Immunisation against gonadotrophin-releasing hormone (GnRH) reduces agonistic behaviours in male rangeland goats

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Abstract

Rangeland goat bucks were used to evaluate the efficacy of a commercially available anti-gonadotrophin-releasing hormone vaccine, Improvac (Zoetis Australia, West Ryde, NSW, Australia). The hypothesis tested was that immunisation would suppress testosterone secretion by the testis and agonistic behaviour between male goats. We also compared intervals of 2 and 4 weeks between primary and booster immunisations and monitored responses over a 2-month period. The 45 goats were split into three groups (n = 15): one group receiving the vaccination booster on Day 14, one group receiving the vaccination booster on Day 28, and the Control group receiving sterile saline injections. Body mass, body condition score and scrotal circumference were measured fortnightly, and blood was collected at 2-week intervals and analysed for testosterone concentration. Behavioural interaction tests of 2-min duration were also conducted fortnightly. There was a significant decrease in paired testicular circumference ($P < 0.05$) and testosterone concentration ($P < 0.01$) in both vaccination groups by the end of the experiment at Day 60, compared with the Control group. Agonistic interactions measured at Day 60 were significantly reduced in both vaccination groups ($P < 0.05$) compared with the Control group. These results support the efficacy of Improvac in reducing agonistic behaviours in rangeland goat bucks and suggest that the use of the vaccine may assist in reducing social stress and possible injury in groups of confined male goats.

Additional keywords: behaviour, goat, testosterone, vaccine.

Introduction

Rangeland goats are a composite breed of goat that are undomesticated and inhabit the rangeland regions of Australia. They are a significant environmental problem (Coblentz 1978), yet concurrently represent a valuable resource for meat production. Agonistic behaviour with new introductions or mixing (Cowley and Grace 1988), and difficulty with husbandry, have proven to be significant challenges to intensive rearing of rangeland goats to overcome seasonal supply problems of captured wild goats, and this is especially so for intact male goats. Entwhistle and Jephcott (2005) reviewed the problem for live export of aggressive behaviour of entire male goats (goats as well as sheep and cattle) and concluded that aggression is a leading cause of mortality in goats and cited that this aggression is most pronounced in feral animals. For the rangeland goat industry in Australia to progress we need methods to avoid the issues associated with aggression to improve husbandry and welfare.

One of the functions of the testis is to produce testosterone, which increases male sexual and agonistic behaviour. For the past 20 years researchers have explored different ways of castrating animals to create maximal production, while also maximising welfare and market

specifications. Sherwood *et al*. (1993) found that structure and function of gonadotrophin-releasing hormone (GnRH) was conserved across species and that suppression of GnRH at the hypothalamic axis, via antibody induction, decreased the concentration of testosterone released and consequently the function of the gonads. Products which induce antibodies to GnRH are now widely used in the agricultural sector, with further applications being investigated for the equine industry and even human medicine (Thompson 2000). In 1996, Godfrey and colleagues completed a study investigating the efficacy of the then commercially available GnRH vaccine Vaxstrate (Arthur Webster Pty Ltd, Castle Hill, NSW, Australia) on rangeland goats and found it to be effective at reducing antagonistic behaviour (Godfrey *et al*. 1996). However, Vaxstrate is no longer commercially available. Since then, little work with rangeland goats has been completed, and with renewed interest in the export industry and new drugs on the market, current efficacy of available products is worthy of investigation.

The GnRH vaccine, Improvac (Zoetis Australia, West Ryde, NSW, Australia) is registered for use in pigs and has proven efficacy on decreasing agonistic behaviours and reducing odour in boars, with the additional benefit of no withholding period (Albrecht *et al*. 2012). We tested the efficacy of Improvac as a method of suppressing agonistic behaviours in Australian rangeland goats. Improvac is currently registered for use in pigs with a protocol of an initial vaccination followed by a booster after a 28-day interval. However, the optimal period required between the primary immunisation and booster anti-GnRH immunisations has not been fully addressed in the literature for any species, but Godfrey *et al*. (1996) found that a 14-day booster protocol was as effective as a 28-day protocol when using Vaxstrate to immunise goats. We examined the efficacy of Improvac in goats with a booster after 14 days in addition to the 28-day interval, with an aim of minimising the time needed for domestication. Our hypothesis was that Improvac would reduce testicular size, decrease circulating testosterone concentration and decrease agonistic behaviour in males.

Materials and methods

Ethical approval was obtained through the Murdoch University Animal Ethics Committee (Animal ethics permit number: R2459/12).

Animals

In late March, 45 Australian rangeland goats (*Capra hircus*), weighing on average 33.2 ± 0.8 kg (±s.e.m.; range: 25.5–44.0 kg), were selected from ~200 goats trapped at a water source over a period of 2 days, using a swinging one-way gate trap, on a station near Mt Magnet in Western Australia. The estimated age of the goats, based on dentition, was between 9 and 15 months. The goats were then immediately transported to the test site at the Murdoch University Veterinary Farm in Perth, Western Australia, an 8-h journey. Prior to transport, goats spent 1 h in a zinc sulfate foot bath. On arrival at the test site at the Murdoch University Veterinary Farm in Perth, Western Australia, goats were given 3 days to recover from travel and acclimatise to new conditions. They received a 1-mL Glanvac 3 in 1 vaccine subcutaneously providing protection against *Clostridium tetani*, *Clostridium perfringens* type D and *Corynebacterium pseudotuberculosis*, a 15-mL Baycox (Toltrazuril; Bayer AG, Leverkusen, Germany) drench for coccidia, administered orally, a 16-mL Cydectin (Moxidectin; Virbac, Milperra, NSW, Australia) for internal parasites, administered orally, and a Clout S (Deltamethrin; Coopers, Sydney, NSW, Australia) backline for lice. A booster dose of 1 mL and 16 mL, for the Glanvac 3 and Baycox, respectively, were given at 4 weeks. The goats were individually identified with numbered ear tags and photographs were taken to allow identification

on the video clips based on their coat colour patterns. They were fed good quality roughage in the form of hay and *ad libitum* feed pellets as used in pre-export feedlots, with free access to water until commencement of the experiment. The pellets contained 92.3% dry matter (DM), 11.9% crude protein and 10 MJ/kg DM of metabolisable energy (ME). The roughage was provided in the form of half a small square bale (~10 kg) of oaten hay in each pen weekly for the first 3 weeks to provide roughage and allow acclimatisation to the pelleted feed. This was then reduced to a quarter of a small square bale (~5 kg) in each pen weekly for the remainder of the trial to promote intake of pellets while still providing some long stem roughage. The hay contained 90.1% DM, 6.4% crude protein, 8.6 MJ/kg DM of ME, 33.6% acid detergent fibre and 63.0% neutral detergent fibre. Group intake in the pens was measured by subtracting the refusal from the amount of pellets given daily. Goats (*n* = 7 or 8 per pen) were kept in 6.6 m × 6.6-m group pens at Murdoch University Veterinary Farm for the duration of the trial, providing 5.4 m^2per animal. There were two feed troughs of 2-m length in each pen, providing at least 0.57 m of trough space per animal. The individual pens were separated from each other by ~2 m, and they were provided with shade cloth around the windward corner of the pen for shelter, which also restricted vision between pens.

Distribution into experimental groups

The 45 individuals were allocated to two experimental groups (E1 and E2: *n* = 15 each) and one Control group (C: *n* = 15). Each group was then further split in two (*n*= 8 and *n* = 7) to increase the sample size for the group behavioural interaction tests, therefore forming groups E1a, E1b, E2a, E2b and Ca and Cb. Animals were placed in groups based on their presentation order when moved into the drafting raceway (an indication of dominance ranking; Houpt 2011), body condition score (BCS) and weight, aiming to produce pens of similar weight and dominance tendency. The treatments for groups during the trial were as follows:

- Experimental group one (E1) received vaccine (Improvac 2 mL, subcutaneously) at Day 0 and Day 14 (Week 2);
- Experimental group two (E2) received vaccine (Improvac 2 mL, subcutaneously) at Day 0 and Day 28 (Week 4); and
- C received 2 mL sterile saline subcutaneously at Day 0, Ca on Day 14 (Week 2) and Cb on Day 28 (Week 4).

Measurements

The BCS, bodyweight and paired testicular circumference were measured fortnightly. To maintain consistency, measurements were always performed by the same assessor. BCS was measured by palpation of the short ribs and spinal processes using the 1–4 scale (1 being 'thin' and 4 being 'fat') of the NSW Department of Agriculture scale (based on Mitchell 1986). Body mass was recorded using battery-operated weigh scales. Scrotal circumference was measured using a measuring tape placed around the widest circumference of the paired testes after manipulating them to the bottom of the scrotum.

Blood (5 mL) was collected via jugular venipuncture using a 24-gauge needle on an EDTA-coated Vacutainer fortnightly. Samples were centrifuged and plasma collected and frozen in Eppendorf tubes to use for measurement of plasma testosterone concentrations using a commerical ELISA kit (Parameter Testosterone, R and D Systems, Minneapolis, MN, USA).

Behavioural interaction was measured weekly by mixing groups of four randomly selected animals selected from each of the Ca, Cb, E1a, E1b, E2a and E2b groups, with the mixing of two groups at any one time, for example Ca versus E1b. Animals were placed in a 4.4 × 4.4-m pen for 2 min and filmed using two video cameras placed at different angles so that all animals could be identified. These films were later reviewed by an individual who was blinded to the groups, and agonistic contacts scored for each animal (head to head, head to body and mounting).

Statistical analyses

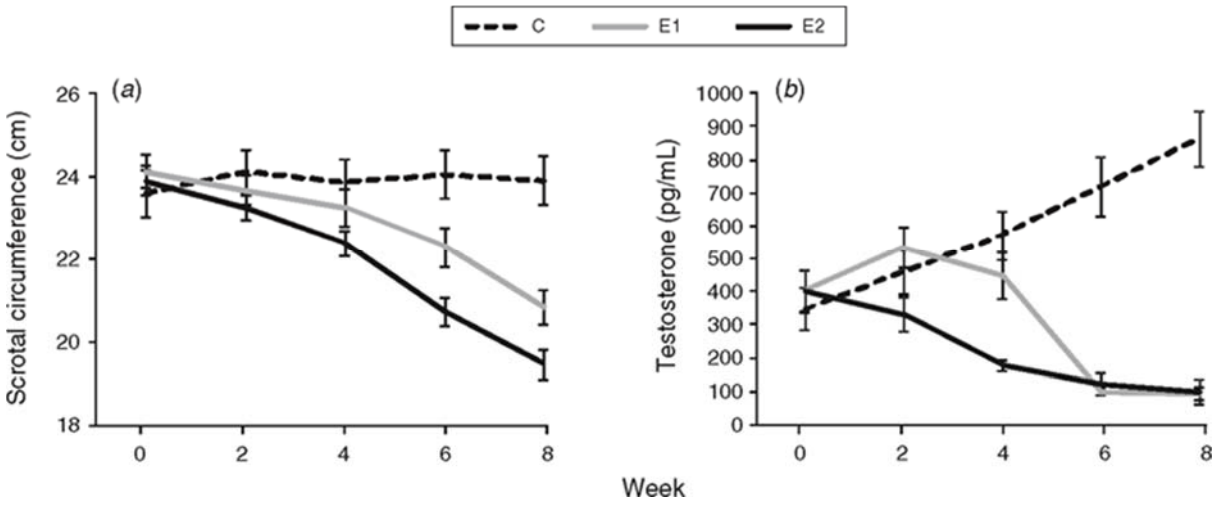
Repeated-measures ANOVA was performed on all data except the agonistic behaviour data, which was analysed by factorial ANOVA. Treatment was included as the fixed factor and animal (age, weight) were included as the random factors. Fisher's protected least significant difference was used for post-hoc analysis.

Results

Testicular size

Overall, there was a significant effect of treatment ($P < 0.0001$), time ($P < 0.0001$) and the interaction between treatment and time ($P < 0.0001$) on testicular size, as measured by scrotal circumference. There was no difference in testes size in the three treatment groups at the start of the experiment or at Week 2, and the testes of the C group did not change in size over the 8 weeks (Fig. 1*a*). At Week 4, the testes of the E2 group were ~6% smaller than those in the C group ($P < 0.05$). At Week 6, the testes of the E1 and E2 groups were both smaller than those in the C group, 5% ($P < 0.05$) and 14% ($P < 0.0001$), respectively, and the E2 group was significantly different to the E1 group ($P < 0.05$). At Week 8, the testes of the E1 and E2 groups were both smaller than those in the C group, 12% ($P < 0.0001$) and 18% ($P < 0.0001$), respectively, and the E2 group was significantly different to the E1 group ($P < 0.05$).

Fig. 1. (*a*) scrotal circumference (cm) and (*b*) plasma testosterone concentration (pg/mL) in the goat bucks in the Control (C: dashed line, $n = 15$), anti-gonadotrophin-releasing hormone (GnRH) vaccine with 14-day booster (E1: grey line, $n = 15$) and anti-GnRH vaccine with 28-day booster (E2: black line, $n = 15$) treatment groups. Values are means ± s.e.m.



Circulating testosterone concentration

Overall, there was a significant effect of treatment ($P < 0.0001$), time ($P < 0.0001$) and the interaction between treatment and time ($P < 0.0001$) on the circulating concentration of testosterone. The concentration of testosterone in the three treatment groups of goats was not different at the start of the experiment or at Week 2 (Fig. 1*b*). Over the 8 weeks there was a 1.6-fold increase of testosterone concentrations in the C group ($P < 0.0001$) and a 1.4-fold decrease in the E1 and E2 groups ($P < 0.0001$). The testosterone concentration in the E1 group was significantly lower than the C group at Weeks 6 and 8 ($P < 0.0001$). The testosterone concentration in the E2 group was significantly lower than the C group at Weeks 4, 6 and 8 ($P < 0.0001$) and lower than the E1 group in Week 2 ($P < 0.05$) and Week 4 ($P < 0.0001$).

Agonistic behaviours

Overall, there was a significant effect of treatment ($P < 0.0001$), time ($P < 0.0001$) and the interaction between treatment and time ($P < 0.0001$) on agonistic behaviour, as measured by number of aggressive physical contacts. At the start of the experiment there were no differences in agonistic behaviour, as measured by number of aggressive contacts, between the C, E1 and E2 groups, with the average number of aggressive contacts in the 2-min interaction period being less than 1 in all three groups (Fig. 2). At Week 2 the agonistic behaviours had increased significantly ($P < 0.01$) in all three groups, averaging ~5 agonistic contacts per 2 min. There was no difference between the three treatment groups at Week 2. At Week 4, there was a non-significant trend ($P = 0.08$) for the number of aggressive contacts to be declining from Week 2, but again no significant difference between the three treatment groups. At Week 6, there was a 1.7-fold decline ($P < 0.05$) in the number of aggressive contacts in the E1 group and a 4-fold decline ($P < 0.001$) in the E1 group compared with Week 2, but the C was not different from Week 2. Both E1 ($P < 0.05$) and E2 ($P < 0.0001$) groups had fewer aggressive contacts compared with the C group at Week 6. At Week 8, there was a 3.8-fold decline ($P < 0.0001$) in the number of aggressive contacts in the E1 group and a 12-fold decline ($P < 0.001$) in the E1 group compared with Week 2 values. There was also a 1.5-fold decline ($P < 0.05$) in the C group compared with Week 2, but both E1 ($P < 0.001$) and E2 ($P < 0.0001$) groups had fewer aggressive contacts compared with the C group.

Feed intake

Overall, there was a significant effect time ($P < 0.001$), but no effect of treatment or the interaction between treatment and time on group pellet intake. Over the 8 weeks of the experiment there was a 3.5-fold increase ($P < 0.001$) in intake in the C group, but the 3.3-fold increase in the E1 group and the 3-fold increase in the E2 group were not significant (Fig. 3). The C group had a higher intake ($P < 0.05$) at Week 2 compared with the E1 and E2 groups, but there was no differences between treatments at any other time.

Body mass and body condition score

Overall, there was a significant effect time ($P < 0.05$), but no effect of treatment or the interaction between treatment and time on body mass. Goats in all treatment groups gained ~3.5 kg over the 8 weeks of the experiment (Fig. 4*a*). There was no significant difference between treatment groups.

Fig. 2. Agonistic behaviour, measured as number of aggressive physical contacts (butting, mounting), during a 2-min interaction period, in the goat bucks in the Control (C: white columns, *n* = 8), anti-gonadotrophin-releasing hormone (GnRH) vaccine with 14 day booster (E1: grey columns, *n* = 8) and anti-GnRH vaccine with 28-day booster (E2: black columns, *n* = 8) treatment groups. Values are means ± s.e.m.

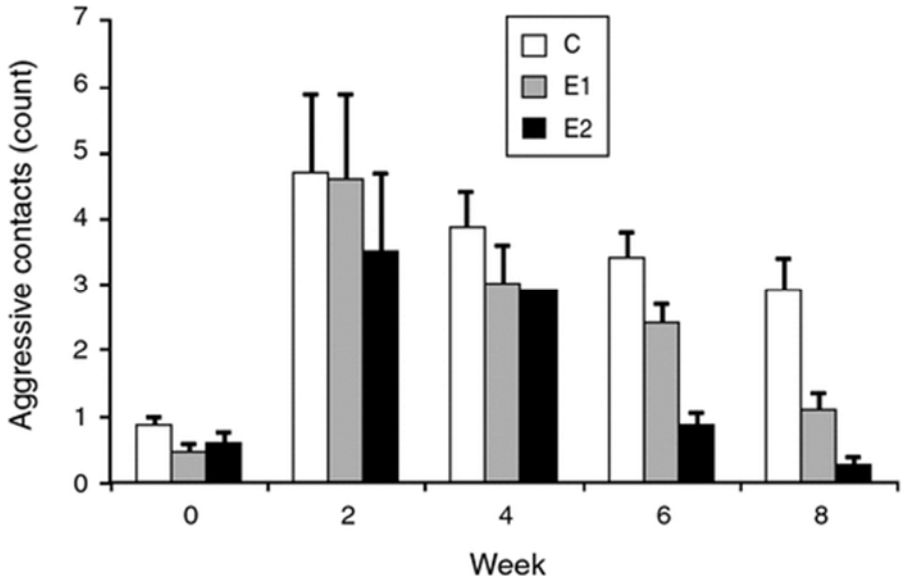


Fig. 3. Pellet intake (kg/week), measured as fortnightly amount per pen, in the goat bucks in the Control (C: dashed line, two pens with *n* = 7 and *n* = 8 goats), anti-gonadotrophin-releasing hormone (GnRH) vaccine with 14-day booster (E1: grey line, two pens with *n* = 7 and *n* = 8 goats) and anti-GnRH vaccine with 28-day booster (E2: black line, two pens with *n* = 7 and *n* = 8 goats) treatment groups. Provision of hay was reduced by half (10 kg/week to 5 kg/week after Week 3). Fortnightly values presented. Values are means ± s.e.m.

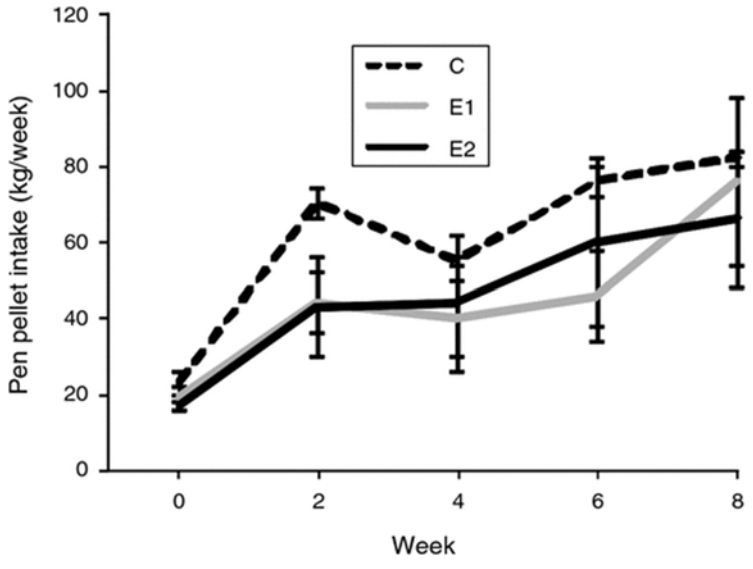
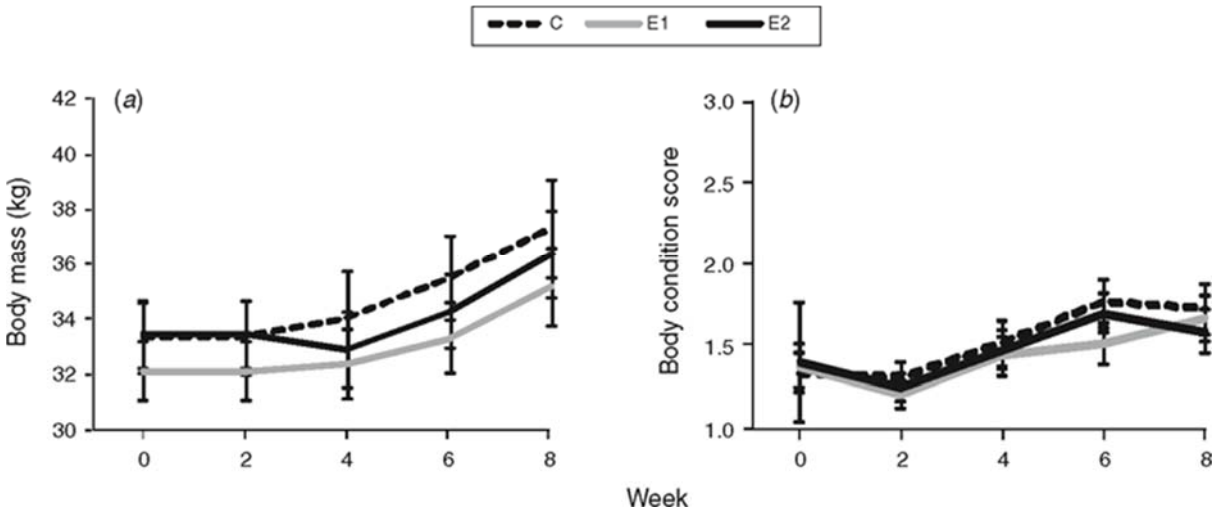


Fig. 4. (*a*) Body mass (kg) and (*b*) body condition score of the goat bucks in the Control (C: dashed line, *n* = 15), anti-gonadotrophin-releasing hormone (GnRH) vaccine with 14-day booster (E1: grey line, *n* = 15) and anti-GnRH vaccine with 28-day booster (E2: black line, *n* = 15) treatment groups. Values are means ± s.e.m.



Overall, there was a significant effect time ($P < 0.001$), but no effect of treatment or the interaction between treatment and time on BCS. Goats in all treatment groups gained ~0.25 BCS units over the 8 weeks of the experiment (Fig. 4*b*). There was no significant difference between treatment groups.

Discussion

The hypothesis that the anti-GnRH vaccine, Improvac, would reduce testicular size, decrease circulating testosterone concentration and decrease agonistic behaviour in males rangeland goats was supported. Therefore, Improvac may be a useful tool to reduce agonistic behaviours in rangeland goat bucks, and this vaccine may assist in reducing social stress and possible injury during transport or intensive rearing.

The Improvac vaccine was effective at reducing testicular size in the rangeland goats, in agreement with effects seen in pigs (Albrecht *et al*. 2012). In a similar study with goats, Godfrey *et al*. (1996) found that another anti-GnRH vaccine, Vaxstrate, stopped the seasonal increase in testicular growth. The magnitude of the effect found in that study, an ~5-cm difference in scrotal circumference over 8 weeks, was similar to the present study. Testicular size is affected by a range of factors, including nutrition and photoperiod (Walkden-Brown and Bocquier 2000; Delgadillo *et al*. 2004). Walkden-Brown *et al*. (1994) found that nutrition and photoperiod affect testicular size in goats by altering the secretion of the gonadotrophins, luteinising hormone (LH) and follicle-stimulating hormone (FSH). The anti-GnRH vaccine would have likely reduced testicular growth and function in the present study by reducing circulating concentrations of LH and FSH, as GnRH directly controls the secretion of these hormones (Wu *et al*. 1987).

GnRH affects testosterone secretion by the testis due to its actions on LH and FSH secretion (Wu *et al*. 1987). LH in particular affects the secretion of testosterone by Leydig cells in the testis (Ewing *et al*. 1983), The average testosterone concentration of the C group of the present study increased over the 8 weeks of the experiment, coinciding with the seasonal (photoperiodic) stimulation of GnRH secretion and increase in sexual behaviour at that time of year (Delgadillo *et al*. 2004). Improvac suppressed testosterone concentrations by the end of the 8-week experiment, even in the face of the normal seasonal increase.

With regard to either giving the booster injection of the vaccine at 14 days (E1) or at 28 days (E2), both treatment groups showed a similar magnitude of suppression of testosterone concentrations by the end of the experiment, but after 4 weeks, the 28-day booster group (E2) had testosterone concentrations comparable to the C group, whereas the 14-day booster group (E1) did not. After 6 weeks both Improvac groups had similar testosterone concentrations. The optimal period required between the primary immunisation and booster anti-GnRH immunisations has not been fully addressed in the literature for any species, but Godfrey *et al*. (1996) similarly found that a 14-day booster protocol was as effective as a 28-day protocol.

Improvac treatment decreased agonistic behaviours from Week 2 to Week 8 in the intact male rangeland goats, with the standard 28-day booster treatment (E2) regimen producing the greatest decrease in agonistic behaviour. However, it must be noted that although the 28-day booster group (E2) had fewer agonistic contacts at Week 8, the time taken to achieve difference to the C was the same for both experimental groups (Week 6). There was a very low level of agonistic behaviour at the start of the experiment in all treatment groups. This may have been because the novelty of the confinement pen where the behavioural tests were undertaken caused a suppression of normal behaviour, as has been seen in behavioural studies of cattle and sheep (Le Neindre 1989; Romeyer and Bouissou 1992). By Week 2, when the goats had been acclimatised to the behavioural testing conditions, agonistic behaviours had increased by ~4–5 times the levels seen at the start of the experiment. Agonistic behaviour also decreased from Week 2 to Week 8 in the C group, but the decline was much smaller than seen in the experimental groups. This small amount of behavioural change probably also indicates an acclimatisation process operating, and probably also establishment of a social hierarchy.

Intake of the feed pellets was not affected by the treatments. It must be noted that the feed intake data is not very robust due to the low level of replication, but the lack of treatment effects was unexpected as it has been previously shown that social hierarchy and level of aggression in goats affects feeding behaviours (Barroso *et al*. 2000). The lack of treatment effect might be due to each pen establishing a stable hierarchy quickly, and because there was *ad libitum* feed and liberal trough space. Therefore, there was less drive for feed competition and once the more dominant goats had finished, those lower in the hierarchy were able to eat sufficiently. The lack of treatment effects on feed intake is also reflected in the lack of difference between groups in body mass and BCS.

In conclusion, Improvac decreases agonistic behaviour in male rangeland goats, reduces testicular size and decreases circulating testosterone concentration. This confirms the efficacy of this commercially available anti-GnRH vaccine developed for pigs to be used on rangeland goats. It was also hypothesised that decreasing the time between primary immunisation and booster might provide faster effects and therefore decrease time needed to reduce agonistic behaviour. Our results showed that a 14-day booster was effective at providing immunocastration effects in rangeland goats, but indicated the 28-day booster was more effective in decreasing overall aggression, but not in reducing the period of time required to effect that change. Therefore, this trial proves efficacy of both 14- and 28-day booster vaccination protocols for use of Improvac in

rangeland goats, but given the more favourable results with reduced aggression we would recommend using the manufacturer's recommendation of a 28-day booster. The encouraging findings of this study indicates that the use of Improvac as part of a domestication protocol to reduce agonistic behaviour in rangeland goats warrants future management trials with a larger number of goats from a variety of sources.

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