Group 7 used an alternative LHRH conjugate prepared at VIAS for use in concurrent experiments (Batch no JS-27) in DEAE-dextran. Each vaccine was administered as a 2 mL dose.

8. LHRH antigen C in DEAE-DEXTRAN
Group 8 used an alternative LHRH conjugate prepared at VIAS for use in concurrent experiments (Batch no JS-31) in DEAE-Dextran.. Each vaccine was administered as a 2 mL dose.

Animals
Two breeds of deer were used; 59 Fallow and 41 Red deer. Both male and female deer were used, ranging in age from 1-2 year olds to mature adults (up to 10 yrs old). The deer were supplied from and remained agisted in four farms in North Central Victoria.

Each formulation, except for Group 3 were tested in both the Fallow and Red deer. The number of deer vaccinated with each formulation is outlined in Table 1. Seven Fallow deer and three Red deer received no treatments and were used as non-vaccinated controls.

TABLE 1. Number of Fallow and Red deer vaccinated with each formulation.

Group No.	Formulation	No. Fallow Deer Used	No Red Deer Used
1	LHRH A - DEAE	8	7
2	LHRH A - Quil A	8	7
3	LHRH A -PMMA	4	0
4	LHRH A - Montanide 50	8	6
5	LHRH A - Montanide 206	8	6
7	LHRH B - DEAE	8	6
8	LHRH C - DEAE	8	6
Controls	not vaccinated	7	3

Vaccination Regime
As outlined in Table 1, except for Group 3, each formulation was tested in both Fallow and Red deer. Group 3 was given only to Fallow deer due to the short supply of this formulation.

Each vaccinated deer was given a primary vaccination at week 0 (8th August 1995) and then a second vaccination at week 4 (5th September 1995). For each dose, the animals received 2 mL of vaccine as a single subcutaneous injection administered at the back of the neck just behind the ear, using a 21 gauge needle and syringe. Group 3 deer received 4 mL of vaccine administered in the same way.

In a previous preliminary experiment (conducted in 1993), a number of site reactions had been observed, particularly in Red deer. In order to avoid unwanted infection, the site for injection in each red deer was first shaved then swabbed with 70% hibitane immediately before vaccination. The fallow deer were not shaved but the site of injection was swabbed with 70% hibitane before vaccination.

Sample collection

Blood samples and weights were collected at week 0 and each fortnight thereafter for a total of 10 weeks (i.e. 6 weeks after the 2nd dose). Blood samples were collected from the jugular vein using 21 Gauge vacutainer needles. Weights were measured using weigh boxes and scales provided by the farmers. A summary of vaccination and sample collection schedules is given in Table 2.

The site of vaccination was also assessed for swelling or abscess and a score was recorded. Site reactions were scored as either 1, 2 or 3 and given a comment and approximate size in centimetres for the actual diameter. A score of "1" was given if the site of injection was slightly swollen and could only be detected by touch. A score of "2" was given if a lump was visible and a score of "3" was given if the reaction had burst open.

TABLE 2: Summary of blood sample collection and vaccination schedules.

DATE	WEEK No.	BLEED No.	VACCINATION
8 August 1995	0	1	Initial
21 August 1995	2	2	
5 September 1995	4	3	Booster
18 September 1995	6	4	
2 October 1995	8	5	
16 October 1995	10	6	

Analyses

Sera collected from the blood samples were analysed for testosterone and anti-LHRH antibody titres. The testosterone analysis was performed using a commercially supplied direct Radioimmunoassay kit (Pantex ^{125}I). The method was modified to include an extraction step whereby serum testosterone was extracted into 90%diethyl ether/10%ethyl acetate. The intra-assay coefficient of variation was <10%. The inter assay coefficients of variation were 12.1, 11.3, 10.0% as calculated from low, median and high pathological standards measured over 28 assays. The assay had a minimum detection limit of 0.5 nM

The anti-LHRH analysis was performed using a direct binding radioimmunoassay whereby doubling dilutions of serum ranging from 1:100 to 1:1600 were incubated with ^{125}I labelled LHRH (supplied by AMRAD (250 μCi) diluted with 0.1M phosphate/saline buffer pH 7.4 until cpm = 7000-7500). Each dilution was incubated at 4°C for 48 hours in a final volume of 400μL 0.1M Na phosphate buffer pH 7.40 containing 1.25 mg/mL human gamma globulin. Following incubation 100μL 1%bovine gamma globulin solution was added. The free LHRH tracer was separated from bound by addition of 1mL 18% polyethylene glycol 6000. The resulting precipitate was separated after centrifugation (3000rpm, 10 minutes) then counted on a Wallace 1410 scintillation counter. Intra-assay variability was determined from measuring three different pig samples up to 9 times in one assay and was found to be 7.0, 3.3 and 4.8% respectively. Inter-assay coefficient of variation was 11.9 and 10.1% calculated from measuring control serum collected from an immunocastrated pig and deer over 55 assays. The titres are expressed at the dilution of serum capable of binding 33% labelled LHRH.

The minimal detectable concentrations as it applies to other analytes is not applicable for our purpose. Any test sera where there is clearly no antisera are reported as having a titre <20. For an animal to be effectively immunocastrated by the LHRH antibodies it needs a titre of at least 400. We interpret any titre of <400 as indication of complete suppression of LHRH.

Results

Site Reactions

All the deer were examined two weeks after each vaccination for any reaction at the site of injection. Several site reactions were observed and are described in Table 3.

TABLE 3. Site Reactions observed in deer two weeks following vaccination.
Site reactions were scored as either 1, 2 or 3. "1" = slightly swollen, only be detected by touch. "2" = a visible lump. "3" = burst abscess.

Group No	**Fallow Deer**			**Red Deer**		
	No. with Score "1"	No. with Score "2"	No. with Score "3"	No. with Score "1"	No. with Score "2"	No. with Score "3"
1. LHRH A - DEAE	0	0	1	0	1	1
2. LHRH A - Quil A	0	0	0	1	1	0
3. LHRH A -PMMA	0	0	0	N/A	N/A	N/A
4. LHRH A - Mont 50	0	0	0	0	1	0
5. LHRH A - Mont 206	0	0	0	0	0	0
7. LHRH B - DEAE	0	0	0	0	0	2
8. LHRH C - DEAE	0	0	0	0	0	1
Non-vaccinated Controls						

Only one site reaction was observed in a total of 59 Fallow deer. This reaction was observed after the first injection and appeared as a burst abscess with a scar approx 2-3 cm in size. Since this was the only reaction seen in any of the Fallow deer, it is assumed that it was caused by a random contamination of the needle or infection of the site following vaccination.

Eight site reactions of various size and severity were observed across 41 Red Deer. The reactions were randomly spread across the different formulations and could not be attributed to a particular adjuvant or conjugate. It would appear that the Red deer are more sensitive than the Fallow to having a reaction at the site of injection.

Anti LHRH Titre

Anti-LHRH serum titres were measured two, four and six weeks after the booster vaccination and results are outlined in Table 4.

TABLE 4: Median anti-LHRH titres in fallow & red deer for each treatment group and non-vaccinated controls.

Treatment Group	**Anti-LHRH titre in Fallow Deer**	**Anti-LHRH titre in Red**

					Deer		
	Bleed 4	Bleed 5	Bleed 6	Bleed 7	Bleed 4	Bleed 5	Bleed 6
1. LHRH A - DEAE	297	160			**775**	353	
2. LHRH A - Quil A	110	60			**460**	246	
3. LHRH A -PMMA	<20	<20			N/A	N/A	N/A
4. LHRH A -Mont 50	187	126			97	41	
5. LHRH A-Mnt 206	116	159			122	164	
7. LHRH B - DEAE	**766**	**604**	180	89	**989**	**526**	216
8. LHRH C - DEAE	**688**	344	135	53	**558**	254	90
Controls	<20	<20	<20	<20	<20	<20	<20

Bleed 4: taken 2 weeks after the booster vaccination
Bleed 5: taken 4 weeks after the booster vaccination
Bleed 6: taken 6 weeks after the booster vaccination
Bleed 7: taken 8 weeks after the booster vaccination

Two weeks after the second dose, the fallow deer treatments 7 & 8 displayed median titres >400 (designated titre indicating significant immune response to vaccination). Titres were at their highest two weeks after the booster vaccination and rapidly decreased to near base levels by 8 weeks after the booster. Group 7 performed marginally better then group 8 since titres remained above 400 up to 4 weeks after the booster. All other groups performed poorly with median titres all below 400. Group 3 vaccinated deer showed no indication of an immune response as anti-LHRH titres did not rise above base level.

In the red deer treatments 1, 2, 7 & 8 all had median titres above 400 two weeks after the booster vaccination. All treatments groups except group 7 showed a decrease in anti-LHRH titre to below 400 by 4 weeks after the booster. Group 7 remained with median titres above 400 four weeks following booster vaccination. Titres were however approaching base levels by 8 weeks after booster vaccination.

Testosterone

Testosterone concentrations were measured for bleeds 1 and 2 to use as pre-immunisation indicators and bleed 4 to determine if immunocastration vaccination had lowered testosterone concentration. The results are presented in Table 5.

TABLE 5: Average serum testosterone concentrations (standard deviation) of fallow & red deer for each treatment group and non-vaccinated controls.

Treatment Group	**Testosterone in Fallow Deer nM**			**Testosterone in Red Deer nM**		
	Bleed 1	Bleed 2	Bleed 4	Bleed 1	Bleed 2	Bleed 4
1. LHRH A - DEAE	1.3 (1.2)	2.1 (3.0)	<0.5 (0.3)	0.6 (0.6)	2.7 (3.2)	<0.5 (0)
2. LHRH A - Quil A	3.8 (3.0)	5.7 (6.4)	1.1 (0.8)	0.8 (1.4)	2 (2.4)	<0.5 (0.1)
3. LHRH A -PMMA	2.9 (3.0)	8.3 (5.4)	4.0 (7.7)	N/A	N/A	N/A
4. LHRH A-Mont 50	1.1 (0.9)	1.3 (1.5)	2.8 (5.2)	<0.5 (0.2)	1.1 (0.6)	<0.5 (0.1)
5. LHRH A-Mnt 206	0.6 (0.5)	2.1 (3.3)	1.2 (1.5)	N/A*	N/A*	N/A*
7. LHRH B - DEAE	2.0 (2.5)	2.3 (3.3)	2.3 (2.7)	N/A*	N/A*	N/A*
8. LHRH C - DEAE	<0.5 (0.1)	2.3 (2.1)	4.7 (6.7)	1.3 (1.0)	<0.5 (0.5)	<0.5 (0.5)
Controls	3.1 (4.5)	6.7 (9.2)	5.0 (5.5)	2.3 (1.6)	1.4 (0.2)	<0.5 (0.1)

N/A* Not applicable since 4 out of 6 animals in these groups were female.

Testosterone concentrations were higher in fallow deer than in the red suggesting that some fallow still had high circulating testosterone concentrations from the rut. Testosterone concentrations in the fallow were variable (as seen by the std devs) at all bleeds including bleed 4 (taken two weeks after the booster). At bleed 4, groups 1, 2, 5 and 7 all had mean testosterone concentrations less than half of that seen in the controls. This may be indicative that for these groups the vaccination had a biological effect causing a reduction in testosterone concentration two weeks post booster vaccination. The results are however complicated by the timing of the experiment which meant vaccinations were given at the end of the rut when testosterone concentrations are naturally changing with the onset of the non-breeding season. The best indicator of biological effect and testosterone response will be able to be determined in the third experiment.

In general most red deer had low testosterone concentrations below the minimum detection level of the radioimmunoassay. These concentrations were observed at bleeds 1, 2 and 4 indicating that the red deer were no longer in rut and had commenced their non-breeding season. For this reason it was not possible to determine if any of the vaccine treatments were specifically producing a decrease in circulating testosterone concentrations.

Weight

The weights of the deer were monitored for the duration of the experiment to determine whether any vaccine formulation resulted in an increased or decreased weight gain compared with the controls. Table 6 outlines the average weight gained over the first 8 weeks of the experiment (i.e. until 4 weeks post booster vaccination) in the Fallow and Red deer for each formulation and control.

TABLE 6: Average weight (Kg) gained over the first 8 weeks (August-October) of the experiment in the Fallow and Red deer for each formulation and control.

Group No	**Fallow Deer**		**Red Deer**	
	Farm*	Weight gain Kg	Farm*	Weight gain Kg
1. LHRH A - DEAE	DD	15.6	BH	16.5
2. LHRH A - Quil A	DD	15.5	BH	18.2
3. LHRH A -PMMA	DD	12.9	n/a	n/a
4. LHRH A - Mont 50	DD	13.8	BH	16.7
5. LHRH A - Mont 206	DD	15.4	BH/AC	14.7 24.6 / 9.7
7. LHRH B - DEAE	KL	7.0	AC	8.9
8. LHRH C - DEAE	KL	0.5	AC	11.8
Non-vaccinated Controls	DD/KL	12.0 15.1 / 8.0	BH/AC	11.2

*Farms are identified by the initials of the owners. Where groups were made up of deer from more than one farm an average is given for each farm (Red deer controls were not separated because only three animals were in the total group).

The experiment was conducted in August - November at a time when pastures were generally poor and all the deer in the experiment were receiving supplement grain feed. With a few exceptions most groups of deer gained more than 10% of weight. The deer at KL farm were the main exception and did not gain weight for the first 6 weeks of the experiment. This was primarily due to the deer being agisted on a poorer and unfamiliar pasture for easier handling throughout the experiment. The deer were later shifted (Week 6) and a weight increase was seen at the next set of measurements. The red deer in group 7 (being from AC farm) also were slightly poorer performers. AC deer however were poorer in weight gain overall when compared to the deer at BH.

The results in Table 6 indicate that for most treatment groups vaccinated deer showed similar weight gain to the non-vaccinated controls.

Conclusion

The aim of this experiment was to identify a conjugate formulation and adjuvant which would stimulate the best immune response in deer with minimal site reactions. To this end three different conjugates and 5 adjuvants were tested. The five adjuvants were all assessed with the LHRH conjugate (LHRH A). This conjugate had proven to induce strong immune responses in other species in previous work and was therefore expected to be immunogenic in deer. It was assumed that if a particular adjuvant produced a particularly strong immune response (when used in conjunction with the LHRH A conjugate) it could also be expected to be immunogenic when used against another antigen of choice. Thus the experimental protocol tested the adjuvants against one conjugate only (LHRH A).

Similarly it was assumed that if a antigen produced a strong immune response using DEAE as adjuvant, the same antigen would also be likely to be immunogenic when combined with another adjuvant. Thus the experimental protocol tested each conjugate against one adjuvant only (DEAE).

Site reactions were minimal in Fallow deer and are unlikely to be a problem for this breed. 20% of Red deer suffered some form of site reaction across two injections. The incidence of site reaction was independent on the type of adjuvant or conjugate.

Red deer have longer hair, are known to wallow in mud and tend as a result to often be quite dirty. The wallowing behaviour is not observed in Fallow deer. It is possible that the increase in site reactions may have been due to infections resulting from the dirtier condition of the deer.

Similar results were observed in the preliminary experiment carried out in the Murray Valley Deer in 1993. In this experiment 7 Fallow and 10 Red deer were vaccinated with a LHRH/DEAE formulation not tested in this survey. Eight red deer showed some reaction to the injection whilst only two fallow deer had site reactions. In order to reduce the high numbers of site reactions for this current experiment, the vaccination sites in the red deer were shaved and swabbed with antiseptic before injection. Whilst the incidence of site reactions in Red deer was reduced from the preliminary experiment (where 80% of deer had site reactions), 20% of the Reds still showed a reaction to the vaccination, 10% presented as large scars being the result of burst abscesses. Although in general the farmers were not concerned about the size or quantity of site reactions, this result may be high when it is considered that in practice farmers/veterinarians will be unlikely to shave and swab the area before injection.

The anti-LHRH titres indicated that none of the five different adjuvants used in combination with the LHRH A conjugate elicited a strong immune response. This result was unexpected since DEAE had, for example, been used as the adjuvant of choice in other deer immunocastration work (Ataja *et al* 1992, Freudenberger *et al* 1991, 1993). Montanide 50 and Montanide 206 were recommended by the manufacturer as adjuvants particularly suitable for use in deer. Whilst Group 1 (LHRH A-DEAE) was the best of the adjuvants producing a mean titre of 775 in red deer, the titres rapidly decreased within the next 2 weeks and did not produce a particularly strong response in the fallow.

The PMMA nanoparticles were particularly non-immunogenic. This adjuvant was tested since it was reported to be the adjuvant of choice out of a total of 24 adjuvants (Stieneker *et al*, 1995). In that study however inactivated HIV-2 split whole virus was used as the antigen.

This type of antigen is significantly larger and different in nature to the small molecular weight immunocastration antigen (LHRH, molecular weight approx 80 000). In preparing the vaccine the LHRH conjugate was poorly adsorbed onto the nanoparticles and a larger volume was injected into each deer to help compensate for the low amount of adsorbed (and thus immunogenic) antigen. Despite the larger volumes all deer vaccinated with this treatment group gave no indication of the presence of LHRH antibodies. Titres were equivalent to the non-vaccinated controls. It is assumed that very little of the conjugate successfully adsorbed to the nanoparticles and this adjuvant is not suitable for "small" molecular weight antigens.

Of the three different conjugates tested Group 7 LHRH B-DEAE was the most immunogenic with titres remaining above 400 for up to 4 weeks following the booster vaccination and as such was selected as the formulation of choice for use in deer. The choice of DEAE-dextran is further supported by the fact that it has been the adjuvant of choice in other deer immunocastration work (Ataja *et al* 1992, Freudenberger *et al* 1991, 1993).

Whilst it is recognised that anti-LHRH titres are not fully indicative of the true efficacy of the vaccine in terms of reducing sexual behaviour, higher titres lasting for a longer period of time suggest that the vaccine may remain efficacious for a longer period. Maximising the duration of vaccine efficacy is of prime importance if the rut is to be successfully suppressed in stags for the full duration of the breeding season.

Experiment 2: Effects Of Dose Of Vaccine On The Immune Response

Aim:

The aim of this experiment was to determine the minimum dose of vaccine which will produce a strong immune response. The results observed in the first experiment indicated that anti-LHRH titres did not remain high beyond 8 weeks after the booster vaccination. An increase in dose may produce a higher or longer sustained titre. It is also possible however that the titre is independent of the dose used and a lower dose may be equally effective. An investigation of dose response will determine the most optimal dose for producing an effective vaccine.

Materials And Methods

Vaccine formulation LHRH B in DEAE-DEXTRAN

An alternative LHRH antigen was prepared at VIAS for the dose response experiment (Batch JS-32) An earlier prepared batch of conjugate tested in the first experiment was used as a positive control (Batch no JS-27).

The following vaccine doses were prepared in DEAE-dextran as follows:
Treatment 1 100% Dose Positive Control, Batch JS-27,
Treatment 2 100% Dose, Batch JS-32,
Treatment 3 200% Dose, Batch JS-32,
Treatment 4 50% Dose, Batch JS-32,
Dose amounts are in terms of the amount of active conjugate added.

Animals

32 Male fallow deer were used, ranging in age from 1-2 year olds. The deer were supplied from and remained agisted in a farm in North Central Victoria. Each dose, except for the positive controls were tested in 8 Fallow deer; positive control in 4 deer.

Vaccination Regime

Each vaccinated deer was given a primary vaccination at week 0 (1st November 1995) and then a second vaccination at week 4 (29th November 1995). For each dose, the animals received 2 mL of vaccine as a single subcutaneous injection administered at the back of the neck just behind the ear, using a 21 gauge needle and syringe. In order to avoid unwanted site reactions, the site for injection was swabbed with 70% hibitane immediately before vaccination.

Sample collection

Blood samples and weights were collected at week 0 and each fortnight thereafter for a total of 9 weeks (i.e. 4 weeks after the 2nd dose). Blood samples were collected from the jugular vein using 21 Gauge vaccutainer needles. Weights were measured using weigh boxes and scales provided by the farmers. A summary of vaccination and sample collection schedules is given in Table 7.

The site of vaccination was also assessed for swelling or abscess and a score was recorded. Site reactions were scored as either 1, 2 or 3 and given a comment and approximate size in centimetres for the actual diameter. A score of "1" was given if the site of injection was slightly swollen and could only be detected by touch. A score of "2" was given if a lump was visible and a score of "3" was given if the reaction had burst open.

TABLE 7: Summary of blood sample collection and vaccination schedules.

DATE	WEEK No.	BLEED No.	VACCINATION
1 November 1995	0	1	Initial
15 November	2	2	
29 November	4	3	Booster
12 December	6	4	
3 January 1996	9	5	

Analyses

Sera collected from the blood samples were analysed for testosterone and anti-LHRH antibody titres. The testosterone analysis was performed using a commercially supplied direct Radioimmunoassay kit (Pantex ^{125}I). The method was modified to include an extraction step whereby serum testosterone was extracted into 90%diethyl ether/10%ethyl acetate. The intra-assay coefficient of variation was <10%. The inter assay coefficients of variation were 12.1, 11.3, 10.0% as calculated from from low, median and high pathological standards measured over 28 assays. The assay had a minimum detection limit of 0.5 nM

The anti-LHRH analysis was performed using a direct binding radioimmunoassay whereby doubling dilutions of serum ranging from 1:100 to 1:1600 were incubated with ^{125}I labelled LHRH (supplied by AMRAD (250 μCi) diluted with 0.1M phosphate/saline buffer pH 7.4 until cpm = 7000-7500). Each dilution was incubated at 4°C for 48 hours in a final volume of 400μL 0.1M Na phosphate buffer pH 7.40 containing 1.25 mg/mL human gamma globulin. Following incubation 100μL 1%bovine gamma globulin solution was added. The free LHRH tracer was separated from bound by addition of 1mL 18% polyethylene glycol 6000. The resulting precipitate was separated after centrifugation (3000rpm, 10 minutes) then counted on a Wallace 1410 scintillation counter. Intra-assay variability was determined from measuring three different pig samples up to 9 times in one assay and was found to be 7.0, 3.3 and 4.8% respectively. Inter-assay coefficient of variation was 11.9 and 10.1% calculated from measuring control serum collected from an immunocastrated pig and deer over 55 assays. The titres are expressed at the dilution of serum capable of binding 33% labelled LHRH.

The minimal detectable concentrations as it applies to other analytes is not applicable for our purpose. Any test sera where there is clearly no antisera are reported as having a titre <20.

For an animal to be effectively immunocastrated by the LHRH antibodies it needs a titre of at least 400. We interpret any titre of <400 as indication of complete suppression of LHRH.

Results & Discussion

Site Reactions

All the deer were examined two weeks after each vaccination for any reaction at the site of injection. No site reactions were observed in any of the deer.

Anti LHRH Titre

Anti-LHRH serum titres were measured two and five weeks after the booster vaccination and results are outlined in Table 8.

TABLE 8: Median anti-LHRH titres in fallow for each treatment group .

GROUP	Bleed 4 12/12/96 2 weeks after 2nd dose	Bleed 5 3/1/96 4 weeks after 2nd dose
Non-vaccinated controls	0	0
Positive control (100%, JS-27)	1108	362
100%, JS-32	1325	354
200%, JS-32	620	169
50%, JS-32	696	143

Titre results for bleed 4 indicate that at the 100% dose the new batch of vaccine (JS-32) had equal efficacy to the previously proven batch (JS-27, tested in experiment 1). The similar titres indicate that the batches of vaccine can be prepared with consistent immunogenic properties.

The median titre for 200% dose was approximately half that seen for 100% dose although it was expected that titres would increase or at least remain similarly high when the dose was increased.

The median titre for 50% dose was approximately half that seen for 100% dose. This result was not unexpected and indicated that the dose of the vaccine could not be decreased without seeing a deterioratioin in resultant titres.

Testosterone

Testosterone concentrations were measured for bleeds 1 and 2 (1st and 15 November) to use as pre-immunisation indicators and are summarised in Table 9. The testosterone concentrations for most samples were below the minimal detectable level of the assay, i.e. below 0.5 nM. The low results are consistent with testosterone concentrations observed in the non-breeding season (Freudenberger *et al*, 1993). Thus testosterone could not be used as an indicator for vaccine efficacy since low or decreasing concentrations could not be attributed to the vaccine over the seasonal effects.

TABLE 9: Average serum testosterone concentrations (+ standard deviation) (nM) for each treatment group.

GROUP	Bleed 1 1/11/96 first dose,	Bleed 2 15/11/96 2 weeks after 1st dose
Non-vaccinated controls n=4	<0.5 (0.09)	<0.5 (0.09)
Positive control (100%, JS-27) n=4	2.2 (4.2)	<0.5 (0.09)
100%, JS-32 n=8	<0.5 (0.3)	<0.5 (0.1)
200%, JS-32 n=8	<0.5 (0)	<0.5 (0)
50%, JS-32 n=8	0.6 (0.1)	<0.5 (0.1)

n= number of animals in each group

Weight

The weights of the deer were monitored for the duration of the experiment to determine whether the vaccine formulation produced an increased or decreased weight gain compared with the controls. Table 10 outlines the average weight gained over the first 8 weeks of the experiment (i.e. until 4 weeks post booster vaccination, 1st November-3rd January) in the vaccinated deer and controls. An analysis of variance indicated that there was no significant difference between groups (P>0.05), thus indicating that vaccination had no effect on weight gain during the experiment.

TABLE 10: Average weights, (+standard deviation) (Kg) and weight gain over the first 8 weeks (November-January) experiment for each treatment group.

GROUP	Bleed 1 (1 Nov 95)	Bleed 5 (3 Jan 96)	Av Weight gain
Non-vaccinated controls n=4	36.6 (1.9)	46.6 (3.3)	10.0
Positive cont (100%, JS-27) n=4	36.8 (2.9)	47.0 (3.4)	10.2
100%, JS-32 n=8	41.0 (4.1)	49.9 (5.9)	8.9
200%, JS-32 n=8	37.1 (3.7)	45.6 (5.6)	8.6
50%, JS-32 n=8	36.3 (4.4)	45.9 (5.2)	9.6

n=number of animals in each group

Conclusion

The aim of this experiment was to determine the lowest dose of vaccine which would produce a strong immune response as measured by anti-LHRH titre.

As indicated in the results, the dose of vaccine administered had no effect of weight gain. This was expected since the experiment was conducted in the non-breeding season when all deer are feeding well and food uptake is not altered by sexual breeding behaviour.

The absence of any site reactions in the fallow deer support the findings of the first experiment that the immunocastration vaccine does not produce infections or swellings at the site of reaction in the fallow deer. Since no red deer were tested in this experiment no further indication of the incidence of site reaction in reds can be provided at this point.

The anti-LHRH titre results indicated that the optimal dose was the 100% dose. Lower titres were observed at both the 50% and 200% scales. This outcome suggests that the titre verses dose curve is bell-shaped with the optimum being at the 100% mark. It is not possible however to conclusively draw this conclusion due to the small range of different doses tested. This situation was unavoidable due to the small amount of peptide available for preparation of the immunocastration conjugate.

It is was observed that the titres were seen to rapidly decrease and were below 400 within four weeks of administering the booster dose. This raises concerns as to whether a two dose vaccine will be sufficient to reduce sexual behaviour and weight loss for the full duration of the breeding season. It may be necessary to administer a third dose in order to maintain titres and efficacy.

The experiment therefore has provided no evidence that increasing or decreasing the dose from the dose of Experiment 1 will improve the immune response as measured by anti-LHRH titre. Thus the dose to be used in the third experiment (major behavioural experiment) will be remain unchanged.

Experiment 3: Efficacy Of Vaccine In Controlling Sexual Behaviour And Weight Loss During The Rut

Aim:

The aim of this experiment was to examine the efficacy of the vaccine to control sexual behaviour and weight loss during the rut. Experiments 1 and 2 had determined the optimal formulation and dose for use in the deer. We wished to examine the effect of the vaccine when administered during the rut, how long it remained efficacious and whether use of the immunocastration vaccine could improve weight condition, reduce aggressive behaviour and improve carcass quality when animals were subsequently slaughtered during the rut.

a)

b) Materials And Methods

Vaccine formulation *LHRH-"B" conjugate in DEAE-DEXTRAN*

An LHRH conjugate was prepared at VIAS for the efficacy experiment (Batch JS-34). The freshly made conjugate solution was aliquoted into four lots in a sterile glass then stored at -20°C until required for use. When required, each batch of vaccine dose was prepared in DEAE-dextran immediately before use then administered 2mL/dose.

Animals

Fallow Deer: 50 Male fallow deer were used, ranging in age from Rising 2-6 year olds. The deer were supplied from and remained agisted in a farm in North Central Victoria (Healesville). 40 Deer were vaccinated with the immunocastration vaccine. 10 were non-vaccinated controls. Of the 40 vaccinated deer, 26 were 2 year old at the beginning of the experiment; the remaining 14 were aged 3 years or more. The controls were randomly selected from the herd on the first vaccination day. Nine of the ten deer were 2 years old; the remaining 1 deer was aged 3 years. The deer were aged by the farmer by inspection.

Red Deer: 31 Male red deer were used, all Rising 2 years of age. The deer were supplied from and remained agisted in a farm in South Central Victoria (Neerim South). 21 Deer were vaccinated with the immunocastration vaccine. The remaining 10 were non-vaccinated controls. The controls were randomly selected from the herd on the first vaccination day.

Administration of copper supplement injections to Red deer

At the first and second farm visits, one red deer (62) was identified as being unsteady on its feet. Following veterinary consultation and continuing deterioration of deer 62, blood samples from the test herd were forwarded for copper analysis. Results indicated that the herd was suffering copper deficiency. At week 8, (Bleed 5) all the red deer were given a subcutaneous injection of "Young's Copper Injection", containing 60 mg/mL copper as the glycinate. Each animal was given a single 0.5 - 1 mL dose.

No other husbandry was required for either the fallow or red deer.

Vaccination Regime

Fallow Deer: Each vaccinated fallow deer was given a primary vaccination at week 0 (17th January 1996) and then a second vaccination at week 4 (14th February 1996). At week 6, four vaccinated deer were actively aggressive within the herd and were removed from the experiment in order to avoid the risk of harm to the other animals since it was apparent they had not responded to the vaccine. One other deer was also removed from the experiment due to suffering a broken leg in the field. In order to maintain the efficacy of the vaccine, the remaining 35 fallow deer received a third then fourth doses at week 10 (27 March 1996) and week 15 (1 May 1996). A total of 150 injections were administered to the herd.

Red Deer: Each vaccinated red deer was given a primary vaccination at week 0 (19th January 1996) and then a second vaccination at week 4 (16th February 1996). The red deer received a third dose only at week 10 (29 March 1996). One deer received only the primary vaccination before dying due to a snake bite. A second deer received only two injections after dying as a result of copper deficiency. Thus a total of 60 injections were administered to the herd.

For each dose, both fallow and red deer received 2 mL of vaccine as a single subcutaneous injection administered at the back of the neck just behind the ear, using a 21 gauge needle and syringe. In order to avoid unwanted site reactions, the site for injection was swabbed with 70% hibitane immediately before vaccination.

Sample collection

Blood samples were collected at week 0 and every two-three weeks thereafter for a total of 27 weeks. Blood samples were collected from the jugular vein using 21 Gauge vaccutainer needles. A summary of vaccination and sample collection schedules is given in Table 11.

The site of vaccination was also assessed for swelling or abscess and a score was recorded. Site reactions were scored as either 1, 2 or 3 and given a comment and approximate size in centimetres for the actual diameter. A score of “1” was given if the site of injection was slightly swollen and could only be detected by touch. A score of “2” was given if a lump was visible and a score of “3” was given if the reaction had burst open.

TABLE 11: Summary of blood sample collection and vaccination schedules.

DATE*	WEEK No.	BLEED No.	VACCINATION
17 & 19th January	0	1	1
31st January, 1st Feb	2	2	
14 & 16th February	4	3	2
28 & 29th February	6	4	
13 &14th March	8	5	
27 & 29th March	10	6	3
17 & 18th April	13	7	
1st & 3rd May	15	8	4
22nd & 23rd May	18	9	
12 &13th June	21	10	
3rd & 5th July	24	11	
29th & 26 July	27	12	

Dates indicate the days that Fallow and Red deer farms were visited respectively

Weight Determination

Fallow Deer: The weight of each fallow deer was taken each time the animal was yarded. Thus the weights of the vaccinated animals were measured at each farm visit, every 2-3 weeks for the duration of the experiment. The weights of the non-vaccinated control deer were measured at the beginning of the experiment and at least once again during the rut.

Red Deer: The weight of each red vaccinated deer was taken every 4-6 weeks. The non-vaccinated controls were weighed at Bleed 1 and Bleed 4 then not again until weeks 21 and 27 (Bleeds 10 & 12).

For both red and fallow deer the weights were measured using weigh boxes and scales provided by the farmers.

Testes Measurement

Fallow Deer: The testes of vaccinated and control fallow deer were measured at Bleeds 3, 4, 5, and 9. The testes of the vaccinated deer was also measured one more time at Bleed 11. The measurement of testes at other visits was omitted due to the deer behaving aggressively within the yards. On these occasions prolonged handling of each individual animal posed a risk to the remainder of the herd held in the holding pens. The width (taken at the widest section) and length (excluding the epididimus) of each testes were measured using a micrometer.

Red Deer: The testes of each vaccinated red deer was measured every 2-3 weeks. The non-vaccinated controls were measured each time they were yarded throughout the experiment. Testes measurement was taken by comparison with an orchidometer set.

Analyses

Sera collected from the blood samples were analysed for testosterone and anti-LHRH antibody titres. The testosterone analysis was performed using a commercially supplied direct Radioimmunoassay kit (Pantex ^{125}I). The method was modified to include an extraction step whereby serum testosterone was extracted into 90%diethyl ether/10%ethyl acetate. The intra-assay coefficient of variation was <10%. The inter assay coefficients of variation were 12.1, 11.3, 10.0% as calculated from low, median and high pathological standards measured over 28 assays. The assay had a minimum detection limit of 0.5 nM

The anti-LHRH analysis was performed using a direct binding radioimmunoassay whereby doubling dilutions of serum ranging from 1:100 to 1:1600 were incubated with ^{125}I labelled LHRH (supplied by AMRAD (250 μCi) diluted with 0.1M phosphate/saline buffer pH 7.4 until cpm = 7000-7500). Each dilution was incubated at 4°C for 48 hours in a final volume of 400μL 0.1M Na phosphate buffer pH 7.40 containing 1.25 mg/mL human gamma globulin. Following incubation 100μL 1%bovine gamma globulin solution was added. The free LHRH tracer was separated from bound by addition of 1mL 18% polyethylene glycol 6000. The resulting precipitate was separated after centrifugation (3000rpm, 10 minutes) then counted on a Wallace 1410 scintillation counter. Intra-assay variability was determined from measuring three different pig samples up to 9 times in one assay and was found to be 7.0, 3.3 and 4.8% respectively. Inter-assay coefficient of variation was 11.9 and 10.1% calculated from measuring control serum collected from an immunocastrated pig and deer over 55 assays. The titres are expressed at the dilution of serum capable of binding 33% labelled LHRH.

Behaviour

Fallow Deer: Behaviour of the Fallow deer was monitored by visual observation of the animals when yarding and handling. Fallow deer were videoed at week 8 (fours weeks following the first booster vaccination). The deer were filmed being yarded and held in the outside pens of the farmer's handling shed. At weeks 24 and 27 the fallow deer were introduced into the interior holding rooms in groups of 6-10. The groups were visually observed in the interior pens by an observer sitting on the top of a dividing wall. Each deer was identified as being either actively participating in aggressive fighting behaviour or remaining passive, before being removed from the group and identified by its ear tag.

Red Deer Visual observation of the red deer was severely limited due to the enclosed nature of the handling yard. Behaviour was more readily observed by the farmer in regards to the ease of being able to lead each deer into the crush.

Slaughter of Fallow Deer

At week 27 the fallow deer were bled, weighed and all passive, non-fighting deer (18 from the herd of 35 animals) were separated for slaughter. All remaining deer were returned to pasture. The eighteen deer were taken to Casticum Brothers, Dandenong, the following day and slaughtered under commercial conditions. Slaughter weights and testes were collected from each deer. The slaughter weights from 16 castrates that were included in the stock for slaughter were also collected for comparison. The castrates were part of the farmer's normal farm stock and had been agisted on the same farm for at least the duration of the experiment. The date of castration is unknown. Testes collected from the slaughter were preserved in formalin and forwarded for histological examination.

Results

Site Reactions

Both red and fallow deer were examined at each farm visit for any reaction at the site of injection.

Fallow Deer: Incidence of site reactions were undetected in the Fallow deer for the first or second injections. Two site reactions in the form of burst abscesses were observed two weeks after the third injection and one further site reaction observed following the fourth injection (See Table 12). Thus out of a total of 150 injections administered to the fallow deer, three site reactions were observed; representing 2% of the injections. All three abscesses were approximately 2x4 cm in size, and healed into hard scabs within two weeks after the injection.

TABLE 12: Number of Site Reactions observed in Fallow Deer at each farm visit.

DATE	BLEED NO	Number Site Reactions Score 1*	Number Site Reactions Score 2*	Number Site Reactions Score 3*
17 January	1 V1	0	0	0
31st January,	2	0	0	0
14th February	3 V2	0	0	0
28th February	4	0	0	0
13th March	5	0	0	0
27th March	6 V3	0	0	0
17th April	7	0	0	2
1st May	8 V4	0	0	2
22nd May	9	0	0	3
12th June	10			
3rd July	11			
29th July	12			

*A score of "1" was given if the site of injection was slightly swollen and could only be detected by touch. A score of "2" was a visible lump. A score of "3" was a burst abscess.

Red Deer: Incidence of site reactions was more common in the red deer compared to the Fallow Deer. The site reactivity results for each individual red deer is illustrated in Table 13. Two weeks after the first injection, 7 site reactions, of varying severity, were detected. Within a further two weeks most of the unburst abscesses could not be detected, and the one burst abscess had healed into a hard scab. Two weeks after the second injection, a further 7 site reactions were detected. Finally two more site reactions were detected after the third injection. Site reactions varied from being small unburst abscesses detectable only by palpitation to burst abscesses healing into scabs up to 8x8 cm in size. In general most burst site reactions had healed within 6-10 weeks. Thus out of a total of 60 injections administered to the red deer herd 16 site reactions were detected, representing 26% of the injections.

Site reactions were also detected as a result of copper supplement injections administered at Week 8 (Bleed 5). The injections were administered after the herd was determined to be suffering copper deficiency. Each deer was administered a single 0.5mL subcutaneous injection on the rear back near the rump. By four weeks after the injections 17 out of the 19

deer (89%) had developed site reactions to the copper injection. Of these 4 presented with unhealed scabs which were bleeding or containing pus. The site reactions were treated with antiseptic spray and healed within 4-6 weeks.

TABLE 13: Site reaction size and score observed in red deer due to the Immunocastration vaccine.

DEER ID	BLD 1 V1	BLD 2	BLD 3 V2	BLD 4	BLD 6 V3	BLD 7	BLD 11
50		0	0	0		0	0
53		0	0	0		0	0
59		0	0	0		0	0
61		3x3cm 2	3x3cm 2	3x3cm 3		0	0
62		0	0	Dead	Dead	Dead	Dead
162		0	0	0		0	0
63		5x5cm 2	5x5cm 2	2x2cm 2		2x2cm 2	0
64		0	0	0		0	0
67		0	0	0		0	0
69		0	0	Dead	Dead	Dead	Dead
70		0	0	1x1cm 2		0	0
75		5x5cm 2	0	2x3cm 2		0	0
76		0	0	0		0	0
91		0	0	0		0	0
93		0	0	0		0	0
94		0	0	0		0	0
99		Faint 1	0	2x2cm 3		2x2cm 3	0
191		5x5cm 1	0	3x4cm 2		0	0
198		0	0	5x5cm 3		2x2 cm 3,3	2x2 cm 3,3
199		5x5cm 2	Faint 1	8x4cm 3		2x2 cm 3,3	0
Or94		3X3cm 3	3X3cm 3	1x2cm 3		0	0

*A score of “1” was given if the site of injection was slightly swollen and could only be detected by touch. A score of “2” was a visible lump. A score of “3” was a burst abscess.

Anti LHRH Titre

Fallow Deer

The range of anti-LHRH titres for each bleed and the median value is given in Table 14. For both groups of vaccinates, the median titres were consistently lower than that observed in earlier experiment. Titres rapidly decreased and were below 400 within 4-6 weeks of each boost. (A median titre of 400 has been observed in other species as the minimum titre to have a likely biological effect.) The 2 yr old vaccinates were widely ranging in the observed LHRH titres indicating that the immune response was variable with each individual. The non-vaccinated controls had no measurable anti-LHRH titres as expected. Median titres are also indicated in Figure 1 against the percentage of samples with testosterone concentrations <2nM. The graph indicates for both groups of vaccinates, the bleeds where median titre was high correlated to when testosterone concentration was low within the group.

TABLE 14: Median anti-LHRH Titres (and range) measured in Fallow Deer at each farm visit.

DATE	BLEED NO	Vaccinates Age 2 yrs nM	Vaccinates Age 3+ nM	Non-vaccinated controls nM
17 January	1 V1			
31st January,	2			
14th February	3 V2			
28th February	4	770 (363-6005)	574 (374-2011)	<20
13th March	5	327 (134-1534)	255 (141-520)	<20
27th March	6 V3	111 (<20-770)	84 (<20-214)	
17th April	7	627 (209-2035)	480 (84-784)	
1st May	8 V4	400 (<20-980)	116 (276-<20)	
22nd May	9	1261 (390->3500)	499 (152-1270)	<20
12th June	10	565 (117-2981)	187 (<20-539)	
3rd July	11	243 (<20-847)	42.5 (<20-184)	
29th July	12	89 (<20-338)	63 (<20-130)	

Red Deer

The range of anti-LHRH titres for each bleed and the median value is given in Table 15. As seen in previous experiments, the median titres were consistently higher than that observed for the fallow deer. The higher titres (and maintenance of lower testosterone concentrations) resulted in three rather than four doses of vaccine being administered to the Red deer. Despite the higher titres the immune response had dropped by the eighth week following vaccination with median titres now below 400. Titre continued to drop thereafter reaching a median base level 4 months after the 3rd vaccination. The non-vaccinated controls had no measurable anti-LHRH titres as expected. Median titres are also indicated in Figure 2 against the percentage of samples with testosterone concentrations <2nM. The graph indicates that, the bleeds where median titre was high correlated to when testosterone concentration was low within the group.

TABLE 15: Median anti LHRH Titres (and range) measured in Red Deer at each farm visit.

DATE	BLEED NO	Vaccinates (reciprocal diln)	Non-vaccinated controls
19th January	1 V1		
1st Feb	2		
16th February	3 V2		
29th February	4	1658 (48->3500)	<20
14th March	5	984 (298->3500)	
29th March	6 V3	732 (130-3218)	
18th April	7	975 (215->3500)	
3rd May	8 V4	525 (139->3500)	
23rd May	9	259 (51-2338)	
13th June	10	177 (<20-1417)	
5th July	11	100 (<20-852)	
26 July	12	<20 (<20-574)	<20

Testosterone

Fallow Deer Testosterone concentrations were monitored as the primary indicator of vaccine efficacy and duration of effect. The mean (plus standard deviation) for each group at each farm visit is given in Table 16. The results are also illustrated in Figure 1 as the percentage of deer with testosterone concentrations below 2 nM, that is indicating inactive testes.

Within two weeks of receiving the booster vaccinations 92% of the two year olds had testosterone concentrations below 2 nM. In comparison 40% of the controls had testosterone concentrations < 2nM (suggesting that not all deer were actually into the breeding season at this stage). Within a further two weeks however, the testosterone concentrations in the vaccinated 2 yr olds had risen dramatically with only 40% of deer having testosterone < 2nM. At this stage 100% of the controls had testosterone > 2nM as expected for the breeding season. The marked rise in testosterone concentration in the vaccinated group, suggested that the vaccine did not hold efficacy beyond 2-3 weeks and deer were moving into rut. Thus a third and fourth dose was given. On each occasion, testosterone concentrations fell within 2 weeks of the booster, but began to rise as a group by the fourth week. Standard deviations indicate there was a varied response between individuals with testosterone concentrations ranging for example from <0.5 nM to 32 nM (Bleed 5).

TABLE 16: Mean Testosterone Concentration (+standard deviation) measured in Fallow Deer at each farm visit.

DATE	BLEED NO	Vaccinates Age 2 yrs nM	Vaccinates Age 3+ nM	Non-vaccinated controls (nM)
17 January	1 V1	2.5 (2.2)	2.2 (2.4)	0.9 (0.4)
31st January,	2	3.0 (2.6)	4.3 (4.0)	2.5 (2.4)
14th February	3 V2	2.2 (2.8)	2.8 (4.3)	9.6 (9.1)
28th February	4	0.9 (0.95)	3.3 (4.0)	6.6 (8.5)
13th March	5	6.3 (7.9)	16.9 (13.2)	18.3 (6.9)
27th March	6 V3	6.1 (9.1)	16.5 (14.0)	NT
17th April	7	2.6 (4.1)	18.3 (14.0)	NT
1st May	8 V4	4.2 (9.8)	10.5 (8.7)	NT
22nd May	9	0.7 (0.8)	3.2 (4.7)	1.7 (1.4)
12th June	10	0.7 (1.2)	3.5 (6.3)	NT
3rd July	11	3.5 (6.4)	5.1 (5.9)	NT
29th July	12	1.7 (2.4)	2.1 (2.0)	NT

Red Deer The mean testosterone concentrations (plus standard deviation) for the vaccinated and non-vaccinated controls at each farm visit are given in Table 17. The results are also

illustrated in Figure 2 as the percentage of deer with testosterone concentrations below 2 nM, that is indicating inactive testes.

Within two weeks of receiving the booster vaccination 80% of the vaccinated deer had testosterone concentrations below 2 nM, in comparison to the controls whereby 100% of deer had testosterone concentrations > 2nM suggesting that the controls had entered into the rut as expected. Within a further two weeks, 79% of the vaccinated deer still had testosterone < 2nM, this result dropping to 52% by the sixth week after the booster vaccination. It was therefore suggestive the vaccine had a longer duration of effect in the red deer in comparison to the fallow. As a result the red deer were only given a third dose to maintain vaccine efficacy. Two weeks after the third dose 84% of deer had testosterone < 2nM. Within 6 weeks after vaccination this percentage had dropped to 36%. Standard deviations indicated there was a varied response between individuals with testosterone concentrations ranging for example from 0.8 nM to 16.7 nM (Bleed 3).

TABLE 17: Mean Testosterone Concentration (+standard deviation) measured in Red Deer at each farm visit.

DATE	BLEED NO	Vaccinates nM	Non-vaccinated controls
19th January	1 V1	2.5 (2.2)	2.2 (2.4)
1st Feb	2	3.0 (2.6)	4.3 (4.0)
16th February	3 V2	2.2 (2.8)	2.8 (4.3)
29th February	4	0.9 (0.95)	3.3 (4.0)
14th March	5	6.3 (7.9)	16.9 (13.2)
29th March	6 V3	6.1 (9.1)	16.5 (14.0)
18th April	7	2.6 (4.1)	18.3 (14.0)
3rd May	8 V4	4.2 (9.8)	10.5 (8.7)
23rd May	9	0.7 (0.8)	3.2 (4.7)
13th June	10	0.7 (1.2)	3.5 (6.3)
5th July	11	3.5 (6.4)	5.1 (5.9)
26 July	12	1.7 (2.4)	2.1 (2.0)

Figure 1: Percentage of fallow deer with testosterone concentration below 2 nM. (Median LHRH Titres are given for each bleed)

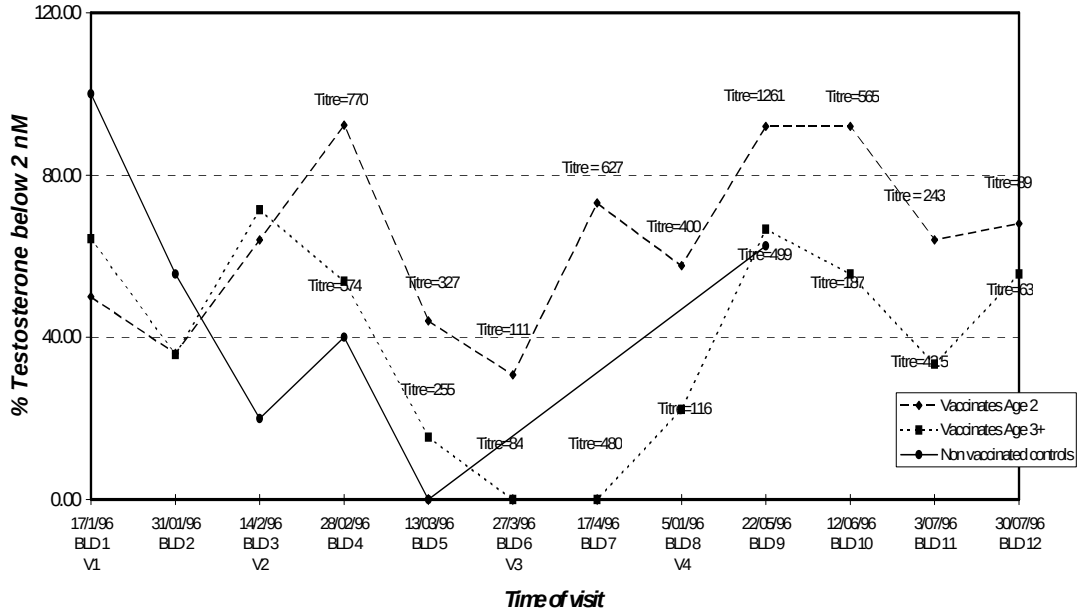
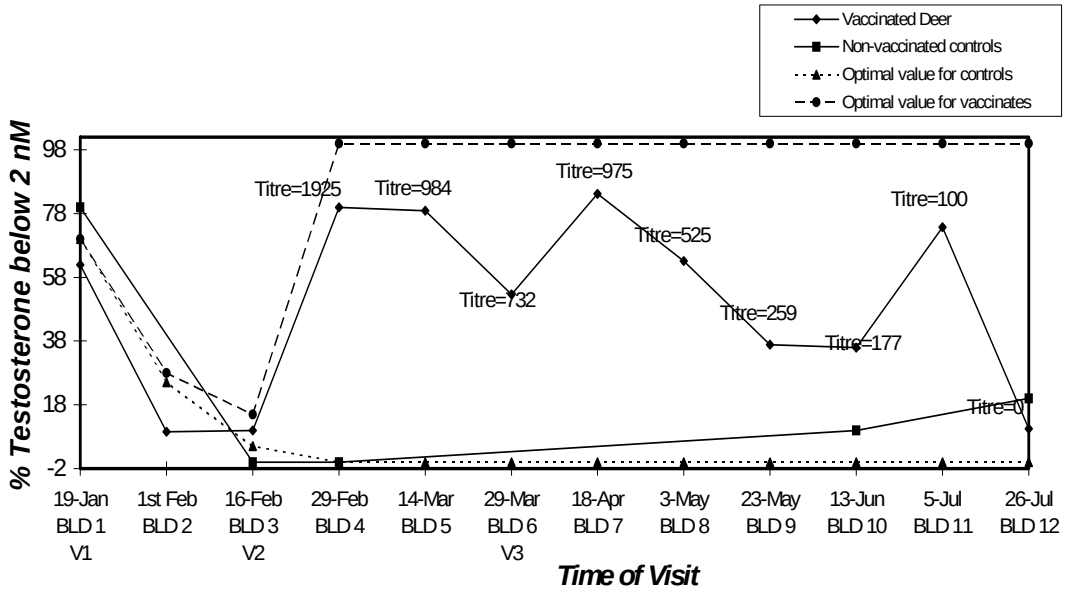


Figure 2: Percentage of Red deer with testosterone concentration below 2 nM. (Median LHRH Titres are given for vaccinated deer)



Testes Measurement

Fallow Deer: The mean length and width of testes measured in the vaccinates (Age 2), vaccinates (Age 3 or more) and non-vaccinated controls are shown in Figure 3. The figure shows that by Bleed 5, four weeks after the second vaccination, the size of testes in the vaccinated deer (Age 2) had significantly reduced in both length and width in comparison to the non-vaccinated controls ($P<0.01$ at Bleed 5 and Bleed 9). The size of the testes in this group remained reduced for the duration of the experiment. In comparison the vaccinated deer aged 3 years or more did not as a group show any evidence of being different to the non-vaccinated controls ($P>0.05$). This may be a reflection of the comparative maturity of these animals. At Bleed 9 16 out of the 35 (46%) vaccinated deer had totally regressed testes; then 12 out of 32 (38%) at Bleed 11.

The testes were collected from seventeen of the vaccinated deer sent to slaughter. The weight of the testes collected varied from 5.7 - 22.8 g, length 30 - 51 cm. Histology examination showed a broad spectrum of degeneration indicating testicular atrophy. Testes were graded from normal to severe testicular atrophy and results are outlined in Table 18. Only one sample was observed as normal by histological examination.

TABLE 18: Scale of testicular atrophy in testes collected at slaughter.

SCALE OF DEGENERATION OF TESTICULAR FUNCTION	NO. OF SAMPLES (SAMPLE ID)
NORMAL. Active spermatogenesis in tubules, all spermatogenic cells present including spermatogonia, spermatocytes, spermatids and sperm.	1 (216)
MILD. A mixture of normal tubules and atropic tubules. Numerous tubules devoid of spermatogenic cells, but many other tubules showing all spermatogenic cells including spermatogonia, spermatocytes, spermatids and sperm	6 (229, 221, 206, 239, 217, 203)
FREQUENT Tubules generally hypocellular. Occasional tubules contain mature spermatozoa. Majority tubules show pyknosis of spermatogonia.	5 (204/227, 225, 208, 238, 207)
SEVERE All tubules devoid of spermatocytes although occasional spermatogonia. No evidence of spermatogenesis, no spermatids or spermatozoa present.	5 (228, 213, 209, 224, 210)

Red Deer: The measurement of testes in the red deer was conducted using a orchidometer since the crush held the deer such that measurement by a micrometer was not possible. The mean volume of testes measured in the vaccinated compared to the controls are shown in Figure 4. The figure indicates (and analysis by single anova supports) that by Bleed 3 (i.e. the time of the second vaccination) the two groups were significantly different ($P<0.01$). By Bleed 4, i.e. two weeks after the second vaccination the average testes sized of the vaccinated deer had further reduced in comparison to the controls ($P<0.01$). The size of the vaccinated group remained low for the remainder of the experiment. The chart also indicates that the average testes size of the control group when measured in July had also decreased, presumably marking the beginning of the non-breeding season for the red deer. By the final visit (Bleed 12) there was no difference between the vaccinates and controls in regards to testes size.

Figure 3: Mean Length and Width of Testes in Fallow Deer throughout Experiment 3.

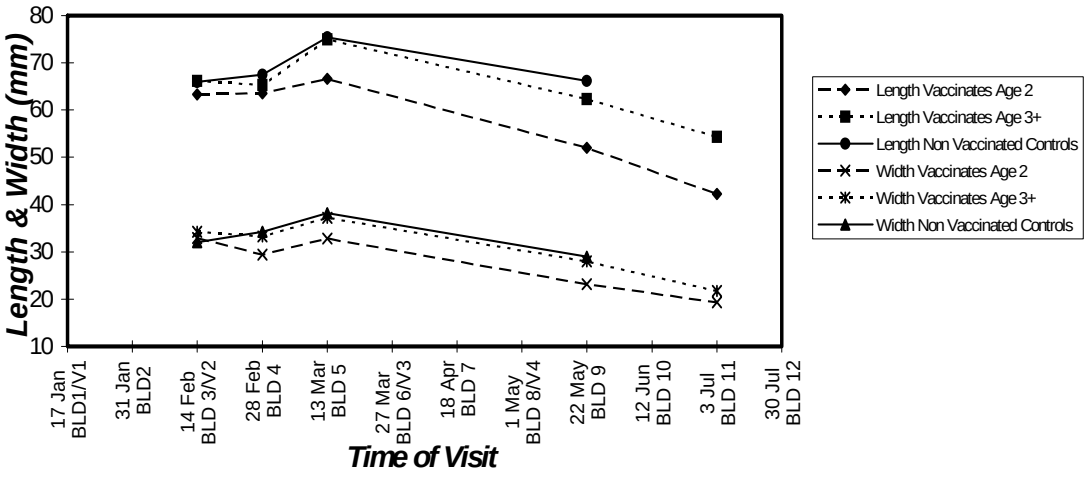
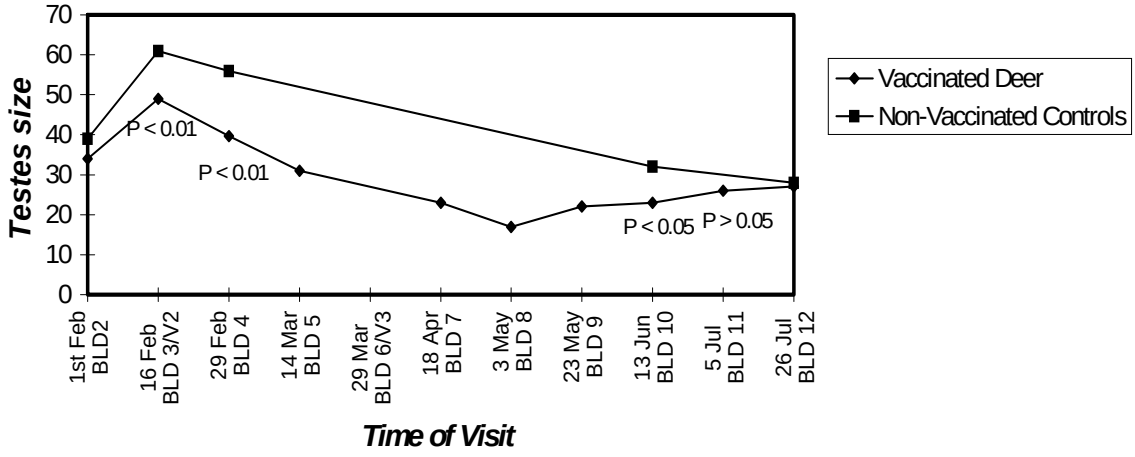


Figure 4: Mean testes size in Red Deer (Orchidometer) throughout experiment 3



Weight gain of Fallow Deer

The weights of the vaccinated fallow deer were monitored for the duration of the experiment. Due to the risk of handling the non-vaccinated control deer during the rut, the controls were weighed each fortnight until Week 8 (13 March) by which time the breeding season was well underway and the deer too aggressive to safely handle. The control deer were weighed once more midway through the rut to provide a possible comparison of weight condition between control and vaccinated deer. The results are outlined in Table 19 and Figure 5.

The group of vaccinated 2yr olds varied spasmodically in weight condition throughout the experiment gaining up to 2.6 Kg from the initial weight measured at the first visit (17 Jan) and losing no more than 1.07 Kg. At week 18 (Bleed 9, 22 May) the deer were 0.5 Kg below their initial weight. The final weight taken at week 27 (Bleed 12, 29th July) was 1.0 Kg above the initial weight. Thus the vaccinated 2 yr old deer as a group maintained their weight condition throughout the experiment within approximately ± 2 Kg.

Comparison between the vaccinated 2yr old deer and controls showed that there was no weight difference between the two groups in the first 8 weeks of the experiment (Bleed 5). The controls were not weighed again until week 18 (Bleed 9, 22 May). At this stage the two groups were significantly different ($P<0.05$), with the controls being 4.6Kg below the vaccinated deer and 4.9 Kg below their initial weight.

The chart also indicates that the vaccinated deer aged 3 years or more, as a group, were 7.4-8.1 Kg heavier than the 2 year old vaccinated deer for the first 8 weeks of the experiment (until Bleed 5). After this time four deer were removed from the experiment since their aggressive behaviour presented serious risk to the other animals in the herd. A fifth deer was also removed due to suffering a broken leg. The difference between the remaining vaccinated deer aged 3 or more years and the 2yr old vaccinates decreased to 3.2-5.3 Kg. This result was most likely due to the loss of heavier animals from the older vaccinated group.

Figure5: Mean Weight in Fallow throughout experiment 3. (Kg)

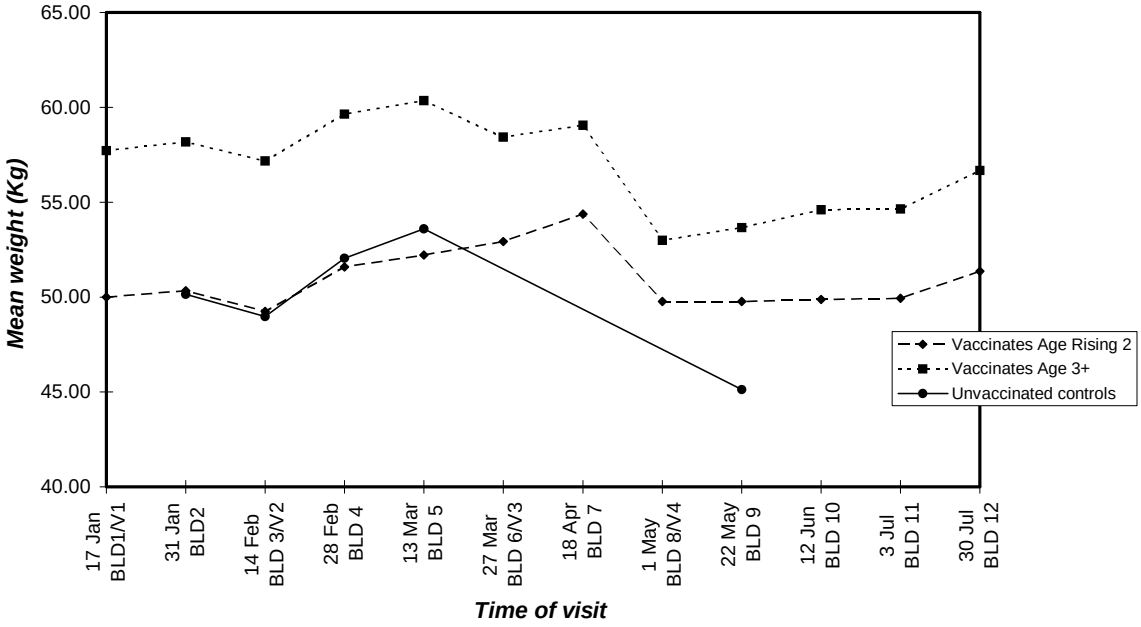


TABLE 19: Average weights (standard deviation) at each farm visit for the "vaccinated Fallow deer aged 2 yrs", "vaccinated Fallow deer aged 3 years or more" and the non-vaccinated controls.

GROUP	Week No	Bleed No.	Vaccinated deer Aged 2 yrs	Vaccinated deer Aged 3 or more yrs	Non-vaccinated controls
17 January	0	1	50.3 (5.1)	57.7 (11.3)	50.0 (8.1)
31st January,	2	2	50.3 (5.5)	58.2 (11.2)	50.1 (7.4)
14th February	4	3	49.2 (4.9)	57.2 (11.1)	49.0 (6.9)
28th February	6	4	51.6 (5.2)	59.6 (11.3)	52.0 (6.5)
13th March	8	5	52.2 (4.8)	60.4 (10.9)	53.6 (6.9)
27th March	10	6	52.9 (5.6)	58.4 (6.2)	NT
17th April	13	7	54.4 (5.1)	59.1 (4.8)	NT
1st May	15	8	49.8 (4.7)	53.0 (4.8)	NT
22nd May	18	9	49.8 (4.7)	53.0 (4.8)	45.1 (4.2)
12th June	21	10	49.9 (4.8)	54.6 (5.8)	NT
3rd July	24	11	49.9 (4.9)	54.6 (5.2)	NT
29th July	27	12	51.4 (5.0)	56.9 (5.4)	NT

Liveweight, carcass weight and dressing out percentages for vaccinated fallow deer.

Eighteen vaccinated fallow deer were sent for slaughter on 1st August 1996. The animals were selected from the herd on the basis of behaviour, i.e. animals which were non-aggressive and quiet. During slaughter, the ear tags of two vaccinated deer were lost and the weight data not collected for those animals. In addition, 15 surgically castrated deer, that had been agisted on the same property were also sent to slaughter on the same day. Table 20 shows the individual and average liveweight, carcass weight and dressing out percentages for the vaccinated and castrated deer. Analysis by single anova indicated that there was no statistically significant difference between the vaccinated deer and castrate group in terms of liveweight, carcass weight or dressing out percentage.

TABLE 20: Liveweight, carcass weight and dressing out percentages for the vaccinated and castrated fallow deer slaughtered 1st August 1996.

Vaccinate ID	Live-weight (Kg)	Carcass weight (Kg)	Dressing out %	Castrate ID	Live-weight (Kg)	Carcass weight (Kg)	Dressing out %
203	51.5	29.4	57.09	R102	58	34.8	60.00
204	51	29	56.86	R153	51.5	23	44.66
207	45.5	25.8	56.70	R155	49	29.8	60.82
208	54.5	29.8	54.68	R157	43	31.2	72.56
209	62.5	36.4	58.24	R158	44	25.4	57.73
213	46.5	27.8	59.78	R159	51	29	56.86
216	45	25.4	56.44	Y010	51.5	31	60.19
217	61.5	31.8	51.71	Y013	50	25.8	51.60
224	51	27.6	54.12	Y015	54	30.2	55.93
225	49	29.2	59.59	Y017	49.5	30	60.61
227	42	24.4	58.10	Y018	47	27.4	58.30
228	49.5	29.4	59.39	Y02	51.5	30.8	59.81
229	52.5	29.2	55.62	Y024	53.5	31.8	59.44
238	54	31.6	58.52	Y03	46	27.2	59.13
239	50	28.2	56.40	Y116	45.5	26	57.14
221*	57	33.4	58.60				
Average	**51.1**	**29.0**	**56.9**	**Average**	**50.2**	**28.2**	**56.8**
Std Dev	**5.6**	**2.9**	**2.2**	**Std Dev**	**4.1**	**4.0**	**8.1**
%CV	**11.0**	**10.0**	**3.9**	**%CV**	**8.2**	**10.6**	**9.9**

*All deer except 221 were aged Rising 2 yr old at the beginning of the experiment. Deer no. 221 was aged by the farmer as a three year old. The average, std dev and %CV for the vaccinates was calculated on the Rising 2 yr olds only.

***Weight gain of Red Deer**:*

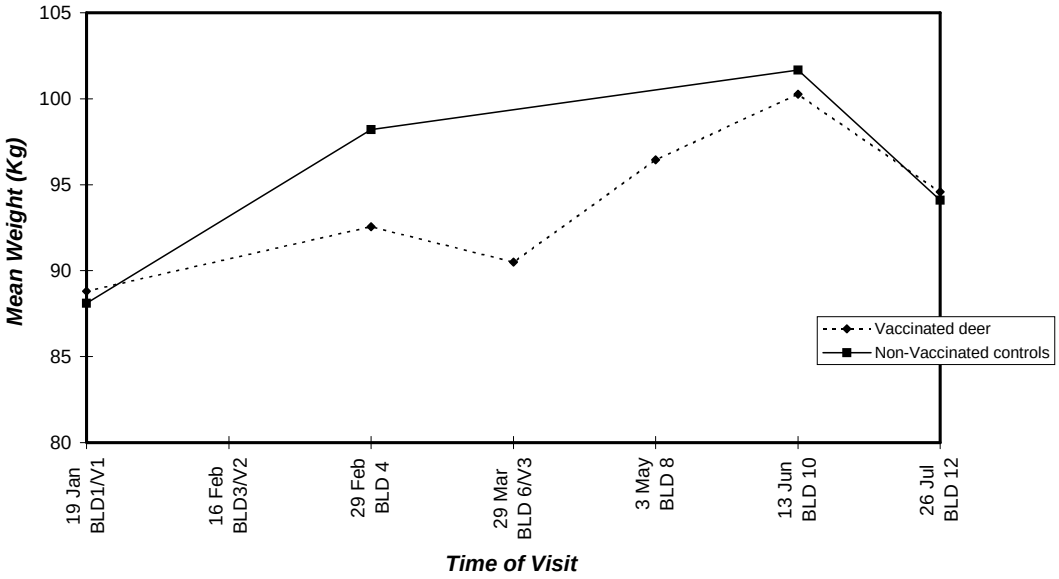
The weights of the vaccinated red deer were monitored every 4-6 weeks for the duration of the experiment. Due to the risk of handling the non-vaccinated control deer during the rut, the controls were weighed at Weeks 0 & 6 (Bleeds 1 & 4) then not again until weeks 21 and 27 (Bleeds 10 & 12). The results are outlined in Table 21 and Figure 6.

The results and analysis by single anovas show that at Bleeds 1, 10 & 12 there was no difference between the vaccinated and non-vaccinated deer ($P>0.05$). Whilst there was a weight difference at bleed 4, this was not observed again. The results thus indicate that there is no evidence that the vaccine had an effect on weight gain or loss in comparison with the non-vaccinated controls.

TABLE 21: Average weights (standard deviation) at each farm visit for the "vaccinated Red deer" and the non-vaccinated controls.

	Week No	Bleed No.	Vaccinated deer	Non-vaccinated controls
19th January	0	1	88.8 (10.4)	88.1 (7.3)
1st Feb	2	2	NT	NT
16th February	4	3	NT	NT
29th February	6	4	92.6 (9.4)	98.2 (8.2)
14th March	8	5	NT	NT
29th March	10	6	90.5 (9.4)	
18th April	13	7	NT	NT
3rd May	15	8	96.4 (8.5)	
23rd May	18	9	NT	NT
13th June	21	10	100.3 (7.5)	101.7 (6.5)
5th July	24	11	NT	NT
26 July	27	12	94.6 (7.4)	94.1 (9.6)

Figure 6: Mean Weight in Red Deer through experiment 3 (Kg)



Behaviour

Fallow Deer

Observation of behaviour was based on the ability to safely yard and hold the vaccinated deer in pens at each visit. Deer were generally brought into the pens in groups of 6-10. Where deer were observed to be fighting, they were separated into smaller groups of 2-3 and the most aggressive deer were handled first then released to pasture. Once the aggressive deer were removed the remaining deer within the pens were always observed to remain quiet and non-aggressive. The fallow deer were filmed being yarded and held in the outside pens at week 8 (four weeks following the second dose of vaccine). In general the vaccinated group was observed to stand quietly in the yards with minimal fighting. The exception was four mature stages that continually fought by head-butting and were removed from the herd. These stags had high testosterone, thicken necks and showed no indication that the vaccine had any biological effect. In order to protect the safety of the remaining herd these deer were removed from the experiment.

At bleeds 11 & 12, (9 and 12 weeks following the fourth vaccination respectively), the vaccinated deer were visually observed in the interior pens by an observer sitting on top of a dividing wall. Each deer was identified as being either actively participating in aggressive fighting or remaining passive. Results are outlined in Table 22 and 23. The tables indicate that behaviour did not totally correlate against testosterone concentration although the deer with the lowest testosterone were generally observed to be quiet and those with the highest testosterone were generally observed to be aggressive. Low anti-LHRH titres were observed in all deer found to be aggressive (no titres > 400 were measured). It should be noted that low titres were also found in "quiet" deer, the LHRH titre in this group ranging from <20 - 705.

Table 22: Behaviour of vaccinated fallow deer as observed at Bleed 11, 9 weeks post the fourth dose, listed in increasing Testosterone concentration.

Vaccinate ID	Age	Testosterone Concn (nM)	anti-LHRH Titre	Behaviour
213	2	0	705	quiet
210	2	0.01	401	quiet
227	2	0.02	493	quiet
207	2	0.06	356	quiet
228	2	0.09	847	quiet
230	2	0.1	112	quiet
222	2	0.2	110	quiet
242	2	0.2	313	
239	2	0.4	<20	quiet
201	2	0.70	222	aggressive
209	2	0.7	74	quiet
229	2	1.1	78	aggressive
217	2	1.7	188	quiet
208	2	1.9	335	quiet
219	2	1.9	21	aggressive
225	2	1.9	561	quiet
238	2	1.9	365	quiet
226	2	5.4	126	aggressive
203	2	5.8	264	quiet
214	2	6.2	114	quiet
224	2	6.2	383	quiet
202	2	10.50	266	quiet
206	2	11.9	<20	aggressive
204	2	28.6	<20	quiet
231	5	0.4	<20	aggressive
221	3	0.5	184	quiet
223	3	1.5	149	quiet
205	3	2.8	<20	quiet
243	3	3.4	175	agg?
237	4 or 5	5.9	<20	aggressive
215	5	7.7	85	aggressive
218	3	18.2	<20	aggressive

Table 23: Behaviour of vaccinated fallow deer as observed at Bleed 12, 12 weeks post the fourth dose, listed in increasing Testosterone concentration.

Vaccinate ID	Age	Testosterone Concn (nM)	anti-LHRH Titre	Behaviour
213	2	0.01	315	quiet
228	2	0.04	145	quiet
210	2	0.1	338	quiet
242	2	0.1	105	aggressive
206	2	0.2	<20	quiet
230	2	0.2	66	aggressive
238	2	0.2	212	quiet
216	2	0.4	198	quiet
219	2	0.4	<20	aggressive
204	2	0.5	<20	quiet
209	2	0.5	90	quiet
227	2	0.5	295	quiet
217	2	0.6	106	quiet
225	2	0.6	61	quiet
208	2	0.7	46	quiet
202	2	0.90	211	aggressive
207	2	2.2	27	quiet
214	2	2.2	96	aggressive
222	2	2.8	27	aggressive
226	2	2.8	44	aggressive
229	2	4.1	<20	quiet
224	2	5.7	89	quiet
239	2	6.9	<20	quiet
203	2	8.2	91	quiet
201	2		<20	aggressive
231	5	0.6	<20	aggressive
218	3	0.8	<20	aggressive
215	5	0.9	<20	aggressive
243	3	1.1	80	aggressive
221	3	1.6	125	quiet
223	3	4.8	63	aggressive
205	3	5.1	130	aggressive

Red deer

Behavioural observation of the red deer was severely limited due to the enclosed nature of the yards and holding pens. Behaviour could only be assessed in terms of the ease for which the deer were yarded and handled. On each farm visit no difficulty was observed in yarding or handling the vaccinates. The only exception was the final bleed where many of the deer were fighting and aggressive toward the farmer. this bleed coincided with 90% of the vaccinates having testosterone greater than 2 nM and a median titre of < 20 (i.e. returned to base levels).

Velvet Production

Velvet production was monitored in the red deer. Both herds of vaccinated red and fallow deer were successful in producing a growth of velvet. Buttons were observed to drop as early as Bleed 5 (14th March, 4 weeks post the second vaccination). Velvet growth was observed in 11 out of 19 vaccinated red deer by Bleed 7 (18th April). The growth was generally a straight spike or of an irregular shape with the absence of defined browtine or baytine. In all cases the velvet was typical castrate growth and graded at “D” class.

Velvet growth was observed again in December 1997 to see if the vaccinated deer resumed “normal” growth expected of an entire stag. All 19 vaccinated stags grew typical 3yr old velvet. Whilst a few individuals were of irregular shape, the farmer believed that the occurrence was in accordance to that expected in a normal herd. The velvet range in length, and width from 0.8 to 1.5Kg and was generally graded as "Class C"..

Meat Quality Studies

The original experimental proposal aimed to complete meat quality studies investigating issues of tenderness, pH and taste. Unfortunately these studies could not be completed since the farmer was not able to let us have whole or part of the deer carcasses since he needed the deer to meet an export order.

Conclusion

The aim of this experiment was to examine the efficacy of the vaccine to control sexual behaviour and weight loss during the rut. Experiment 1 and 2 had determined the optimal formulation and dose for use in the deer. The immunocastration vaccine used in this experiment was prepared at VIAS using the formulation and dose shown to be most optimal in these previous experiments. In experiment 3, we wished to examine the effect of the vaccine when administered during the rut, how long it remained efficacious, whether use of the immunocastration vaccine affected velvet growth, improved weight condition, and subsequent carcass quality when animals were slaughtered during the rut.

Site reactions were virtually undetected in the fallow deer. In contrast, and consistent with the findings in earlier experiment, the incidence of site reactions in the red deer was much higher, representing 26% of all injections. The reactions ranged from small unburst abscesses to burst abscesses up to 5x5 cm in size. Of interest was the findings that copper supplement injections administered to the red deer through the experiment produced similar reactions in 89% of the injections. This finding suggests that the red deer are not reacting to a particular ingredient of the formulation but may be generally sensitive to infection after subcutaneous injections. Discussions with a number of farmers regarding vaccinations highlighted that farmers rarely need to yard deer after velveting or administering routine vaccinations**.** Thus the incidence of site reactions in routine practice is largely not noticed.

Whilst the immune response is measured by the anti-LHRH antibody titre, the primary biological effect of the immunocastration vaccine was the suppression of testosterone production, reduction in testes size and subsequent reduction in aggressive sexual behaviour during the rut. In general, a testosterone concentration of >2nM is considered as being indicative of active testes function. Results in this study indicated that the immunocastration vaccine was effective in reducing testosterone concentrations within two weeks of a booster vaccination. In the 2 yr old fallow vaccinates 80 - 92% of the herd had testosterone < 2nM two weeks following each boost. In the vaccinated red deer 80 - 84% of the herd had testosterone concentrations < 2nM two weeks after each boost. In comparison the non-vaccinated controls were well within the breeding season with testosterone > 2nM. On each of these occasions, the vaccinated deer were generally quiet in the yards and holding pens with minimal fighting and generally low risk of injury to the animals. However achieving duration of effect proved difficult as testosterone concentrations increased to active levels within 4-6 weeks of each boost in at least 50% of the herds.

The red deer had a slightly longer duration of vaccine efficacy with low testosterone concentrations being maintained in the majority of the herd for up to 6 weeks after each boost. This effect was also supported with higher anti-LHRH titres consistently being measured in the red deer over the fallow deer. The stronger immune response and reduction in testosterone in the red deer compared to the fallow deer has been a consistent observation in all previous studies conducted at VIAS. This outcome is unusual in that both breeds received the same dosage of vaccine although the fallow deer were of average 50 Kg weight compared to the Red deer with an average weight of 95Kg. Thus the red deer actually had a lower dose/weight compared to the fallows and yet always stimulated a stronger immune response.

A second direct biological effect of the vaccine was seen in the reduction of testes size. Vaccinated 2 yr old fallow deer showed a significant reduction in testes size compared to the controls and remained reduced for the duration of the experiment. By the last weeks of the

experiment approximately 50% of the herd had regressed testes as would be expected for the non-breeding season. Of all the vaccinates sent to slaughter only one testes appeared normal by histological examination. All others showed varying degrees of testicular atrophy. Similar results were also observed in the red deer with the testes in the vaccinates being significantly reduced in size in comparison to the controls within 2 weeks after the first booster and remained smaller for the duration of the experiment. Despite the rapidly changing testosterone concentrations, the testes remained reduced and non-functional to varying degrees suggesting that fertility may be poor despite rising testosterone concentrations as the vaccine efficacy wore off.

Despite the reduction in testes size in the fallow deer, the control of aggressive behaviour in the herd was difficult to maintain and seemed to reflect the rapidly changing testosterone concentrations. Although a direct correlation between observed fighting and testosterone concentrations could not be established in the fallow deer, both farmer and scientific staff observed that the herd was more difficult to safely yard and handle as testosterone concentration increased. Control of aggressive sexual behaviour however remains essential if deer are to be taken to slaughter during the breeding season. Of the 35 vaccinated fallow deer remaining in the experiment at the end of July, 18 were identified as being quiet and suitable for transporting to slaughter. The 18 selected deer remained quiet within the holding yards and were easily transported without incident. On a practical level however the need to sort through a herd to find suitably quiet deer for slaughter would be prohibitive for most farmers. A commercially viable vaccine would need to suppress and hold aggressive sexual behaviour in virtually 100% of the herd.

Apart from controlling sexually aggressive behaviour, another aim of the immunocastration programme was to improve the liveweight condition of stags throughout the breeding season. The vaccinated 2yr fallow deer as a group maintained their weight condition throughout the experiment within approximately $\pm$ 2 Kg. Comparison between the vaccinated 2yr old deer and controls showed that there was no weight difference between the two groups in the first 8 weeks of the experiment (Bleed 5). The controls were not weighed again until week 18 (Bleed 9, 22 May). At this stage the two groups were significantly different ($P<0.05$), with the controls being 4.6Kg below the vaccinated deer and 4.9 Kg below their initial weight. The weight data suggests that the immunocastration vaccine was highly successful at improving liveweight gain in comparison to the controls in 2 yr olds. This finding however is based on the single weight comparison at Week 18 between vaccinates and controls and cannot be used as a definitive result. We wish to address these suggestions by attempting to obtain more weight data on non-vaccinated controls in a subsequent experiment to be scheduled for the next rut.

In contrast, the weight data for the red deer showed that there was no difference between the vaccinated and non-vaccinated deer ($P>0.05$) with both groups increasing in weight through the experiment by an average of 6 Kg. Thus there was no evidence that the immunocastration vaccine had an effect on weight gain or loss in comparison with the non-vaccinated entire deer. The outcomes could be attributed to a number of factors including the relative abundance of feed at the red deer farm, the generally quieter nature of this herd of red deer (even the entires) or a basic breed difference whereby weight loss is not as severe in 2 year old red deer in comparison to the fallow breed.

Of the eighteen vaccinated fallow deer and 15 surgically castrated deer sent for slaughter on 1st August 1996, results indicated that there was no statistically significant difference between the vaccinated deer and castrate group in terms of liveweight, carcass weight or

dressing out percentage. The surgically castrated animals therefore were able to maintain the same weight condition as that seen in the immunocastrates. This result may be expected if the deer were surgically castrated in the more recent months prior to slaughter. In this circumstance the castrates would have had a feed-intake similar to the vaccinated immunocastrates and therefore expected to have a similar weight condition. In addition, the short time that the deer were castrated would reduce the likelihood of increased fat deposit (typical of surgical castrates) since it would not have had time to develop. Since the surgically castrated deer were not part of the experiment and were simply part of the slaughter herd, we have no information to indicate the date of castration. Previous studies by other researchers have indicated that surgical castration of young stags leads to greater fatness (Drew *et al* 1978). Surgical castration at an older age (closer to slaughter) may provide the same weight gain benefits throughout the rut as would be anticipated for immunocastrated deer. However the practice of castrating older deer has welfare implications and is of concern to the industry. Where immunocastration could provide maintenance of weight condition throughout the rut, this is an easy and "welfare conscious" alternative.

Velvet production was monitored in the red deer. Both herds of vaccinated red and fallow deer were successful in producing a growth of velvet as early as April. Growth was generally a straight spike or of an irregular shape with the absence of defined browtine or baytine. In all cases the velvet was typical castrate growth and graded at "D" class. Velvet growth was observed again in December 1997 to see if the vaccinated deer resumed "normal" growth expected of an entire stag. All 19 vaccinated red stags grew typical 3yr old velvet. Whilst a few individuals were of irregular shape, the farmer believed that the occurrence was in accordance to that expected in a normal herd. These results indicate that any biological effect of the vaccine in regards to the development of velvet growth will be negligible by the onset of the following non-breeding season and typical growth and returns could be expected.

Difficulties were however encountered with mature fallow stags aged 3 years or more since they generally responded poorly to the vaccine. The majority of mature stags showed no reduction in testosterone, testes size and many became and stayed aggressive with the onset of the breeding season. Typically the mature stags were noticeable for thickened necks throughout the trial. These results showed that the immunocastration vaccine would need to be restricted in use to 2 year old stags. This circumstance however should not prove to be a limitation to the proposed market since the vaccine has always been aimed for use in venison deer who are generally kept only up to their 3rd year. Most mature stags are kept on farms for the purpose of breeding and therefore are not candidates for the immunocastration vaccine.

The third experiment aimed to answer basic questions in relation to vaccine efficacy, control of weight loss, carcass quality, antler growth and behavioural changes. The short duration of effect for the vaccine and the difficulty in obtaining continuous weight data, however meant that further clarification of the weight data would be valuable through a subsequent trial to be held in the next breeding season. At this time we were given the opportunity to test an immunocastration vaccine developed by a commercial company. This experiment was aimed specifically at providing additional data regarding behaviour and weight loss during the rut and enabled us to compare the performance of a commercial prototype vaccine against the VIAS deer immunocastration vaccine we had previously identified as being optimal.

Experiment 4: Further investigation of weight loss during the rut.

Aim:

Experiment 4 aimed specifically at providing additional data regarding behaviour and weight loss during the rut and gave us the opportunity to compare the performance of a commercial prototype vaccine (Vaccine I) supplied by a commercial company against the laboratory preparation we had previously identified as being "optimal" for use in deer.

Materials And Methods

Positive Control: VIAS Vaccine formulation

Experiment 4 was conducted using the remainder of vaccine left from Experiment 3.

Commercial Prototype "Vaccine I"

Vaccine I was supplied from a commercial vaccine company as Batch BT-27 (14/1/97) for use in Experiment 4.

Animals

Fallow Deer: 34 fallow stags aged "Rising 2" were used. The deer were supplied from and remained agisted in a farm in Western Central Victoria (Creswick). 20 Deer were vaccinated with Vaccine I. Four were vaccinated with the VIAS positive control and 10 were left as non-vaccinated controls. The controls were randomly selected from the herd on the first vaccination day. During the experiment one of the non-vaccinated control deer died overnight in the pasture. A second non-vaccinated control deer, injured due to fighting was removed from the trial whilst recovering. All deer were agisted in one pasture until the fourth farm visit. At this time the non-vaccinated controls were well into the rut, actively aggressive and were a threat to the quiet vaccinated deer. The non-vaccinated controls were therefore separated into an adjoining paddock.

All deer received the same diet. This consisted of pasture supplemented with wheat grain, turnips and hay.

Vaccination Regime

Each of the Vaccine I vaccinated fallow deer were given a primary vaccination at week 0 (23rd January 1997), second vaccination at week 4 (19th February 1997), then a third vaccination at week 10 (3rd April 1997).

The VIAS positive control deer received a primary vaccination at week 0 (23rd January 1997) then second vaccination at week 4 (19th February 1997). Due to the limited supply of vaccine, there was only sufficient formulation to give two of the four the third dose at week 10 (3rd April 1997).

Sample collection

Blood samples were collected at week 0 and every two-three weeks thereafter for a total of 27 weeks. Blood samples were collected from the jugular vein using 21 Gauge vaccutainer needles. A summary of vaccination and sample collection schedules is given in Table 24.

The site of vaccination was also assessed for swelling or abscess and a score was recorded. Site reactions were scored as either 1, 2 or 3 and given a comment and approximate size in centimetres for the actual diameter. A score of "1" was given if the site of injection was slightly swollen and could only be detected by touch. A score of "2" was given if a lump was visible and a score of "3" was given if the reaction had burst open.

TABLE 24: Summary of blood sample collection and vaccination schedules.

DATE*	WEEK No.	BLEED No.	VACCINATION
23rd January 1997	0	1	1
6th February	2	2	
19th February	4	3	2
6th March	6	4	
18th March	8	5	
3rd April	10	6	3
17th April	13	7	
1st May	15	8	
15th May	18	9	
29th May	21	10	
12th June	24	11	
26th June	27	12	
10th July	29	13	
24th July	31	14	
7th August	33	15	
21st August	35	16	
11th September	38	17	
2nd October	41	18	

Weight Determination

The weight of each fallow deer was taken each visit (each fortnight). The weights were measured using a weigh box and scales provided by the farmers.

Testes Measurement

The testes of vaccinated and control fallow deer were measured each fortnight. The width (taken at the widest section) and length (excluding the epididimus) of each testes were measured using a micrometer.

Analyses

Sera collected from the blood samples were analysed for testosterone and anti-LHRH antibody titres. The testosterone analysis was performed using a commercially supplied direct Radioimmunoassay kit (Pantex ^{125}I). The method was modified to include an extraction step whereby serum testosterone was extracted into 90%diethyl ether/10%ethyl acetate. The intra-assay coefficient of variation was <10%. The inter assay coefficients of variation were 12.1, 11.3, 10.0% as calculated from low, median and high pathological standards measured over 28 assays. The assay had a minimum detection limit of 0.5 nM

The anti-LHRH analysis was performed using a direct binding radioimmunoassay whereby doubling dilutions of serum ranging from 1:100 to 1:1600 were incubated with ^{125}I labelled LHRH (supplied by AMRAD (250 μCi) diluted with 0.1M phosphate/saline buffer pH 7.4 until cpm = 7000-7500). Each dilution was incubated at 4°C for 48 hours in a final volume of 400μL 0.1M Na phosphate buffer pH 7.40 containing 1.25 mg/mL human gamma globulin. Following incubation 100μL 1%bovine gamma globulin solution was added. The free LHRH tracer was separated from bound by addition of 1mL 18% polyethylene glycol 6000. The resulting precipitate was separated after centrifugation (3000rpm, 10 minutes) then counted on a Wallace 1410 scintillation counter. Intra-assay variability was determined from measuring three different pig samples up to 9 times in one assay and was found to be 7.0, 3.3 and 4.8% respectively. Inter-assay coefficient of variation was 11.9 and 10.1% calculated from measuring control serum collected from an immunocastrated pig and deer over 55 assays. The titres are expressed at the dilution of serum capable of binding 33% labelled LHRH.

Behaviour

Behaviour of the Fallow deer was monitored by visual observation of the animals when yarding and handling. Visual observations were limited due to the enclosed nature of the pens. Where possible, the identification number of each fighting deer was recorded. It should be noted however that the yards were designed to allow deer to be separated and minimise fighting. This reduced the incidence of fighting but had the advantage that it allowed non-vaccinated deer to be handled throughout the rut. Fallow deer were videoed at week 6 (two weeks following the first booster vaccination). The deer were filmed being yarded and held in the pens of the farmer's handling shed.

Results

Site Reactions

Only one incidence of site reactions was detected in this experiment. This occurred with the first dose of Vaccine I and presented as a 3 x 4 cm burst abscess (score 3). The abscess was healed within 6 weeks.

Anti LHRH Titre

The range of anti-LHRH titres for each bleed and the median value is given in Table 25. Median titres are also displayed in Figure 7. For both groups of vaccinates, a positive immune response was seen within two weeks after the second dose. Consistent with Experiment 3, titres however rapidly decreased and were below 400 within 6 weeks of the boost. (A median titre of 400 has been observed in other species to the minimum titre to have a likely biological effect.) A increased titre was observed in response to the third injection. Titres held above 400 for 6 weeks after the third injection. This response encouraging as it was an improvement on results seen in Experiment 3. By 8 weeks following the third dose, titres steadily decreased and were near-to-baseline by Bleed 12 (12 weeks after third dose).

The vaccinated positive control group gave titres consistent with that seen in Experiment 3 and consistent with titres observed with Vaccine I. The non-vaccinated controls had no measurable anti-LHRH titres as expected.

TABLE 25: Median anti-LHRH Titres (and range) measured in Fallow Deer at each farm visit.

GROUP	Week No	Bleed No.	Vaccine I deer	Positive Controls	Non-vaccinated controls
23 Jan 1997	0	1/V1			
6 February	2	2			
19 February	4	3/V2			
6 March	6	4	882 (<20-1848)	1010 (463-1228)	<20
18 March	8	5	468 (<20-953)	527 (320-749)	<20
3 April	10	6/V3	165 (<20-415)	197 (102-261)	<20
17 April	13	7	1600 (<20->3200)	982 (356-2614)*	<20
1 May	15	8	858 (<20-1620)		<20
15 May	18	9	456 (1164)		<20
29 May	21	10	264 (<20-820)		<20
12 June	24	11	156 (476)		<20
26 June	27	12	144 (<20-293)		<20
10 July	29	13	28 (<20-222)		<20
24 July	31	14	<20 (<20-201)		<20
7 August	33	15	26 (<20-100)		<20
21 August	35	16	<20 (<20-129)		<20
11 Sept	38	17	<20 (<20-95)		<20
2 October	41	18	NT		<20

*****No anti-LHRH data has been reported for this group following this bleed since only 2 out of the 4 deer were able to receive a third dose. The results here indicate that the two deer receiving the third dose gave a positive response consistent with Vaccine I group.

Testosterone

Testosterone concentrations were monitored as the primary indicator of vaccine efficacy and duration of effect. The mean (plus standard deviation) for each group at each farm visit is given in Table 26. The results are also illustrated in Figure 8 as the percentage of deer with testosterone concentrations below 2 nM, that is indicating inactive testes.

Testosterone concentrations for the non-vaccinated controls were as expected for the breeding season. As the trial commenced testosterone concentrations increased and 100% of the non-vaccinated groups had testosterone concentrations above 2 nM (indicating active testes) by 18th March. Testosterone concentrations remained high until the end of May whereby episoidal waves of high and low testosterone numbers for the group were measured. By the end of the trial 80% of the herd had decreased testosterone, below 2nM, indicating inactive testes as expected for the start of the non-breeding season.

In contrast the Vaccine I group displayed low testosterone within two weeks of the second vaccination (at bleed 3). At this time, 75% of the herd had testosterone below 2nM (compared to only 10% of the non-vaccinated controls). Within a further two weeks however, the testosterone concentrations in the Vaccine I group had risen dramatically with only 30% of deer having testosterone < 2nM. The marked rise in testosterone concentration in the vaccinated group, suggested that the vaccine did not hold efficacy beyond 2-3 weeks and deer were moving into rut. These results were consistent with the positive control groups and results seen in Experiment 3. Thus a third dose was administered. Testosterone concentrations fell within 2 weeks of the booster now with 90% of the herd having testosterone concentrations below 2nM. The Vaccine I group continued to have low testosterone as a group (with 80-90% <2nM) then began to rise as a group by the tenth week after the third dose. This result was highly encouraging since testosterone response to vaccine efficacy was much shorter in Experiment 3 (using the VIAS laboratory formulation).

As the vaccine efficacy decreased, the Vaccine I group began to have increasing concentrations of testosterone. At bleeds16 and 17 (21st Aug, 11th Sept) only 20% of the herd had inactive testosterone concentrations. This suggested that the herd had moved into a delayed rut once the vaccine efficacy had worn off. Testosterone concentrations remained high even though the non-vaccinated controls now had low testosterone as expected for the emerging non-breeding season. The impact of this outcome is most evident in the weights whereby the Vaccine I group showed significant weight loss for the same period.

(Note that the data for the positive control group was not included in Figure 8. The small size of the group and the absence of the third dose for all four deer meant that interpretation of the testosterone averages was potentially unreliable.)

TABLE 26: Mean Testosterone Concentration (nM) (+standard deviation) measured in Fallow Deer at each farm visit.

GROUP	Week No	Bleed No.	Vaccine I Vaccinated deer	Positive Controls	Non-vaccinated controls
23 January 1997	0	1/V1	2.6 (2.0)	1.8 (0.6)	1.7 (1.0)
6 February	2	2	3.4 (3.3)	1.70 (0.6)	3.6 (3.2)
19 February	4	3/V2	6.3 (5.8)	0.66 (0.1)	8.8 (6.2)
6 March	6	4	2.2 (1.8)	10.4 (13.9)	15.6 (12.9)
18 March	8	5	2.8 (2.3)	2.3 (1.9)	18.2 (7.0)
3 April	10	6/V3	8.1 (5.4)	12.2 (3.6)	45.1 (15.0)
17 April	13	7	2.7 (2.1)	5.4 (7.1)	35.6 (20.5)
1 May	15	8	1.1 (0.6)	5.8 (8.5)	38.0 (16.8)
15 May	18	9	1.6 (1.0)	5.1 (3.8)	0.8 (0.3)
29 May	21	10	1.5 (1.6)	0.6 (0.2)	7.1 (9.9)
12 June	24	11	1.6 (1.6)	1.0 (0.7)	20.9 (12.5)
26 June	27	12	3.1 (3.2)	4.3 (5.5)	14.5 (13.3)
10 July	29	13	3.3 (3.6)	2.4 (3.0)	3.5 (3.5)
24 July	31	14	1.8 (1.3)	1.5 (1.4)	6.7 (5.4)
7 August	33	15	NT	NT	NT
21 August	35	16	5.3 (3.7)	7.1 (6.7)	5.0 (3.7)
11 Sept	38	17	6.1 (4.1)	5.6 (6.6)	7.2 (9.3)
2 October	41	18	NT	NT	NT

Figure 7: Median anti-LHRH Titre through Experiment 4.

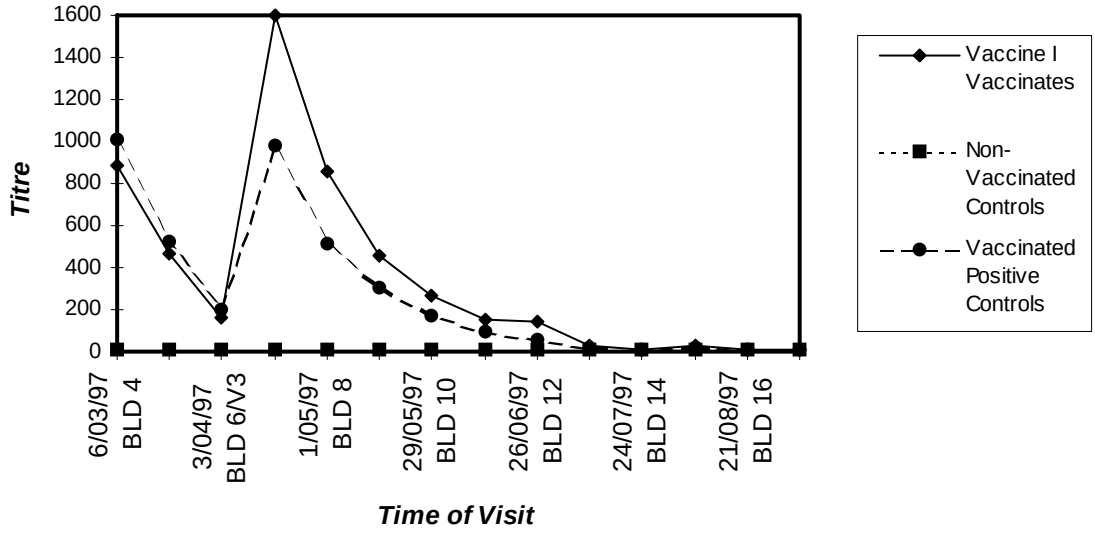
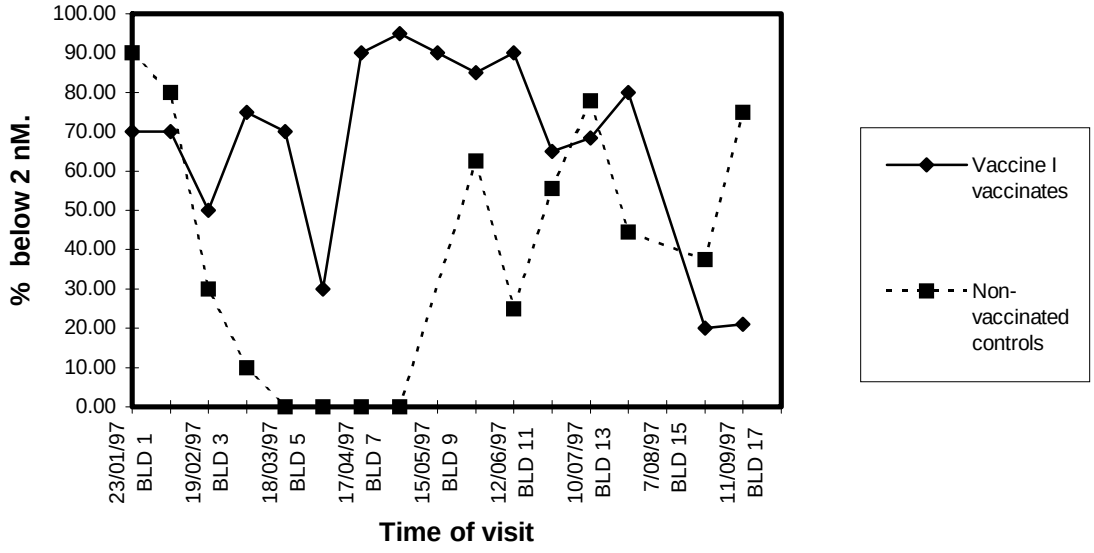


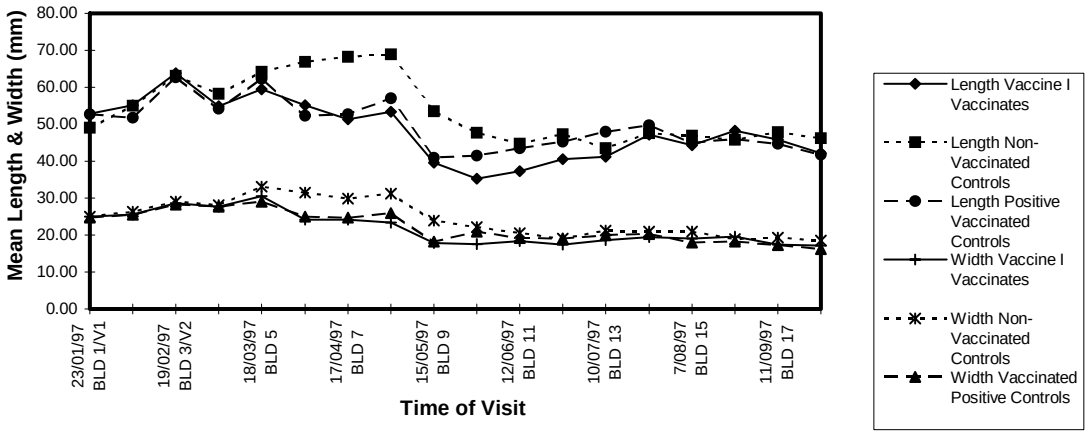
Figure 8: Percentage of deer in Exeriment 4 with testosterone concentration below 2 nM.



Testes Measurement

The mean length and width of testes measured in the Vaccine I group, vaccinated positive controls and non-vaccinated controls are shown in Figure 9. The figure shows that for the duration of the experiment both the Vaccine I group and positive controls were very similar in response to the vaccine. Testes in all groups increased in size in the first few weeks as the rut began (but before vaccine had started to have effect). By Bleed 6, six weeks after the second vaccination, the size of testes in both groups of vaccinated deer had significantly reduced (P,0.05) in both length and width in comparison to the non-vaccinated controls. As vaccine efficacy continued, the size of the testes in this group remained reduced, and in most cases fully regressed, until bleed 13 (10th July). By this stage and then for the remainder of the trial testes length and width for the vaccinates were not significantly different from the non-vaccinated controls (which by now were decreasing in size in response to the upcoming non-breeding season).

Figure 9: Mean Length & Width of Testes through Experiment 4.



Weight gain of Fallow Deer

The weights of the vaccinated fallow deer were monitored for the duration of the experiment. The results are outlined in Table 27 and Figure 10.

At the outset of the experiment the deer were randomly separated into Vaccine I group, VIAS positive controls and non-vaccinated control groups. Analysis by singly anova indicated that the three groups were not statistically different in regards to weight. With the exception of Bleed 6 (3rd April), Bleed 9 (15th May), Bleed 17 (11th September) and Bleed 18 (2nd October), the mean weights of the three groups remained close and were not statistically different . Despite the statistical closeness of the results, there were some marked differences between the vaccinated and non-vaccinated groups.

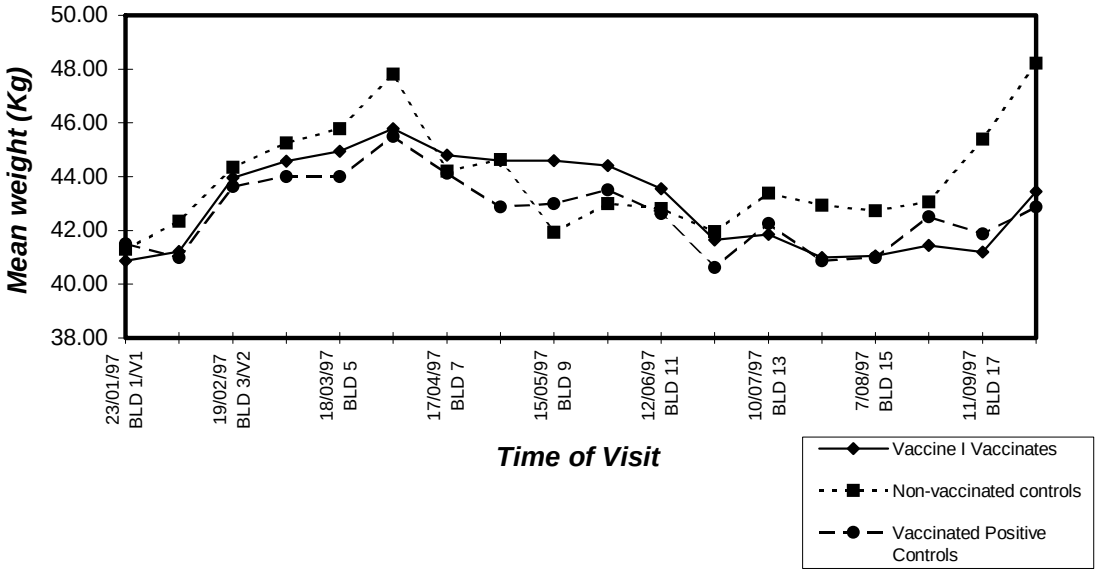
The non-vaccinated controls had a mean weight of 41.3 Kg at the outset of the trial. This group continued to increase in weight peaking at a mean of 47.8 Kg at Bleed 6 (3rd April). Over the following 6 weeks the group lost weight and with the lowest mean of 41.9 Kg at Bleed 9(15th May) presumably due to the effect of the rut. The group maintained weights of 41-43 Kg over the following weeks as the breeding season continued. A significant increase in weight and body condition of the non-vaccinated control group was observed at Bleed 17 (11th September). This coincided with the lower testosterone concentrations as the deer emerged into the non-breeding season. The group had a final mean weight of 48.2 Kg at 2nd October 1997.

Both the vaccinated groups similarly began the trial with increasing weights peaking at Bleed 6 (3rd April) at 45.8 and 45.6 Kg for the Vaccine I group and Positive Controls respectively. Weight loss in subsequent weeks was not as rapid as observed in the non-vaccinated control groups This is presumably due to the efficacy of the immunocastration vaccine with low testosterone concentrations also being observed for this period. Both vaccinated groups however did slowly continue to loose weight over the subsequent weeks of the trial, the lowest levels observed at Bleed 12 (26th June) at 41.0 Kg for both groups. Improving body condition and weight typical of the non-breeding season and warmer temperatures was not observed in the vaccinated groups until Bleed 18 (2nd October). This late response meant the Vaccine I and positive control vaccinated groups ended the trial with mean weights of 43.4 and 42.9 Kg respectively. These final weights were statistically significantly below the non vaccinated control group.

TABLE 27: Average weights (standard deviation) at each farm visit for the "Vaccine I" deer, vaccinated positive controls and the non-vaccinated controls.

GROUP	Week No	Bleed No.	Vaccine I Vaccinated deer	Positive Controls	Non-vaccinated controls	Significant difference between three groups
23 January 1997	0	1/V1	40.9 (2.2)	41.5 (1.8)	41.3 (1.2)	P>0.05
6 February	2	2	41.2 (2.7)	41.0 (2.5)	42.3 (1.9)	
19 February	4	3/V2	43.9 (2.4)	43.6 (2.1)	44.3 (1.8)	
6 March	6	4	44.6 (2.4)	44.0 (2.0)	45.3 (1.8)	
18 March	8	5	44.9 (2.3)	44.0 (0.41)	45.8 (2.2)	P>0.05
3 April	10	6/V3	45.8 (2.2)	45.5 (2.3)	47.8 (2.5)	P>0.05
17 April	13	7	44.8 (2.0)	44.1 (1.5)	44.2 (2.3)	
1 May	15	8	44.6 (2.2)	42.9 (1.3)	44.6 (2.6)	
15 May	18	9	44.6 (2.4)	43.0 (1.4)	41.9 (1.7)	P<0.05
29 May	21	10	44.4 (1.8)	43.5 (2.0)	43.0 (2.0)	
12 June	24	11	43.5 (2.2)	42.6 (2.3)	42.8 (3.4)	
26 June	27	12	41.6 (1.9)	40.6 (1.4)	41.9 (2.0)	
10 July	29	13	41.8 (1.9)	42.2 (1.6)	43.4 (1.8)	
24 July	31	14	41.0 (2.0)	40.9 (1.9)	42.9 (2.0)	P>0.05
7 August	33	15	41.0 (2.5)	41.0 (0.9)	42.7 (2.0)	P>0.05
21 August	35	16	41.4 (2.4)	42.5 (1.3)	42.5 (1.3)	P>0.05
11 Sept	38	17	41.2 (2.2)	41.9 (1.4)	41.9 (1.4)	P<0.01
2 October	41	18	43.4 (2.7)	42.9 (2.6)	42.9 (2.4)	P<0.01

Figure 10: Mean weights during Expt 4.



Behaviour

Observation of behaviour was based on the ability to safely yard and hold the vaccinated deer in pens at each visit. Deer could be viewed with some limitation in the pens and fighting deer were identified where possible. Where deer were observed to be fighting, they were separated into smaller groups of to minimise risk. Table 28 details the observed behaviour of the Vaccine I group and non-vaccinated control groups in relation to the percentage of deer with testosterone concentrations below 2nM. The onset of aggressive behaviour could not be directly related to individual testosterone concentrations or even testosterone concentration of the group. However Table 28 does indicate that in general periods of aggressive behaviour were linked to periods whereby most of the group (>50%) had testosterone concentrations above 2 nM (indicative of active testes).

The non-vaccinated controls as expected became aggressive and actively fighting each other as the deer moved into the rut and testosterone concentrations increased. The group however displayed a much quieter behaviour by the beginning of May. In the following weeks, fighting within the controls was often less aggressive to that observed in the vaccinated group. A contributing factor however had to be the lower stocking density in the pens; 10 control deer compared to 24 in the vaccinated group. In the last two visits of the trial the controls showed a reduction in aggression presumably marking the beginning of the non-breeding season.

The Vaccine I group were easily handled and yarded in the 8-10 weeks following the third dose of vaccine. The only exception at each of these visits would be two or three deer (not alway the same individuals) which would push and head butt the other deer in the pens. Given that these deer could not be identified as the same individuals at each visit it was not possible to determine if this was the direct result of a poor response to the vaccine. The final

weeks of the trial saw an increase in the aggression of the fighting within the vaccinated herd. Most deer were actively aggressive and soon panting after a few minutes within the pens. This aggressiveness corresponded with decreasing LHRH titres, increasing testosterone concentrations and subsequent weight loss as the vaccine efficacy deteriorated.

Velvet Production

Fourteen out of the 20 Vaccine I vaccinated deer were successful in producing a growth of castrate velvet. Buttons were observed to drop as early as Bleed 7 (17th April, 2 weeks post the third vaccination). The growth was generally a straight spike growing up to 200 mm in length, typical of castrate growth. The velvet was harvested over 8 weeks from Bleed 9 (15th May)
By Bleed 16 the herd all had hard buttons as testosterone concentrations increased.

TABLE 28: Behaviour and mean testosterone concentration (nM) of Vaccine I vaccinates and non-vaccinated control groups at each visit.

GROUP	Week No	Bleed No.	Vaccine I group % Testos. . below 2nM	Vaccine I group: Behaviour	Non vaccinated controls % Testos below 2 nM	Non-vaccinated controls Behaviour
23 Jan 1997	0	1/V1	70	quiet	90	quiet
6 February	2	2	70	quiet	80	quiet
19 February	4	3/V2	50	quiet	30	quiet
6 March	6	4	75	quiet	10	quiet
18 March	8	5	70	general fighting. 7 identified	0	general fighting
3 April	10	6/V3	30	aggressive fighting 5 identified split herd	0	aggressive fighting
17 April	13	7	90	quiet except for 3 individuals	0	aggressive fighting
1 May	15	8	95	quiet except for 2-3 individuals	0	quiet
15 May	18	9	90	quiet except for 3 individuals		quiet
29 May	21	10	85	quiet except for 3 individuals	62	quiet
12 June	24	11	90	quiet except for 3 individuals	25	quiet
26 June	27	12	65	quiet except for 3 individuals	55	
10 July	29	13	68	aggressive fighting	78	general fighting
24 July	31	14	80	aggressive fighting	44	aggressive fighting
7 August	33	15	N/A	fighting	N/A	aggressive fighting
21 August	35	16	20	aggressive fighting	37	aggressive fighting
11 Sept	38	17	21	aggressive fighting	75	general fighting
2 October	41	18	N/A	some fighting much reduced in aggression	N/A	much quieter

Conclusion

The aim of this experiment was to gain additional weight and behavioural data from the use of immunocastration vaccines and to examine the performance of a commercial prototype immunocastration vaccine ("Vaccine I") supplied by a commercial company in comparison to the VIAS laboratory preparation.

In general the Vaccine I displayed a slight improvement in terms of producing anti-LHRH titres and decreasing testosterone concentrations. The experiment showed that 90-95% of the herd produced a strong immune response to the vaccine. Titres remained above 400 for 6 weeks after vaccination and testosterone concentrations below 2 nM in more than 80% of the herd for up to 10 weeks after the third dose. This compares with Experiment 3 whereby 80% of the herd showed a strong biological response to the vaccine; and efficacy was shorter with titres dropping below 400 with 4 weeks of the boost in 50% of the herd. These differences seen between Experiment 3 & 4 are considered to be slight since such differences may equally be effected from the different farm locations, nutrition, season temperatures and herd.

In terms of controlling weight loss during the rut, the Vaccine I produced some positive results in comparison to the non-vaccinated controls in the initial part of the trial. Weight loss in the 8-10 weeks following vaccination was significantly less than that observed in the non-vaccinated group which were at that stage actively in rut.

This advantage was however negated at the end of the trial (September-October) when the vaccinated group moved into an apparent delayed rut as antibody titre declined. At this stage the non-vaccinated controls were rapidly increasing in weight with the emergence of the non-breeding season and new spring pasture growth. In contrast the vaccinated group showed deterioration in weight despite equal access to fresh pasture growth. This same period coincided with increasing testosterone concentrations, and decreased anti-LHRH titres to baseline levels. The vaccinated deer became increasingly difficult to yard or handle. This aggression and deterioration in body condition continued at least through to our final visit in mid-October.

The emergence of a delayed rut, with resultant weight loss and aggression offers little benefits to farmers unless they decide to slaughter during the rut, whilst the antibody titre remains high. If farmers however are unable to fulfil slaughter expectations (e.g. due to inaccessibility of abattoir facilities, personal constraints) they will most likely experience a significant loss in weight and body condition in the vaccinated herd. In consulting with farmers throughout the project, it is apparent that they are often forced to modify and delay their management plans including slaughter times. Thus keeping to a strict immunocastration plan may not be feasible for many.

The onset of a "delayed" rut following immunocastration has also been observed by others (Ataja, 1992; Freudenberger *et al*, 1991, 1993). Thus it is likely that "immunocastration" at least in the form of an LHRH antigen vaccine, will only ever act to delay (rather than prevent) the effects of the rut. The results as observed in Experiment 4 are therefore likely to occur with any proposed LHRH immunocastration vaccine regardless of vaccine formulation or dose.

Slaughtering during the rut is however of high importance if the industry is to provide a “year-round” supply of venison. It is with this aim that an immunocastration vaccine may in the future be of use. Experiment 4 clearly indicated that the vaccinated group were quiet and

easily handled in the 8-10 weeks following the third vaccination. This provides a period of time from April- June whereby stags of good weight condition could be sent to slaughter.

The exact cost benefit to farmers in terms of body condition and weight loss is however difficult to predict from the findings of this research. In experiment 3, the fallow vaccinated deer were 5Kg heavier than the non-vaccinated entires by the end of May. In experiment 4 the fallow deer only maintained a 2-3 Kg advantage over the non-vaccinated stags in the period that the vaccine was efficacious (April-May). In experiment 3, the red deer showed no weight advantage over the non-vaccinated controls at any stage of the experiment. It is possible that for 2 year old stags, weight loss during the rut may be equally governed by the poorer pasture conditions as by changes in metabolism and behaviour. It is therefore likely that the cost benefits to farmers using the immunocastration will be highly variable. It should be noted that previous research into immunocastration in other countries also found a variable response in regards to weight gain/loss (Ataja, 1992; Freudenberger *et al*, 1991, 1993). This again supports the suggestion that the results observed in this project are typical of "immunocastration" rather than being specific to any particular vaccine formulation used here.

One concern however is that the vaccine was not effective in 5-10 % of the herd. Some individuals could therefore pose some risk during transportation if they are in rut. In this trial one deer produced no measurable immune response despite a total of three injections. This result is consistent with the findings in the previous experiments whereby a portion of the herd (up to 20%) had a poor biological response to the vaccine.

The vaccine efficacy experiment showed that both vaccines were effective in suppressing testosterone, reducing testes, minimising weight loss and aggressive behaviour. The results of Experiment 3 and 4 indicate that the vaccine would be most successfully used in 2 year old stags targeted for slaughter during the earlier part of the breeding season.

In practice however the vaccine is limited in four ways:

- firstly by its short duration of effect which therefore requires the use of multiple doses,
- secondly by the fact that 5-10% of the herd do not respond immunologically or biologically to the vaccine and therefore present problems in terms of behaviour and handling;
- thirdly the cost benefit to farmers in terms of weight and body condition have been shown to be highly variable;
- fourthly the vaccine acts to delay the rut not prevent it, thus limiting farmers to the necessity of sending animals to slaughter that season so to avoid significant weight loss and body condition.

In its current formulation the vaccines tested in this project are not viewed as being suitable for commercialisation for use in deer.

Technical Summary:

1. An immunocastration vaccine was developed using a formulation seen as being most optimal for use in deer.

2. This vaccine and another vaccine supplied by a commercial company have been shown to be effective in reducing testicular function in stags in up to 95% of the herd. Whilst antibody titres remain high, rut induced weight loss and aggressive behaviour can be reduced.

3. The vaccines have been shown to produce no adverse site reactions in fallow deer. The vaccines produced site reactions in the form of burst abscesses or swellings in approximately 26% of the red deer. Given that the red deer also suffered severe site reactions to copper injections, it is suspected that this finding may be a factor of the species rather that being due to a specific component of the immunocastration vaccine.

4. Minimising weight loss and improving productivity was highly variable throughout the trials. No weight benefit was observed for immunocastrated red deer. Immunocastrated fallow deer were 2-5 Kg heavier than non-vaccinated stags whilst the antibody titres remained high.

5. Immunocastration is most effective in 2 year old stags and not appropriate for fully mature stags.

6. Limitations of the vaccines include a short period of vaccine efficacy of 8-10 weeks. The use of the immunocastration vaccine does not act to prevent the onset of the rut but rather delays it. Once vaccine efficacy deteriorates, aggressive behaviour and weight loss can be severe at a time when pastures are good. The net result is that if vaccinates are not slaughtered during the rut, the entire non-vaccinated stags will ultimately be of a higher liveweight and better body condition in comparison to immunocastrates.

7. Other work reported in the literature for immunocastration in deer found similar results in regards to variable weight response and that immunocastration acts to delay the rut rather than prevent it. These reports support the conclusion that these key findings are not specific to any formulation tested here but is an outcome pertaining to any form of LHRH immunocastration.

Implications and Recommendations

1. Immunocastration offers the potential for supplying venison in the earlier months of the breeding season (April-June). The benefits to the farmer is that the opportunity to slaughter is extended until May.

2. Economic gain in terms of liveweight may be highly variable and vaccination may not be of cost benefit to farmers in terms of producing a heavier carcass. Benefits may be in terms of easier handling of deer and having a longer opportunity to send deer to slaughter.

3. In practice the vaccine is limited in four ways:
   - firstly by its short duration of effect which therefore requires the use of multiple doses,
   - secondly by the fact that 5-10% of the herd do not respond immunologically or biologically to the vaccine and therefore present problems in terms of behaviour and handling;
   - thirdly the cost benefit to farmers in terms of weight and body condition have been shown to be highly variable;
   - fourthly the vaccine acts to delay the rut not prevent it, thus limiting farmers to the necessity of sending animals to slaughter that season so to avoid significant weight loss and body condition.

1. In its current formulation the vaccines tested in this project are not viewed as being suitable for commercialisation for use in deer.

2. It is recommended that the industry investigate other avenues for making the provision of year-round venison viable. This may include:
   - Establishing and improving abattoir facilities so that the overheads associated with slaughter of immature stags are minimised.
   - Continued research into the feed quality during the rut and its effect on minimising weight loss especially in the relatively immature 2 year old stags.
   - Continued education for farmers on nutrition and supplementary feeding in particular during the breeding season.

Description of the Project Intellectual Property

Prior Intellectual Property was owned by VIAS and Agriculture Victoria Services Pty Ltd. This project has not generated any subsequent intellectual property.

Total funds and other Contributions:

Corporation funds

	1995/96	*1996/97*	*Total*
Salaries	15,624	15,624	31,248
Travel	1,250	1,250	2,500
Operating	12,330	15,850	28,180
Capital	0	0	0
TOTAL	29,204	32724	61928

Total funds

	1995/96	*1996/97*	*Total*
Total Corporation Funds	29,204	32,724	61,928
Research Organisation	51,666	51,666	103,332
Industry	25,000	25,000	50,000

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Immunocastration in Emus

A report submitted to Rural Industries Research and Development Corporation .

Based on a work project submitted by Tamara Hauke

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2nd Semester

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AIM OF THIS PROJECT

The aim of this project is to determine the effect of immunocastration in emus; to determine if a measurable immune reponse is produced in emus injected with an immunocastration vaccine; to determine if immunocastration has a biological effect on the emus causing a reduction in testosterone, and aggressive sexual behaviour.

INTRODUCTION

Emus are native to Australia and are a flightless bird which are farmed here as an alternative stock rather than the traditional farming of cattle or sheep. Emus produce meat, oil, leather, feathers and offal. The meat is high in protein and very low in fat. The oil from the emus is used for treating a range of ailments. The leather is very characteristic and the leg skin is especially valuable for making jackets, vests and wallets (Cahill 1995).

Emus in Australia have a breeding season from April to September; rather than Spring - Summer as most birds do. The cues used by emus to begin reproduction are largely unknown but are expected to be stimulated by nutritional cues; the shorter daylight hours and cooler temperatures as Autumn/Winter starts. These environmental changes set in action a complex of hormone mechanisms that cause the birds to come into the mating season (Minnaar 1994, Martin, 1994). At the start of the breeding season there are behavioural changes in the bird which are controlled by the change in hormone concentrations. In particular during this time the testosterone concentration increases. The breeding age is between two to three years old (Minnaar 1994).

Most of the fighting and chasing occurs between mid to late summer (Minnaar 1994). Aggression in emus is seldom directed at humans, but usually at other emus, as attempts are made to establish dominance (Minnaar 1994). Some of the fighting may also be due to frustration (Minnaar 1994). During this time the farmers can not touch the birds, yard them or put them in a truck to transport them to be slaughtered because the sexually induced aggression will result in the animals fighting, kicking and pecking each other. Thus handling and transporting the birds presents a high risk of harm to both farmer and the bird and the quality of the carcasses and feathers. Once the breeding season has started most of the mature birds are laying, there is generally very little fighting.

Castration and controlling fertility

Animals are generally castrated for several reasons; to control fertility, to manage and handle more easily, and to eliminate aggressive and/or undesirable behaviours (Sali 1991). Castration for the livestock producer enables easier manageability of male animals and allows females to be marketed

without carcass loss due to pregnancy (Sali 1991). Loss of appetite associated with development of sexual maturity can have significant effect on the weight of carcasses. Castration is often considered as a means of avoiding seasonal changes in appetite (Martin 1994). Castrates also deposit more fat than entire animals - a factor of potential importance to the emu industry where oil is a valuable commodity. For economic and ethical reasons, surgical gonadectomy is not suitable tool for commercial emu farmers (Martin 1994). Castration by use of immunocastration vaccine maybe a more feasible option.

The endocrine system

There are a number of major hormones which control reproductive organs in male and females. In regards to males in particular the sequence of the hormones is as follows:

The hypothalamus releases luteinizing hormone - releasing hormone (LHRH) into the pituitary gland. The pituitary gland synthesises and releases luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH then act on the reproductive organs (testes) to release steroids responsible for spermatozoa development and maturation and for sexual behaviour as shown in **Figure 1** (Sali 1991). The complete sequence of hormones is required for males to become sexually active and fertile. If the sequence is interrupted at any point the end result is that testosterone is not produced, testes do not develop, the animal is infertile and all sexual activity and behaviour including aggressive behaviour will stop. At the Victorian Institute Animal Science (VIAS) we have developed a vaccine which interferes in the hormone sequence by interrupting the production of LHRH. This is the first hormone in the reproduction sequence.

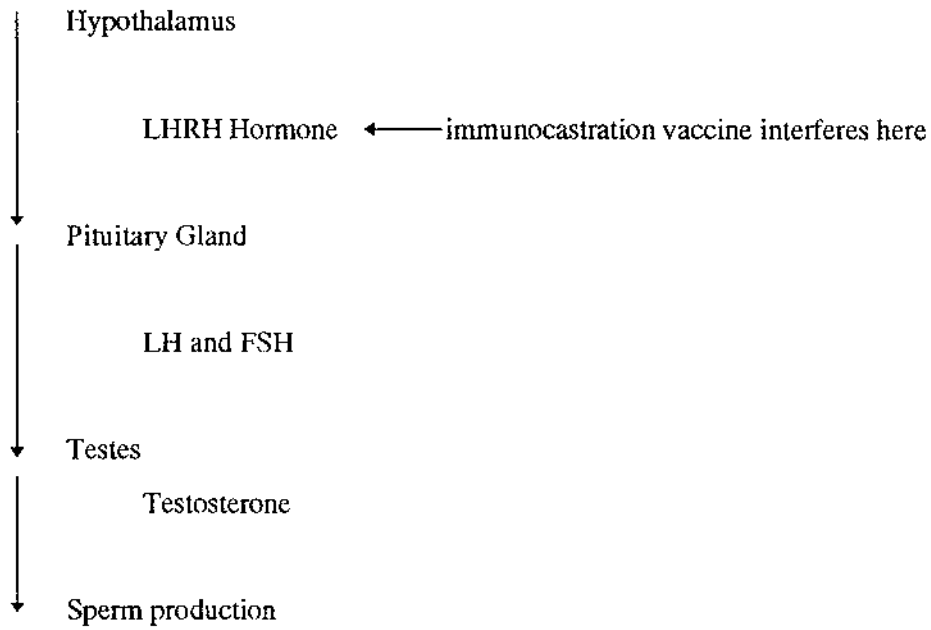


FIGURE 1: Reproduction sequence of hormone production.

In regards to this project, stopping the LHRH hormone will control fertility and sexual behaviour. The result should be that the emus will settle down as if they are in the non-breeding season so that they could be handled or transported.

How the immunocatration vaccine works

Vaccines work by stimulating the body to produce antibodies against the active ingredients of the vaccine. Once produced, antibody production will continue if the same active ingredients are presented in the body. In the case of the immunocastration vaccine, antibody production is stimulated against LHRH hormone. LHRH is a natural hormone which circulates around the body, never normally inducing an antibody response. This is because LHRH is a small molecule, and not big enough to be recognised as a "foreign body".

In order to induce an immune response the LHRH is chemically bound to a large protein molecule. The LHRH - protein "conjugate" is mixed then with suitable adjuvants and is ready for injection. The large conjugate is recognised as "foreign" and immune response is stimulated. Antibodies are produced against the LHRH-protein conjugate, some in particular targeting the LHRH portion. These antibodies will then also interfere with the naturally circulating LHRH breaking it down. LHRH is therefore removed and the reproduction hormone sequence is therefore interrupted.

This project aimed to investigate the immunocastration vaccine for emus.

In this study we aimed to vaccinate a group of farmed emus and investigate the efficacy of the vaccine:

1. To produce an immune response in the emus (ie. induce production of antibodies)
2. To cause the hormonal sequence to be sufficiently interrupted such that testosterone concentration decreases.
3. To cause aggressive sexual behaviour to stop.

MATERIAL AND METHODS

Animals

Sixteen male birds were picked for use in the trial. The emus were supplied by a private farm north east of Victoria (near Healesville). There were eight vaccinated birds and eight non-vaccinated controls, the birds remained agisted on the property. The sex of the bird was determined by the farmer by palpitation of the genitals.

Vaccination Regime

The vaccine was prepared at VIAS in the Endocrinology Department. The vaccine was based on a LHRH-protein conjugate in adjuvant. Each vaccinated emu was given a primary vaccination week 1 (17th January), the second vaccination was given at week 5 (14th February 96) and the third vaccination was given at week 11 (27th March). Before each vaccine was given, the area was swabbed with 70 % hibitane. Each bird received 2.0 ml of vaccine given as a single subcutaneous injection on the right hand side at the top of the drum, using a 21 gauge needle and syringe.

Blood sampling and handling

Each bird had a blood sample taken every 2-3 weeks for a total of 9 weeks. The sample was taken from the jugular vein with a 23 gauge needle and 5.0 ml syringe. The schedule for each bleed and injection is given in **Table 1.** The emus were generally weighed every fortnight in a weigh box provided by the farmers.

The emus were also checked for any site reactions generally every fortnight after vaccination. The site of vaccination was assessed for swelling or abscess and a score was recorded. Site reactions were scored as either 1, 2 or 3 and given a comment and approximate size in centimetres for the actual diameter. A score of "1" was given if the site of injection was slightly swollen and could only be detected by touch. A score of "2" was given if a lump was visible and a score of "3" as given if the reaction had burst open.

TABLE 1: Vaccination and Bleed Schedule

DATE	WEEK	BLEED	VACCINATION
17/1/96	1	1	1st Dose
31/1/96	3	2	
14/2/96	5	3	2nd Dose
28/2/96	7	4	
13/3/96	9	5	
27/3/96	11	6	3rd Dose
10/4/96	13	7	
24/4/96	15	8	
22/5/96	19	9	

Analyses

Sera collected from the blood samples were analysed for Testosterone and anti-LHRH antibody titres. The testosterone analysis was completed using a commercial kit supplied from Pantex (Pantex Testosterone Direct I^{125} cat no. 135). The commercial testosterone assay was modified to include an extraction step to remove any species specific matrix effects.

The anti-LHRH analysis was performed using a direct binding radioimmunoassay whereby doubling dilutions of serum ranging from 1:100 to 1:1600 were incubated with Tritium H^3 labelled LHRH. The titres are expressed as the dilution of serum capable of binding 33% of labelled LHRH.

Testosterone assay method

All reagents were brought to room temperature. 100µl of standards, QCs or samples were pipetted into appropriate tubes. 1 ml of extraction fluid was added (90% Diethyl ether/ 10% ethyl actetate) and the tubes sealed immediately. The tubes were shaken for 15 minutes and from each tube, 250µl of extraction fluid (upper layer) was transferred in triplicate for the standards or duplicate for QCs and samples into a second set of tubes taking care not to disturb the lower phase. The samples were dried under nitrogen for 1 minute.

50µl of I^{125} Tracer was added to all tubes and 100µl of NSB buffer (Non specific binding) to the NSB tubes. First antiserum 100µl was added to the rest of the tubes except the TC's (Total Counts) or the NSB's. The reaction mixture in each tube was vortexed and put into the waterbath for 30 minutes at

37°C then allowed to equilibrate to room temperature for 10 minutes. 250µl of the second antiserum was added to all tubes except the TC's then 500µl of 20% PEG (Polyethylene Glycol 4000). The tubes were mixed and centrifuged for 15 minutes at 2500 rpm (except TC's). The tubes were transferred into foam racks and the supernatant poured off. The radioactivity in each tube was counted on a Gamma Counter for 1 minute per tube. A standard curve was constructed using "Riacalc" software program. The minimal detectable concentration of the assay was 0.27±0.16nM calculated by Riacalc over 28 assays. Intraassay variation is <10%. Interassay variation is monitored by the use of 3 lyophilised human serum commercial controls (Bio-Rad, Series 40030). Data collected from 28 days show the following coefficients of variation.

	QC1	**QC2**	**QC3**
Mean ± SD	3.12 ±0.38	19.21± 2.18	45.20 ± 4.50
%CV	12.11	11.34	9.96

Anti-LHRH assay

The ANTI-LHRH assay was performed using a direct bind radioimmunoassay. In the assay a number of serial dilutions (1:100 to 1:1600) were prepared then pipetted (200µl) in duplicate into 5 ml flat bottom tubes. 100µl of tracer (Tritium H^3) then 100µl Human Gamma Globulin were added to each tube. The tubes were incubated for 48 hours at 4°C. After the incubation was complete 100µl of Bovine Gamma Globulin and 1ml of the 18% PEG (Polyethylene Glycol 6000) were added. The tubes were spun at 3000 rpm for 10 minutes . The tubes were poured off and 500µl 0.1M NaOH was added. The tubes were mixed and 4 ml scintillant was added. The tubes were capped then counted on the Wallace 1410 scintillation counter, 1 minute for each tube. The percentage of tracer bound against the serum dilution is plotted using Excel microsoft computer package and the reciprocal dilution at which 33% of the tracer is taken as the anti-LHRH titre. The intraassay precision is <7%. Interassay precision was monitored by use of 2 inhouse QC sera. Over 55 assays the following coefficients of variation were recorded.

	QC1 titre % binding	**QC1Titre**	**QC2 Titre**
Mean ± SD	60±3.0	1135±135	939±95
% CV	5.7	11.9	10.1

The minimal detectable concentrations as it applies to other analytes is not applicable for our purpose. Any test sera where there is clearly no antisera are reported as having a titre <20. For an animal to be

effectively immunocastrated by the LHRH antibodies it needs a titre of at least 400. We interpret any titre of <400 as indication of complete suppression of LHRH

RESULTS

Behaviour and Handling emus

The emus were yarded by putting a little bit of feed out for them along the lane way. The farmer then slowly walked behind the birds to encourage them along the path. This way of moving them created no problems on the trial because they would always willingly walk down the lane. For the first few visits the emus were handled outside in a holding yard. Each bird was caught and then restrained by the farmer so that a blood sample and /or vaccination could be given.

The emus were handled by the farmer who would grab the bird quickly and firmly from the side or behind and then hold the bird firmly against his own body, keeping one arm across the breast bone and holding the emu around the chest, the other hand holding onto a wing. This method generally kept the birds still long enough for vaccinating and bleeding. In addition a second person would hold the emus head while the blood sample was. However within Bleed 3 Week 5 the birds were becoming familiar with the procedure and less inclined to remain still. At this point it was decided to utilise the weigh-box in order to prevent the emu from trying to run away. The weigh-box (one actually designed for purpose of weighing deer) had a lid which when opened let the emus stand in the box with their neck and head sticking out. After weighing each bird in the box, the farmer would also stand in the box and restrain the bird. The confines of the surrounds resulted in most birds being much quieter and we were able to easily bleed the birds from the outside of the box.

Two emu in particular (Y18 and Y14) were difficult to handle from the beginning of the trial. These birds were extremely sensitive to touch and often would jump as soon as they were touched by the bleeding needle. At several farm visits no blood sample could be taken from these birds. In general the other emus in the trial could be handled without difficulties. On some visits a blood sample was not taken if the emu would not remain still and the emu was at risk of becoming stressed or developing a haematoma. **Table 2** shows for each bleed which blood samples could or could not be obtained for either the vaccinates or control groups. The table indicates that the control group was in general more difficult to bleed.

Although the emus had a tendency to jump or kick whilst restrained they showed no other aggression to the farmer or handlers. No aggression between the emus was observed through the trial when the birds were being yarded or left together in the pens.

TABLE 2: Blood sample Taken or Not Taken from each Trip

Group	Leg Tag	Bld 1 17/1 V1	Bld 2 31/1	Bld 3 14/2 V2	Bld 4 4/3	Bld 5 13/3	Bld 6 27/3 V3	Bld 7 18/4	Bld 8 1/5/	Bld 9 22/5
VACC	Y124	Y	Y	NO	Y	Y	Y	Y	Y	Y
VACC	Y11	Y	Y	Y	Y	Y	Y	Y	Y	Y
VACC	Y0130	Y	Y	Y	Y	Y	Y	Y	Y	Y
VACC	B18	Y	Y	Y	Y	Y	Y	Y	Y	Y
VACC	Y19	Y	Y	Y	Y	Y	Y	Y	Y	Y
VACC	Y13	Y	Y	Y	Y	Y	Y	Y	NO	Y
VACC	R0007	Y	Y	Y	Y	Y	Y	Y	Y	Y
VACC	Y18	Y	Y	Y	NO	Y	NO	NO	NO	NO
CONT	Y14	Y	Y	Y	NO	NO	Y	NO	NO	NO
CONT	Y16	Y	Y	NO	NO	Y	Y	Y	Y	Y
CONT	RL15	Y	Y	Y	NO	Y	Y	Y	NO	Y
CONT	Y17	Y	Y	Y	NO	Y	NO	Y	Y	Y
CONT	Y15	Y	Y	Y	Y	Y	Y	Y	NO	Y
CONT	B17	Y	Y	Y	Y	Y	Y	Y	Y	Y
CONT	R6	Y	Y	Y	Y	Y	Y	Y	Y	Y
CONT	R4	Y	Y	NO	NO	Y	Y	NO	NO	Y

Y= Blood Taken; No= No Blood Taken

The blood samples were taken from the right hand side jugular vein of the neck since it is substantially bigger than the jugular vein found on the left hand side of the neck. To occlude the vein the skin was pulled down tight and the vein occluded with the thumb in the groove of the neck.. A small gauge needle (size 23) and syringe was essential as the emus were prone to haematomas. In addition the hole in the skin created from bleeding (and vaccination) was slow to close and had to be encouraged by rubbing the area with the finger.

Site reactions

No site reactions were detected when all emus were checked two weeks (Week 3, Bleed 2) after the first vaccination. The emus were thereafter randomly checked each farm visit for the presence of a reaction (burst or unburst abscess) at the sites of injection. No site reactions were detected at Bleeds 2, 3, 4 and 5. Refer to **Table 3.**

At Bleed 6, Week 11, when the birds were given their third vaccination, one site reaction was found in the form of a burst abscess which had healed into a hard scab. At Bleed 8, Week 15, four weeks after the 3rd vaccination, 7 of the 8 emus had site reactions. This result was unexpected since only one site reaction had been detected from any of the previous injections. The same batch of vaccine was also given to the fallow deer on the same farm, and out of 40 deer only 2 deer had site reactions.

TABLE 3: Site reactions observed in emus at each farm visit.

Group	Leg Tag	Bld 1 17/1 V1	Bld 2 31/1	Bld 3 14/2 V2	Bld 4 4/3	Bld 5 13/3	Bld 6 27/3 V3	Bld 7 18/4	Bld 8 1/5/	Bld 9 22/5
VACC	Y124	N/A	0	NT	NT	NT	3a	3b	3b	3b
VACC	Y11	N/A	0	NT	NT	NT	0	0	3b	3b
VACC	Y0130	N/A	0	NT	NT	NT	0	0	3b	3b
VACC	B18	N/A	0	NT	NT	NT	0	0	0	3b
VACC	Y19	N/A	0	NT	NT	NT	0	0	3b	3b
VACC	Y13	N/A	0	NT	NT	NT	0	0	0	0
VACC	R0007	N/A	0	NT	NT	NT	0	0	3b	3b
VACC	Y18	N/A	0	NT	NT	NT	0	0	3b	3b
CONT	Y14	N/A	0	NT	NT	NT	0	0	0	0
CONT	Y16	N/A	0	0	0	0	0	0	0	0
CONT	RL15	N/A	0	0	0	0	0	0	0	0
CONT	Y17	N/A	0	0	0	0	0	0	0	0
CONT	Y15	N/A	0	0	0	0	0	0	0	0
CONT	B17	N/A	0	0	0	0	0	0	0	0
CONT	R6	N/A	0	0	0	0	0	0	0	0
CONT	R4	N/A	0	0	0	0	0	0	0	0

N/A = Not applicable; NT = Site Reaction Not taken; 0 = No site reaction; 1 = Unburst abscess, not visible, can be felt by palpating area; 2 = Unburst abscess, visible; 3 = Burst abscess; A = 8x10 cm size; B = 8x5 cm size.

Weight

The weights of the emu were monitored for the duration of the trial to determine whether there was a weight gain or decrease between the vaccinated birds and the controls.

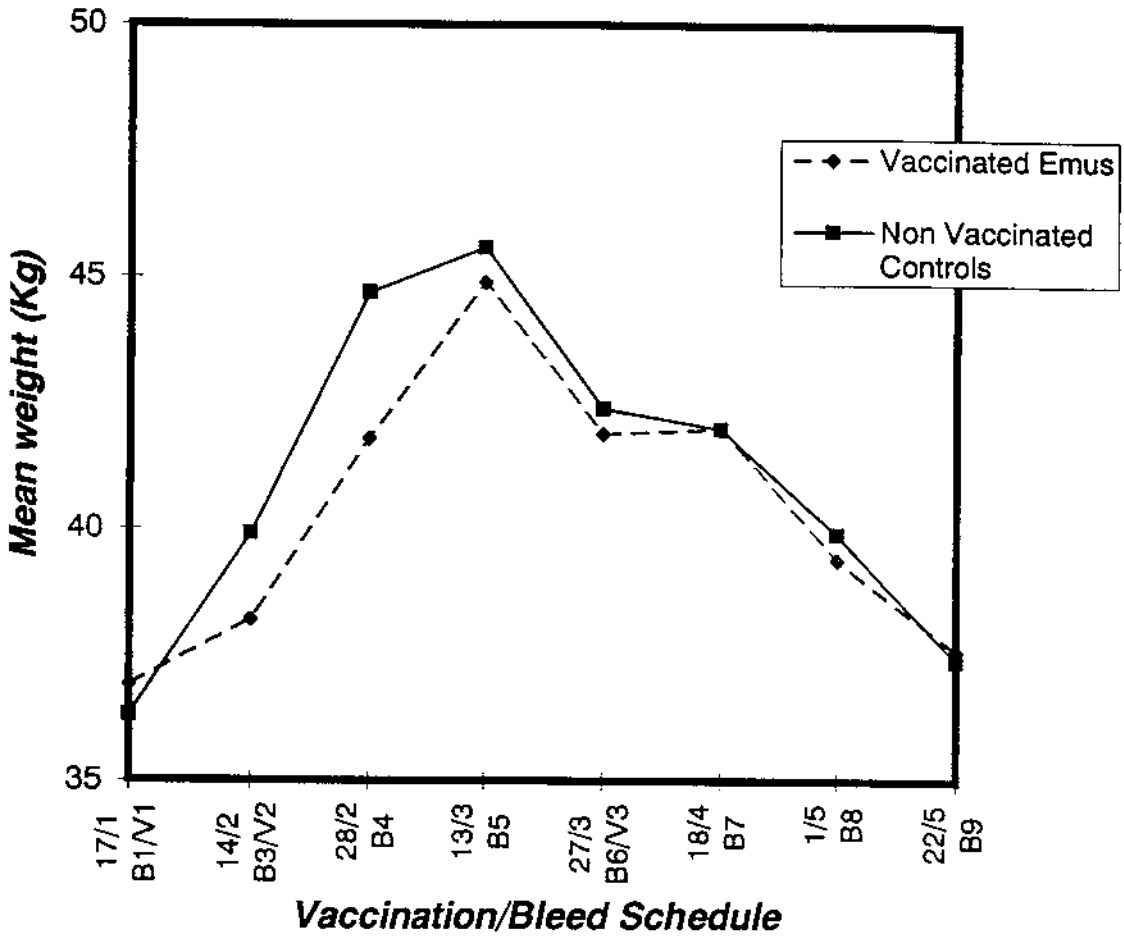
Table 4 shows all the weights taken in the trial plus mean and standard deviation for each week. The mean weights of the vaccinates and controls are illustrated in **Figure 2**. The graph shows that for most weeks no weight difference could be detected between vaccinates and controls. The possible exception was weeks 3 and 4. A single anova was done on Bleeds 3, 4 and 5. It showed that there was no statistically significant difference between the vaccinates and the controls for these weeks ($P>0.05$ for all groups). Thus the vaccine had no effect on the weight gain or loss.

Table 4: Fortnightly weights (Kg) taken at the farm.

Group	Leg Tag	Bld 1 17/1 V1	Bld 2 31/1	Bld 3 14/2 V2	Bld 4 4/3	Bld 5 13/3	Bld 6 27/3 V3	Bld 7 18/4	Bld 8 1/5/	Bld 9 22/5
Vacc	Y124	34.5	NT	35	41.5	41	36.5	38	34.5	34.5
Vacc	Y11	38	NT	39.5	40	49.5	46	45.5	43	41
Vacc	Y0130	37	NT	38.5	41	43.2	40.5	40	39	37
Vacc	B18	39	NT	40	45.5	46	43	43.5	40	38.5
Vacc	Y19	37.5	NT	38	38	44	41	42	39	37
Vacc	Y13	36.5	NT	38.5	45.5	46.5	44	44	40	38
Vacc	R0007	37.5	NT	42	45	49	46	46	43.5	42
Vacc	Y18	35.5	NT	34	38	40.5	38	37	36.5	33
Cont	Y14	41.5	NT	44	48	48.5	43	NT	45.5	37
Cont	Y16	37.5	NT	41.5	44.5	47	43.5	44.5	40.5	41
Cont	RL15	33	NT	37	43	42	40.5	40	37.5	35
Cont	Y17	31	NT	34	38	39.5	36	35.5	33.5	31
Cont	Y15	38.5	NT	40.5	43	44.5	41	42	40	37.5
Cont	B17	36	NT	39.5	44.5	44.5	40.5	41	38.5	36.5
Cont	R6	35.5	NT	41	48.5	48	47.5	45	41.5	40
Cont	R4	37.5	NT	42	48.5	51	47	46	42.5	41
Av VACC		36.94	NT	38.19	41.81	44.96	41.88	42.00	39.44	37.63
Std Dev		1.42	NT	2.60	3.17	3.38	3.51	3.37	3.01	3.01
Av CONT		36.3	NT	39.94	44.75	45.63	42.38	42.00	39.94	37.38
Std Dev		3.26	NT	3.13	3.60	3.74	3.76	3.62	3.58	3.39

NT= Weight Not Taken

Figure 2: Mean Weight of Emus Through The Trial



Testosterone Results

All blood samples were analysed for testosterone concentrations, results are outlined in **Table 5**. Testosterone concentrations were expected to rise in the controls as the birds moved into the breeding season. Active testes should produce circulating testosterone concentration above 1.8 nM (Martin, Date Unknown). We had also anticipated that immunised birds would have a lower testosterone concentration compared with the controls as the vaccine began to take effect. Contrary to these expectations, apart from the random result, the controls at no stage of the trial gave any indication of achieving circulating testosterone above 1.8 nM. Similarly all vaccinates also had low testosterone for the full duration of the trial. The testosterone results of the vaccinated emus could not be used to determine the effectiveness of the vaccine throughout the trial, since the controls also had low testosterone concentrations as well.

TABLE 5: Testosterone concentrations (nM) in Vaccinated and Non-vaccinated control emus.

Group	Leg Tag	Bld 1 17/1 V1	Bld 2 31/1	Bld 3 14/2 V2	Bld 4 4/3	Bld 5 13/3	Bld 6 27/3 V3	Bld 7 18/4	Bld 8 1/5/	Bld 9 22/5
Vacc	Y124	<0.5	0.9	NT	1	0.7	<0.5	<0.5	<0.5	0.7
Vacc	Y11	<0.5	<0.5	<0.5	0.5	0.5	<0.5	<0.5	<0.5	<0.5
Vacc	Y0130	<0.5	<0.5	<0.5	1.2	0.5	0.7	<0.5	<0.5	<0.5
Vacc	B18	<0.5	<0.5	1.98	<0.5	<0.5	<0.5	1	<0.5	0.5
Vacc	Y19	<0.5	<0.5	<0.5	<0.5	<0.5	1.2	1.1	<0.5	<0.5
Vacc	Y13	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	NT	<0.5
Vacc	R0007	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0.9	<0.5	<0.5
Vacc	Y18	<0.5	<0.5	1.53	NT	<0.5	NT	NT	NT	NT
Cont	Y14	<0.5	<0.5	<0.5	NT	NT	1.5	NT	NT	NT
Cont	Y16	<0.5	<0.5	NT	NT	0.8	0.8	0.8	3.2	0.8
Cont	RL15	<0.5	<0.5	<0.5	NT	<0.5	0.8	<0.5	NT	3
Cont	Y17	<0.5	<0.5	<0.5	NT	<0.5	NT	0.95	<0.5	<0.5
Cont	Y15	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	NT	<0.5
Cont	B17	<0.5	<0.5	1.06	0.6	<0.5	<0.5	1.8	<0.5	1.1
Cont	R6	<0.5	<0.5	0.64	0.6	3.8	1.3	0.6	1.1	5.3
Cont	R4	<0.5	<0.5	NT	NT	0.5	1	NT	NT	<0.5

NT= Blood Sample Not Taken

Anti-LHRH Results

LHRH antibody titres give an indication of the strength of the immune response to vaccination. On the basis of previous work done in this laboratory on dogs, pigs and deer a titre of 400 is considered as the lower limit for a meaningful antibody response (ie immune response capable of producing a lasting biological effect).

Where animals have not been vaccinated and no antibodies are detectable, a titre of <20 is recorded. The anti-LHRH titres measured for each emu are given in **Table 6.** All controls tested had titres <20 as expected. In general the immune response in the vaccinated emus after 2 doses of vaccine was weak. Although measurable titres were recorded most results were below 400 (ie between 100-400). Seven out of the eight emus showed a titre above 400 two weeks after the 3rd vaccination. This is 100% of all the blood samples taken. No blood sample was able to collected from the eighth bird due to handling difficulties. Three birds still maintained titres above 400, at 6 weeks after the 3rd vaccination indicating that 3 injections were required to induce a strong titre in any of the emus.

TABLE 6: Anti-LHRH titres measured in Vaccinated and Non-Vaccinated emus.

Group	Leg Tag	Bld 4 4/3	Bld 5 13/3	Bld 6 27/3 V3	Bld 7 18/4	Bld 8 1/5/	Bld 9 22/5
Vacc	Y124	204	182	107	634	379	234
Vacc	Y11	164	163	77	>3500	2417	783
Vacc	Y0130	402	377	233	2695	981	442
Vacc	B18	161	115	<20	407	269	118
Vacc	Y19	<20	320	<20	632	<20	549
Vacc	Y13	190	382	43	499	NT	88
Vacc	R0007	273	423	132	775	488	226
Vacc	Y18	NT	832	NT	NT	NT	NT
Cont	Y14	NT	NT	<20	NT	NT	NT
Cont	Y16	NT	<20	<20	<20	<20	<20
Cont	RL15	NT	<20	<20	<20	<20	<20
Cont	Y17	NT	<20	NT	<20	<20	<20
Cont	Y15	<20	<20	<20	<20	<20	<20
Cont	B17	<20	<20	<20	<20	<20	<20
Cont	R6	<20	<20	<20	<20	<20	<20
Cont	R4	NT	<20	<20	NT	NT	NT
Median Titre		**197**	**348**	**107**	**633**	**488**	**234**

NT = Blood Sample Not taken

DISCUSSION

The aim of the trial was to see if emus would respond to the immunocastration vaccine. This response was to be quantified by measurement of serum anti-LHRH antibody titres, any resulting biological effect was to be monitored by testosterone concentrations and observation of behaviour.

The use of emus as an alternative farming stock is still relatively uncommon in Australia. As a result, research into emus and associated farm practices is also uncommon and no experienced animal experimenters for emus existed at VIAS. In addition most farmers are not equipped to frequently handle the birds nor are the emus used to frequent yarding and handling. Thus the project presented some unique difficulties in simply completing the tasks required. The problem of inexperience was overcome by receiving training at the first farm visit by Dr David Middleton, a veterinarian who has specialised in care of native Australian species. Dr Middleton provided training and advice on holding, vaccinating and bleeding the emus.

All the emus were farmed as a single herd and most were untagged at the beginning of the trial, resulting in the farmer having no records on the exact age of each bird selected. The farmer determined the sex of each bird by palpating the genital area. Unknown to us at the beginning of the trial, the age of the birds would be of high importance since measurable testosterone is not seen in male birds until they are more than 2 years old.

The trial was conducted at the start of the breeding season at a time when the testosterone concentrations in mature males should be high. It was anticipated that the vaccine would have a biological effect on the emus causing a reduction in testosterone concentrations compared with the controls. Contrary to our expectations neither vaccinates or controls through the trial had high testosterone concentrations indicating active testes. Without evidence of an active testes we therefore had no evidence of the suppression of the testes in response to the vaccine. The reasons for this outcome are not known. It is likely however that the birds were immature (<2 Years Old) and thus not yet capable of having high circulatory testosterone concentrations. This suggestion was presented to the farmer who confirmed that it was possible for the birds to have been younger than anticipated. Thus whilst the testosterone should have been an indicator of the vaccine working, in this case, because the birds were not mature and their testosterone concentrations were low, we could not use testosterone as an indicator of the vaccine efficacy.

Monitoring the biological effect of the vaccine was therefore limited to observation of behaviour and weight. There was however no strong evidence that the control emus were more aggressive or difficult

to handle in comparison to the vaccinates. The emus were easily yarded since they readily followed a trail of seed left in the yarding lanes and were easily coxed to walk forward if the farmer slowly followed them from behind. All the emus were consistently reluctant to be held or pushed into the weigh box. Manageability could only be monitored in regards to the ease of taking blood samples. Some indication was given that the control group were more difficult to bleed than the vaccinated group and that this difference noticeably emerged once the emus were well into the breeding season. It is not possible on the basis of this single observation, however to conclude that the vaccine did produce a biological effect and reduce aggressive behaviour. Each emu's response to being handled changed each week possibly because of other stresses such as being handled, yarded or even outside environment controls (Temperature, presence of pests within the farm). These are examples of the uncontrollable factors which can make individual emus more difficult to handle on any particular occasion. We did not observe otherwise any aggression by the emus towards ourselves; an observation supported by Minnaar (1994) who states that aggression is seldom directed at humans. In addition we did not observe within the yards or field any fighting or aggressive behaviour between any of the emus. Thus the controls were observed to have the same relatively passive behaviours as the vaccinates.

In regards to weight control the vaccine did not effect the emu's weight shown by the fact that the vaccinated and non-vaccinated emus had no statistically significant difference in their weights. Both groups showed weight gain until mid-March whereby weights steadily decreased over the next 2 months in line with expectations for the breeding season and poorer pastures. The results indicates that the immunocastsration vaccine is not likely to be a useful tool for managing weight loss over the breeding season.

Despite the lack of biological effect, the vaccines did however induce a measurable immune response in the vaccinates with 3 out of 8 showing a sustained titre for 6 weeks after 3 injections. Whilst such results are promising a successful commercial vaccine would require vitally 100% response by the herd for the full breeding season. This was not indicated in this trial. Secondly the need for 3 injections is unsuitable to farmers, because the birds would have to be handled 3 times each. Alternatively if the vaccine could be made into a slow release vaccine, this would be better for the farmers.

The high incidence of site reactions after the third injection compared with virtually none being observed after the previous 2 injections remains unexplained. All doses of vaccine had also been administrated to fallow deer as well and virtually no site reactions were detected at any stage in any of the fallow. The only difference observed between administration of the third dose compared with the first and second injections is in regard to the positioning of the birds. In the first and second injections the birds were

held outside in the yards. Access to the drum as the bird was vaccinated was good and the emus could be easily swabbed. The third injection was given with the birds in the weigh box. It was necessary for the scientist to lean down over the weigh box wall to give the injection. It maybe possible that the extra difficulty in swabbing the area, lifting the skin to give a subcutaneous injection and then ensuring the hole closed over, had an undefined effect on the development of infection. It is not possible however to draw a conclusion at this point.

CONCLUSION

In this project we set out to investigate if we could get an immune response in emus to the immunocastration vaccine developed at VIAS. The results indicated that an immune reponse (i.e production of anti-LHRH antibodies) was produced in all vaccinated emus after 3 injections and that three out the eight emus held a good titre for at least six weeks after their third vaccination. These results were very promising since the immunocastration vaccine has never been tested in any avian species including emus. The project also aimed to determine if the immunocastration vaccine could produce a biological effect and cause a reduction in testosterone and aggressive behaviour. The testosterone results remained low for the duration of the trial most likely because the emus were not mature and their testosterone levels were not where a mature bird (bird with active testes) testosterone levels are, at least 1.8nM. The emus never showed any aggression to each other or to the handlers apart from some difficulty in bleeding. Biological effect in regards to aggression was also difficult to monitor since the testosterone levels were low. This project was aimed as a preliminary trial investigating the use of immunocastration vaccine in emus. A positive reponse would enable the Institute or commercial partner producing the vaccine to embark on more extensive research with more emus and farms. These results provide a suggestion of success although a commercial vaccine will need to capture 100% reponse lasting for at least 3-4 months. Most likely a single administrative slow release vaccine would be most suitable to farmers to minimise handling of the emus. These results will therefore be presented to our funding body and commercial partner for their consideration and will form the basis for future research in emus if it is decided that the vaccine has potential.

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