MEASUREMENT OF BEHAVIOUR AND HEART RATE TO ASSESS THE AVERSIVENESS OF HANDLING TREATMENTS USED FOR RED DEER

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INTRODUCTION

The welfare status of agricultural animals is important, not only because of a well-established association between improved welfare and productivity (Barnett & Hemsworth, 1990), but also because a growing number of people are becoming concerned with the experiences imposed on the animals utilised by man (Rollin, 1990). Attempts to assess the effects of handling treatments on the welfare of animals have included measures of during- and/or post-treatment activities (Baldcock & Silby, 1990; Gentle *et al*, 1990), physiological responses (Hargreaves & Hutson, 1990a; Minton & Blecha, 1990), and preferences for, or aversion to, particular treatments (reviewed by Rushen, 1990) In Experiment 1 of the present study, pre-, during- and post-treatment behaviour and heart rate were measured to determine whether, for yearling red deer stags, velvet antler removal was a more aversive experience than restraint in a mechanical crush In Experiment 2, a different group of stags was used to evaluate the potential of preference testing for comparing different methods of antler removal

METHODS

Experiment 1

Animals and Management

Nine yearling red deer stags, with growing velvet antlers, were kept at pasture except during handling and observation periods. The stags were fitted with numbered plastic collars for individual identification.

Procedure

The experiment was carried out over a period of six weeks, which began with a five-week period of habituation to a handling procedure. During habituation the deer were handled daily, starting at 8 30 a m , on three successive days (during the first and second weeks) and then four successive days (third, fourth and fifth weeks). Individuals were always handled in the same order, by the same three people (except for Weeks 2 and 3 when one of the handlers was replaced temporarily) and exposed to the same sequence of events.

The test animal was brought into the octagon (Figure 1), and fitted with a nylon harness which held a heart rate monitor During fitting of the harness, two other stags were present in the octagon whenever possible (as numbers of remaining untreated animals allowed), and then removed once the heart rate monitor was switched on. The test stag was released at the start of the course through the deer yards (Figure 1), which was designed to facilitate measurement of avoidance behaviour. The stag was followed slowly by one handler until it entered the weigh scales. The back door of the scales was closed and the stag was held for 30 seconds (s), then the front door opened and the stag was given 10 s to move out of the scales If the stag did not move, the door behind it was opened and the stag was given a further 10 s, then followed through the scales. The stag was also given 10 s to move from the scales to across Line A (Figure 1), before being followed into the octagon.

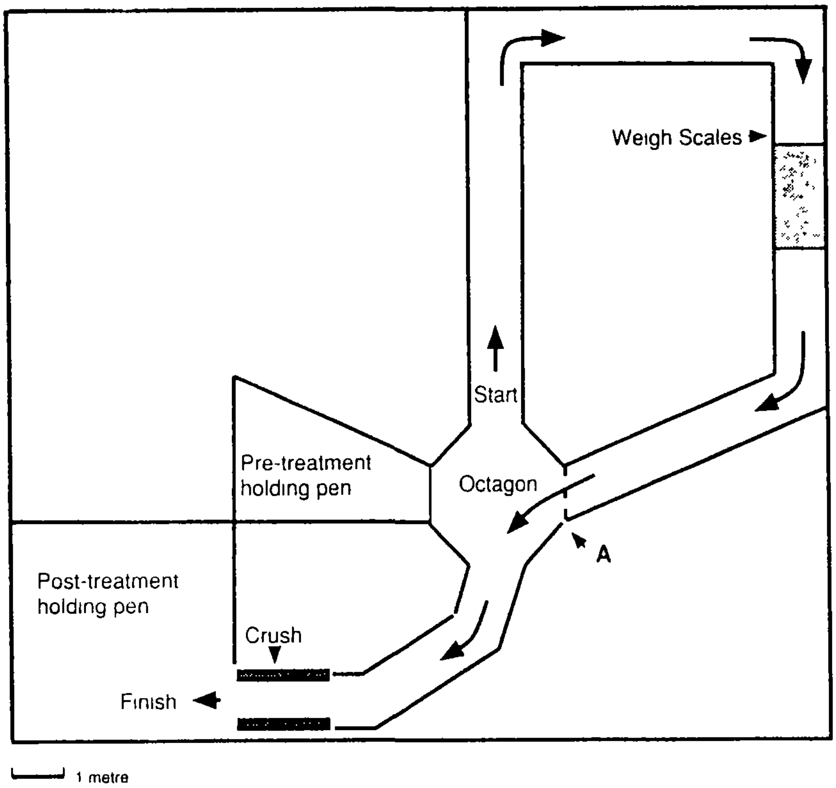

Fig. 1 Layout of handling area for Experiment 1

From the octagon the stag was run into a post-treatment holding pen, and from Week 3 onwards the animals were required to move through a mechanical crush before reaching the holding pen. During Weeks 4 and 5 the deer were restrained briefly (40 s) in the crush Harnessess were removed after the first four, and then the second five, stags had been run through the course.

Once all of the stags had been handled, the group was shifted to an indoor pen where water and hay were provided *ad libitum*. Deer nuts (555 g/head) were also supplied upon entry to the pen and 3 hours later. The deer were released back to pasture after 3 5 hours of confinement.

Treatments

Two treatments were added to the handling procedure in Week 6
Treatment V: One antler was removed during restraint in the crush The stag's head was tied down to the front of the crush, then local anaesthetic (5 ml "Lopaine", applied as a nerve ring block, with an 18-gauge needle) and a rubber tourniquet were applied to the antler. Three minutes following completion of application of the anaesthetic, the antler was removed using a surgical saw.
Treatment C: The stag was restrained in the crush for six minutes (approximately the same time as required for completion of Treatment V).

Five animals were given Treatment V on the first and second days of the week (Group 1), while the remaining four (Group 2) received Treatment C, then the treatments were reversed for the following two days. The deer were run through the same procedure on the fifth day during Week 6, but were given Treatment C only.

Measurements

During Weeks 5 and 6, measurements of behaviour and heart rate were made during specific intervals throughout the handling treatments (described in Table 1), and behaviour was also measured during the first minute immediately after handling, at 15-minute periods at 0, 1 and 3 hours after the group was introduced to the indoor pen, and at 2 (afternoon) and 6 hours (evening) following release into the paddock (Table 2). All handling and indoor measurements were taken from videotapes and covered the full period of observation. During the field observations, behaviour was recorded using interval sampling. Each individual in turn was observed in a randomised order. The activities of each animal over 30 seconds, and its behaviour and distance from its nearest neighbour at the end of the 30 s interval, were recorded. This procedure was carried out four times, at 14-minute intervals, for each observation period.

Table 1. Behavioural and heart rate measurements made during the handling procedure

Octagon → Scales	In Scales	Scales Door Opened → Octagon	In Crush
<u>Heart Rate</u> (i) recorder on additional stags out of octagon (ii) additional stags out of octagon entered start race	<u>Heart Rate</u> (i) 1st 10s (ii) 2nd 10s (iii) 3rd 10s	<u>Behaviour</u> (i) time to reach octagon <u>Heart Rate</u> (i) → octagon	<u>Behaviour</u> No struggles* made during treatment** (i) before injection of anaesthetic (ii) before injection of anaesthetic (iii) during application of tourniquet (iv) following application of tourniquet 30s (v) 30s prior to antler removal (vi) during antler removal (vii) following antler removal → 10s <u>Heart Rate</u> (i)-(vii)during the above intervals

* individual struggles were defined as those not followed by struggling for at least 5 seconds
** struggles and heart rate during handling in C were counted over the time intervals corresponding with the components of V

Heart rate was recorded using Equine Heart Rate Monitor Model HR-8AE (Respironics Ltd, Kowloon, Hong Kong) fitted with a transmitter and a 15 cm aerial. The monitor produced a series of "beeps" which corresponded with the test animal's heart beats. These were transmitted to an FM recorder and recorded simultaneously with each stags' behaviour on videotape.

Table 2. Behavioural measurements made following the handling procedure

Immediately Post-Treatment	0, 1 and 3 hours following confinement in indoor pen	2 and 6 hours following release into paddock
during 1 minute	during 15 minutes	in 4 x 30 s
(i) no steps	(i) time spent eating	(i) whether paced
(ii) no paces*	(ii) time spent sitting	(ii) whether groomed self
(iii) no times nosed or chewed wall or door	(iii) time spent standing inactive	(iii) whether instigated an aggressive interaction
(iv) no times nosed or chewed self or harness	(iv) no times approached feed trough	(iv) whether stag has recipient of an aggressive interaction
(v) no times nosed or chewed other deer	(v) no times changed from sitting to standing position	(v) no flicks of ears
(vi) no head shakes	(vi) no times instigated an aggressive interaction	at end of 4 x 30 s (vi) activity (standing, sitting grazing, pacing or walking)
(vii) no body shakes	(vii) no times stag was recipient of an aggressive interaction	(vii) distance from nearest neighbour (0-4 m, >4-10 m >10 m)
(viii) no flicks of ears	(viii)-(xv) measures i-viii described in the 1-minute observations	
* pacing was defined as walking parallel to within 0.5 m of, the wall or fence		

Data Analysis

Individual animal heart rate profiles for each day generally followed a well-defined pattern of increase when the additional stags were removed from the octagon, a decrease during the period in the scales, followed by an increase when the scales door opened. Orthogonal polynomial contrasts up to cubic for each profile were analysed separately for each week by analysis of variance, fitting group, day and their interaction with the effect of day nested within animal, assuming that there was no correlation from day to day within weeks.

The time taken to leave the scales was analysed by least squares, fitting animal and day for each individual for Weeks 5 and 6. The number of struggles made per minute throughout the time in the crush was also analysed by least squares, fitting treatment after adjusting for animal.

For each stag, heart rate during specific periods in the crush during the first and second days of V and C were compared. For this analysis the difference between the first and second days of the first treatment, plus the difference between the first and second days of the second treatment, was calculated and analysed by least squares, with treatment group fitted to the model. Data for periods in the crush when many animals struggled (during application of local anaesthetic and antler removal) were not analysed because this activity was likely to have elevated heart rate.

The number of times various activities were performed during the first minute post-treatment, and during the 15-minute indoor observations, were analysed by least squares, fitting treatment after adjusting for animal for each activity. For the 15-minute observations, differences between the first and third observations (when the stags were fed deer nuts) were calculated and analysed in the same way.

After a preliminary inspection of the data from the paddock observations, the data for whether or not each stag was grazing was analysed using a generalised linear model, with binomial error and log link function, with treatment fitted after day, adjusting for time, and their interactions, and animal. For all analyses, significance was assessed at the 5% level.

Experiment 2

Animals and management

Nine yearling red deer stags were used. The stags were housed indoors, starting one week before the experiment, to accustom them to the indoor environment, and avoid the time and stress involved with yarding. They were fed deer nuts and hay *ad libitum*. During the week before the experiment, the deer were given several hours in the test area to become familiar with its layout.

Procedure

Eight of the deer were given a series of weekly trials, in which they were treated individually, always in the same order Trials were carried out in a maze (Figure 2), in which the stags were exposed to one of two treatments, depending upon whether the stag turned left or right. A different pair of treatments was used for each trial, which consisted of two days' forced exposure to the treatments (one given in the morning and one in the afternoon on each day), followed over the next two days by four choice tests, then one forced exposure to each treatment, then four choice tests Testing of an individual ceased when it had chosen the same pen three times in succession

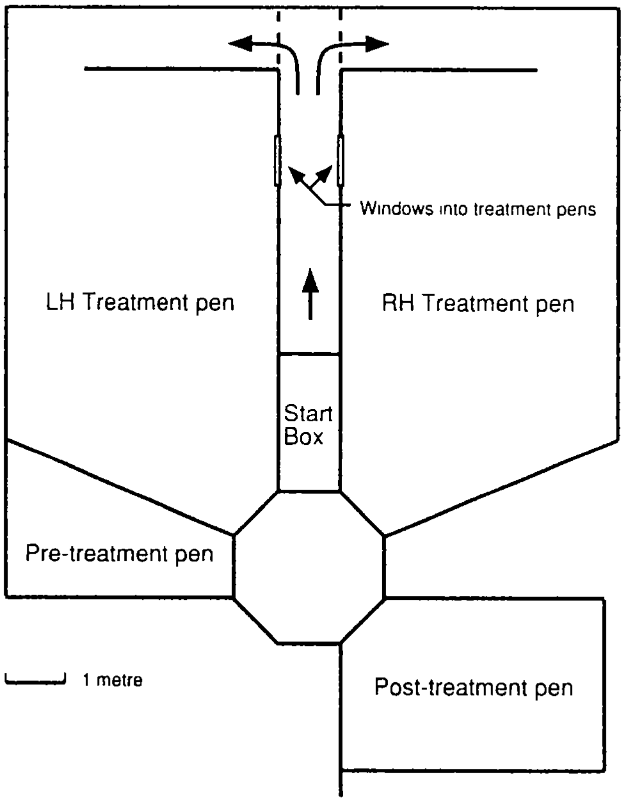

Fig. 2 Layout of preference test area

During testing each stag was held for 20 s in the start box (Figure 2) and then released. Stags not entering a pen within 1 minute were approached slowly from behind until one of the pens was entered. Windows were provided to enable the deer to see into the treatment pens before turning left or right. From the treatment pens each individual was run into a post-treatment holding pen, containing an additional stag (this was to avoid isolation of the first stag to be tested).

Treatments

Logistical problems with presenting the deer with a choice between specific variables were encountered in some of the trials. The pairs of treatments, and pen-treatment combinations used in three of the trails, carried out in the first, second and fifth weeks of the experiment, were as follows:

Trial A: empty pen and crouching person (empty pen on right for every second stag)
Trial B. object in pen and empty pen (empty pen on previously non-preferred side)
Trial C: crouching person (who stood up after the stag entered) and stationary person (crouching person on previously preferred side for 1/2 of the stags)

RESULTS

Experiment 1

Heart rate profiles varied during the handling procedure, showing an increase when the additional stags were removed from the octagon, a decline during the period in the scales, then an increase when the scales door was opened (Figure 3). The analysis of heart rates averaged over profiles showed a significant decline in heart rate over the course of both Weeks 5 and 6 (Figure 4), with no evidence of differences between groups

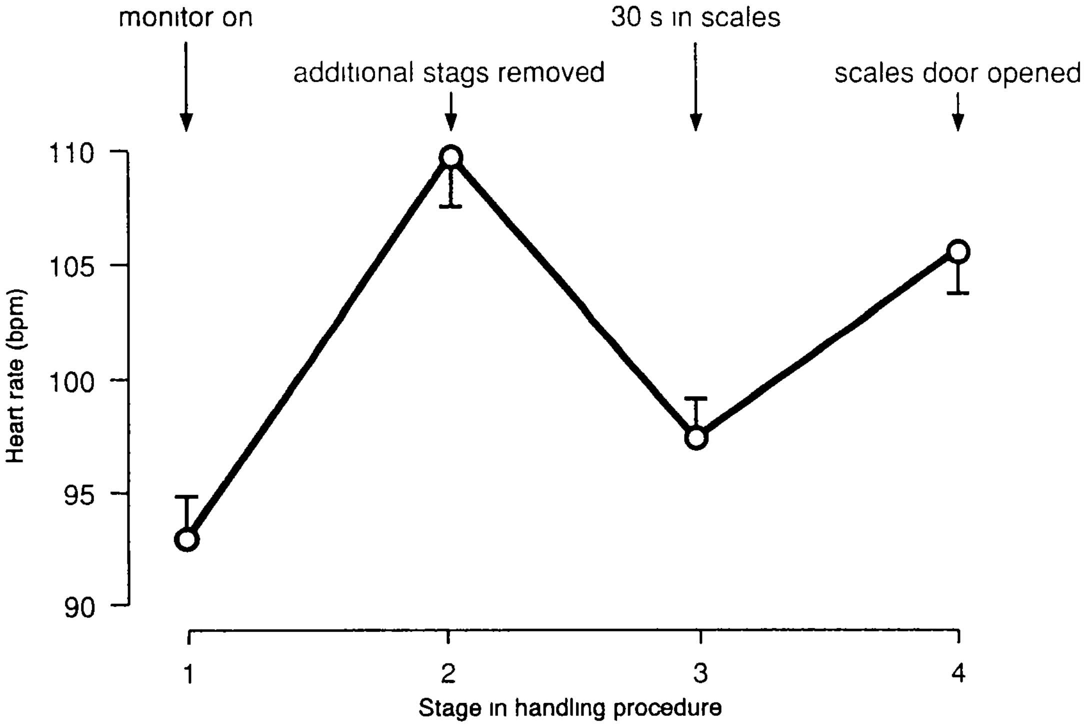

Fig. 3 Mean heart rate of stags during stages in the handling procedure (pooled data for Groups 1 and 2, and Weeks 5 and 6)

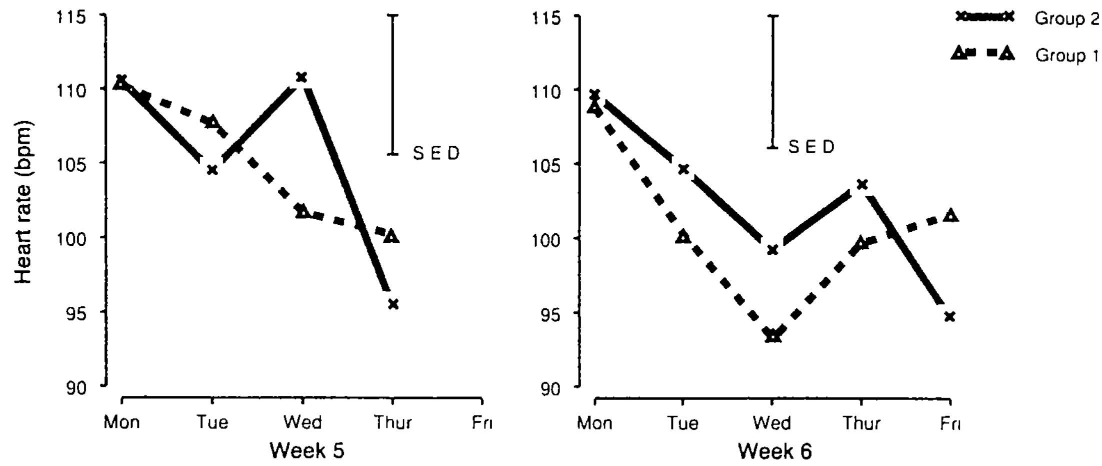

Fig. 4 Mean heart rates for Groups 1 and 2, during handling in Weeks 5 and 6

The increase in heart rate seen when the scales door opened (Figure 3, Stage 4) was not solely due to an increase in activity, because it was seen irrespective of whether stags remained in the scales for a further 10 s after the front door opened. Five of the nine stags consistently remained in the scales for 10s, and showed a mean increase in heart rate of 7 0 (S.E.=1.05) bpm. The time taken to leave the scales did not differ between treatments, and there was no evidence of an increase over Week 6.

When heart rates during restraint, prior to antler removal (and during the equivalent time periods for Treatment C), for the first and second days of each treatment were compared, there was a trend towards heart rate on Day 2 being higher than on Day 1 for Treatment V, while it was significantly lower than on Day 1 for Treatment C (Table 3).

Table 3. Differences in heart rate (bpm) in the crush between first and second days of treatment, for V and C (*indicates a significant difference between Days 1 and 2)

Stage of treatment[1]	Differences between 1st and 2nd days of treatment (bpm)			
	Treatment V	SE	Treatment C	SE
1 in crush, prior to tourniquet	- 3 4	4 09	7 9*	3 40
2 tourniquet on	- 2 0	3 22	4 7*	1 99
3 30s after tourniquet on	- 1 1	2 96	6 7*	2 29

[1] Stages described were for Treatment V, and were compared with the equivalent time periods during C

During restraint in the crush, stags receiving Treatment V struggled on average 0 99 times per minute, significantly more than 0.39 (S.E.D.=0.214) times per minute for Treatment C. The number of struggles made per minute during antler removal (mean=1.54) was not significantly greater than the number made during the corresponding time interval in Treatment C (mean=0.58, S E.D =1.01).

During the immediate post-treatment observations, V stags shook their heads and flicked their ears more than C stags (Table 4). This difference in behaviour persisted throughout the 0-3 hr indoor observation period, during which V stags also groomed themselves more, and ate less. The only behaviour which changed during the 0-3 hr period was sitting (with head up), which increased in V stags but not in C stags (Table 4). During the paddock observations, the only difference between C and V stags was a difference in grazing behaviour. Grazing occured in 58% of the evening observations compared with 7% of the afternoon observations (S.E.D=5%), and during the evening, V stags were observed grazing more than C stags (66% of observations compared with 50%, S.E.D=6 9).

Table 4. Activities which differed significantly (p<0.05) following Treatments C and V

Activity		Treatment		
		C	V	SED
Immediate observation period	head-shaking (no /min)	0 3	2 9	1 06
	ear-flicking (no /min)	0 2	2 6	0 58
0 - 3 hrs post-treatment (mean values)	head-shaking (no /hr)	1 7	6 8	2 19
	ear-flicking (no /hr)	12 7	18 9	9 59
	grooming self (no /hr)	8 9	15 6	2 11
	eating (min/hr)	32 8	26 8	2 16
0 - 3 hrs post-treatment (difference over time)	sitting (min/hr)	3 1	11 0	2 86
9 hrs post-treatment	grazing (% of observations)	50	66	6 9

Experiment 2

All stags except one chose the empty pen during each of the three choice tests in Trial A. The stag which did not chose the empty pen chose the pen containing the human in all three tests. In Trial B, in the first choice test only two stags chose the empty pen. However in the subsequent three tests, all but one of the stags chose the empty pen each time This stag chose repeatedly to enter the pen with the object In Trial C, seven stags consistently chose the pen with the stationary person, after 0 (n=3), 2 (n=3) and 3 (n=1) choice tests The remaining stag consistently chose the pen with the crouching person, which was the pen he had avoided in the previous trial. The stag which did not behave in the same way as the rest of the group was different in all three trials

DISCUSSION

Measurement of heart rate during the pre-treatment handling procedure did not reveal any differences between Treatments V and C, but there were aspects of the handling procedure which had interesting effects. Heart rate in test stags increased when the additional stags were removed from the octagon, probably because the stags became visually isolated from other deer. The same response has been found in sheep (Baldock & Silby, 1990). A second increase was seen when the door of the scales opened, regardless of whether the animal moved out of the scales. This increase probably occurred because the potential to gain control over the situation had increased. In humans, heart rate varied with a subject's perception of control over receiving a noxious stimulus, with acceleration being found when the subject was able to avoid the stimulus, and deceleration occuring when the stimulus could not be avoided (Malcuit, 1973).

The increase in heart rate prior to removal of the second antler, in contrast to the decrease in heart rate seen over the same time period for stags receiving restraint only, was indicative of an anticipatory response to antler removal. Anticipatory responses were also seen in a study on cattle, in which animals which had previously been electroimmobilised had higher pre-treatment heart rates, and were slower to re-approach the treatment area, compared with control animals which were not immobilised (Pascoe & McDonell, 1986).

More struggling occured during Treatment V than Treatment C, indicating that V was more aversive to the deer, and the post-treatment behaviour indoors demonstrated that velvetting continued to affect the stags for several hours In particular, the depression in appetite seen in V stags and the increase in sitting was suggestive of post-treatment pain. Pain in animals is generally characterised by inactivity, aggression, an abnormal posture, restlessness (moving constantly or repeatedly getting up and lying down), and/or reduced food or water intake (Flecknell, 1985) However these effects were not apparent during the later paddock observations, and in the evening observation period V stags appeared to be compensating for their previously reduced food intake by grazing more. During the post-treatment observations, there was no indication that removal of antlers, which are important indicators of social status in red deer (Bubenik, 1966), resulted in individuals being the recipient of aggression from stags with intact antlers.

Aversion to re-approaching the treatment area was not seen in the present study. This may have been because the period of habituation, which was intended to make the handling procedure predictable so that when the two treatments were applied the deer could distinguish between them, resulted in the deer behaving in the same way despite anticipating an aversive treatment. (The older the habit, the harder it is to re-shape (Wiepkema, 1987)). Alternatively, the deer may not have anticipated the treatments, or (despite differences in pre-treatment heart rate and during- and post-treatment behaviour) Treatments C and V did not differ in aversiveness. An alternative approach to assessing aversion to antler removal could be to use more animals and only run them through the procedure three times (removing one antler on the first two occasions for Treatment V). In studies of sheep (Hargreaves & Hutson, 1990b, Rushen, 1986; Rushen & Congdon, 1986) and cattle (Pascoe & McDonell, 1986) effects of different handling treatments on avoidance of a treatment area were becoming apparent after only two or three exposures to the treatments.

In the preference tests, most of the deer avoided the additional stimuli in the pens, and reversed their choices when the position of the aversive stimulus was changed, rather than choosing their previous route out of the test situation. The choice of the stationary person over the crouching person indicated that the deer could choose between two aversive stimuli The rapid establishment of a consistent preference in Trial A indicated that preference testing may have the potential to assess the relative aversiveness of different velvetting treatments

In conclusion, pre-, during- and post-treatment measurements indicated that antler removal was more aversive than restraint in the crush only, and caused a temporary depression in appetite and activity While reluctance to re-approach the treatment area was not found in the present study, this measure may prove to be useful for comparing aversion to treatments in naive animals or for comparing treatments which can be applied several times. Preference testing may be useful in future experiments to compare different methods of antler removal.

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