Post-Velveting Stress in Free-Ranging Red Deer

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Abstract
The behavioural, heart rate and plasma cortisol measures prior to, during and following velveting were determined. Animals were mechanically restrained for velveting (under local analgesia) and subsequently returned to pasture. Recently developed remote blood sampling and heart rate recording equipment was used to assess stress in the normal outdoor environment, thus avoiding many of the confounding stress effects of repeated handling or confinement of earlier studies. Preliminary analyses of the data indicate that velveted stags had similar behaviour patterns and physiological stress responses to animals subjected to the same handling procedures but not velveted.

Introduction

Several studies presented in the series of Deer Courses for Veterinarians in recent years have used various physiological, endocrinological, and behavioural indices of stress to determine how stressful the process of velveting, as performed under the terms of the Code of Conduct, might be (Matthews and Cook, 1991; Matthews *et al.*, 1990; 1992; Pollard *et al.*, 1991; 1992; 1993). The results have generally indicated that the velveting process can affect some of these parameters, both at the time of tissue removal (behaviour and heart rate), and shortly thereafter (mainly behaviour), suggesting that velveting can cause some stress (Matthews *et al.*, 1990; 1991; 1992; Pollard *et al.*, 1991; 1993). In addition, the effects of velvet removal on welfare in the immediate post-operative period have been studied. In a study by Pollard and co-workers, stags given additional analgesic were found to differ in the incidence of several behaviours from stags given local anaesthetic alone, suggesting that velveting results in pain (Pollard *et al.*, 1992). However, these same studies have also shown that the manifestation, extent and duration of the behavioural changes caused by velveting can be subtle, variable, and transient.

Matthews and co-workers have consistently reported that for many physiological, endocrinological, and behavioural indices of stress there were no significant differences in the post-operative period between velveted animals and controls - animals that are yarded, drafted, crushed, and injected with local anaesthetic, but not velveted. Thus, using these indices of stress, the actual velveting procedure was apparently no more stressful to the stags than the handling operations required to undertake it (Matthews *et al.*, 1990; 1991). However, these data have been, of necessity, gathered in experiments carried out on animals that have been handled in ways that could be sufficiently stressful to confound the actual responses to velveting *per se*. Such handling included repeated yarding and restraint to obtain blood samples, and the use of small, recently formed, groups of animals.

In the present study, to reduce such possible confounding effects, we have utilised recently developed remote blood sampling and heart rate recording devices to determine physiological and endocrinological stress responses to velveting in free-ranging stags. Behavioural, physiological and endocrinological data were gathered before, during and after the velveting treatment; post-operative measures continued beyond the period when the local anaesthetic would be effective (approximately 2 hours; Matthews *et al.*, 1992). In

addition, animals were maintained in stable social groups of twelve and kept in familiar paddocks at all times at pasture.

Materials and Methods

Thirty-six rising 2 yr red deer stags from the Ruakura deer unit were used in this study. The stags were divided into three weight-matched replicate groups of twelve animals several weeks before the study began. Within each group three animals were assigned to receive local anaesthetic only (controls), three were to be velveted (velvets), and the remaining six were included to maintain a large group size (dummies). The experimental procedure was carried out over three weeks (one group per week).

Fitting Backpacks

Six stags in each group were sedated with Rompun (1.2 mg xylazine/kg) and a double lumen catheter was inserted into the jugular vein. At the same time animals were fitted with an elastic girth strap fitted with two surface ECG electrodes and a canvas backpack. The remaining six stags in each group were also sedated but were fitted with a canvas backpack only. The sedative was reversed with Yohimbine (0.25 mg/kg i.v.) and the animals were returned to pasture.

Experimental Procedure

The experimental procedure was carried out over two successive days beginning two days after the backpacks were fitted; the experimental days were termed Days 1 and 2 respectively. The control and velvet groups were exposed to exactly the same procedure on Day 1 (yarding, drafting, crush, local anaesthetic) and then released to paddock. On Day 2 the controls were treated exactly as on Day 1; velvets, however, also had their velvet removed by sawing (see Table 1).

Table 1. Experimental procedure.

	Treatment Group	
Day	Controls (n=9)	Velvets (n=9)
1	local anaesthetic	local anaesthetic
2	local anaesthetic	local + velvet

Fitting Blood Sampler and HR Recorder: The twelve stags were yarded at 6am on both Days 1 and 2. Each of the six cannulated stags was restrained in a pneumatic crush whilst a remote blood sampling device ("Dracpac") was connected to the jugular catheter (see Ingram *et al.*, 1994), and a heart rate (HR) recording device (Polar Sport Tester) was attached to the surface electrodes; the two devices were enclosed by the backpack. Each Dracpac device was programmed to sample blood to waste (at 0.15 ml/min) until 1pm at which time it would begin collecting a series of eleven continuous thirty-minute samples at a rate of 0.5 ml/min. The blood samples were stored in PVC bags in an ice-cooled insulated pouch within the canvas backpack. The HR recording devices measured HR at 1

min intervals from the time they were fitted to the animal. After the six cannulated animals had been crushed and thus equipped, all twelve animals were returned to the paddock (between 9:40 - 11:10am). Observations of behaviour (lying, standing, grazing, walking, or fence walking) were made on every animal at pasture at 2.5 min intervals from a hide.

Experimental Treatment: At exactly 1:30pm the animals were run back to the yards and each of the six cannulated stags was crushed and 20 -25 ml of local anaesthetic (lignocaine hydrochloride "Lopaine 2%") was injected as a ring block around each antler pedicle. On Day 2 velvet was removed from stags assigned to the velvet group using a surgical saw 4 min after local was administered. Bleeding was restricted by an elastic band around the pedicles. After 10 min of restraint animals were released from the crush. When all six cannulated animals had been crushed the whole group was returned to the paddock (approximately 3:15pm). Behaviour was again recorded at 2.5 min intervals while the stags were at pasture.

At exactly 6:30pm (when the last blood sample had just been collected), observations ceased, and all the animals were again yarded and blood samplers and HR devices were removed in the crush. On Day 1 animals were returned to the paddock for the night (still wearing the backpacks). On Day 2 the backpacks were removed.
Blood plasma was immediately separated by centrifugation and frozen prior to assay for cortisol (assay details in Ingram *et al.*, 1994). HR data were downloaded into a computer for later analysis.

Analysis of Data: The results presented here are preliminary only; data concerning plasma levels of several metabolic parameters, detailed analysis of HR and behavioural data, and statistical examination have still to be completed. The data shown here have been pooled across replicates within treatments.

Results

Plasma cortisol levels in each treatment group were similar at the beginning of the daily sampling series (ie. when the animals had been undisturbed in the paddock for at least 1.75 hours). The levels for both groups were approximately 11 ng/ml on Day 1, and 22 ng/ml on Day 2 (Figs. 1a & b). The process of yarding, drafting, crushing and injection of local anaesthetic caused an increase in mean plasma cortisol levels to 50 - 70 ng/ml. There was no difference in peak cortisol levels between treatment groups. Peak cortisol levels and timing of the response were similar in animals which were crushed first (2pm) and last (3pm) in each group (data not shown). These levels began to decrease when the animals were returned to the paddock (from 3:30pm). Upon return to the paddock cortisol levels returned to initial values within 1 hr and thereafter remained at these levels. There was no difference in cortisol levels between velvets and controls at pasture in the pm on either Days 1 or 2; however, levels on Day 2 were higher than on Day 1 in both treatment groups.

The HR data for velvet and control animals (averaged within treatments) on Days 1 and 2 are shown in Figs. 2a & b, respectively. The two profiles on each Day follow each other closely, though there are a few regions where the lines diverge; for example, from 1:00 - 1:30pm on Day 1 and from 6:00 - 6:30pm on Day 2.

Mean HR during the three major phases of the experiment (paddock am and pm, and crushing) on Days 1 and 2 are shown in Table 2.

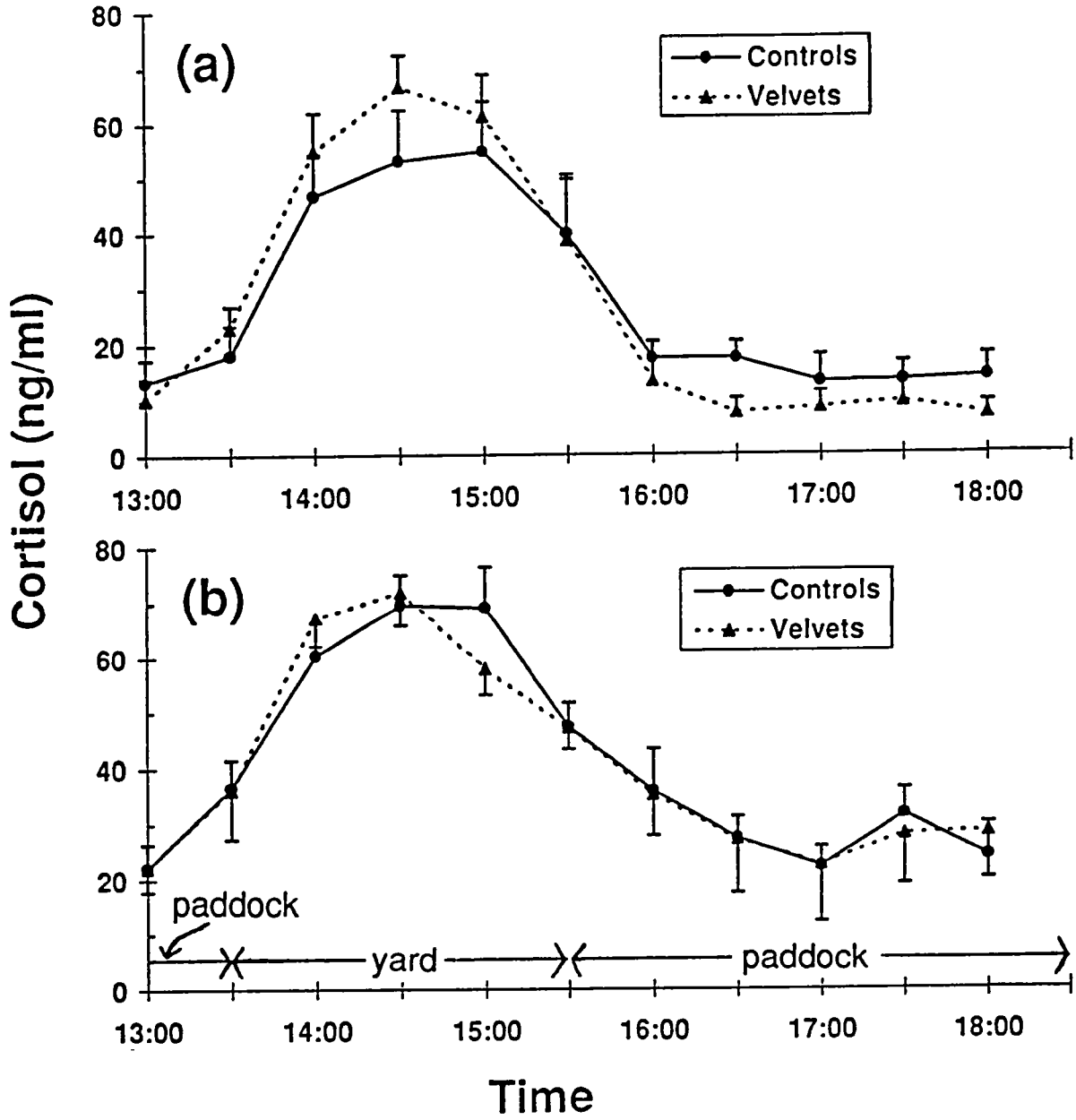

Figure 1. Plasma cortisol levels (ng/ml) of control and velveted stags on (a) Day 1 and (b) Day 2 of the experiment. Means ± sem are shown (n=6-8). Velveting occurred during yarding on Day 2. The data points are plotted on the hr or half hr according to when the blood sample began to collect (each blood sample was a continuous collection over 30 min). The periods when the stags were in the paddock or yard are indicated.

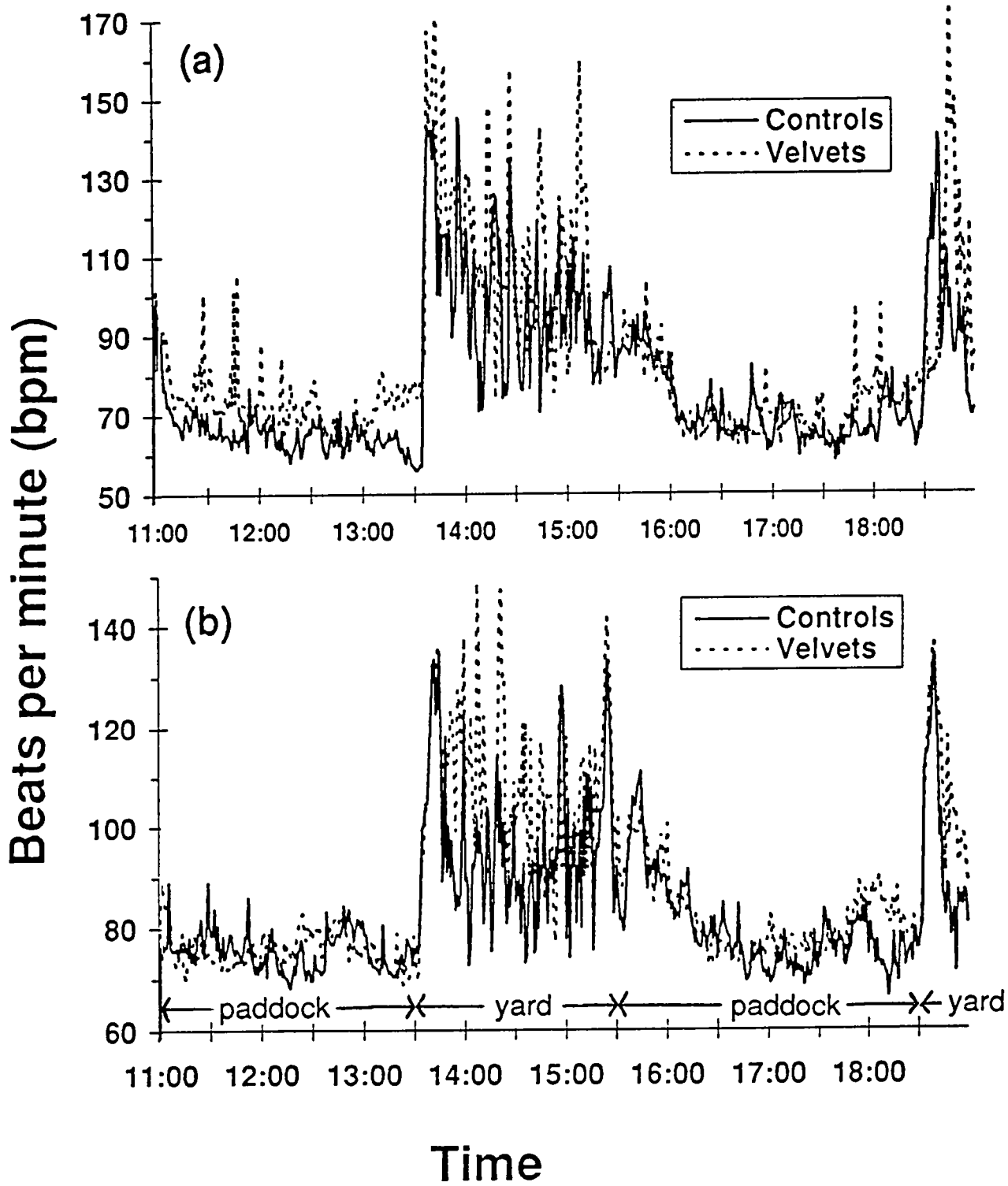

Figure 2. Heart rates (beats per minute) of control and velveted stags on (a) Day 1 and (b) Day 2 of the experiment. Means ± sem are shown (n=5-8). Velveting occurred during yarding on Day 2. The periods when the stags were in the paddock or yard are indicated.

Table 2. Mean heart rates (beats per min) of velveted and control stags during the morning (11:15am-1:30pm) and afternoon (3:30pm-6:30pm) paddock observation periods, and during yarding (including crushing, drafting, injection of local anaesthetic, and, on Day 2, velveting). Mean value ± sem is shown (n=5-8 for each group).

Day	Group	Paddock am	Yarding	Paddock pm
1	Controls	64.5 ± 2.3	98.7 ± 4.8	73.7 ± 5.2
	Velvets	74.2 ± 4.4	110.7 ± 3.2	72.7 ± 4.0
2	Controls	75.4 ± 4.5	96.3 ± 1.9	79.4 ± 4.3
	Velvets	72.8 ± 4.7	108.1 ± 3.9	79.7 ± 5.3

HR values in velveted animals were higher than in controls during the period in the yards on both experimental Days, however there was no difference in treatment group HR levels between Days. Although HRs were different between treatment groups at pasture on Day 1 am, there were no other differences between velvets and controls during other periods in the paddock. Mean HRs at pasture were highest in both groups on the afternoon of Day 2.

The overall proportion of time controls and velvets spent in each activity are shown in Table 3.

Table 3. Proportion (%) of time spent in each activity for each treatment group in the paddock on Days 1 and 2 of the experiment. Percentages are to nearest integer, sem is shown (n=9 for each group).

	Day 1		Day 2	
Activity	Controls	Velvets	Controls	Velvets
Lying	41 ± 8	46 ± 6	51 ± 7	52 ± 5
Standing	43 ± 9	36 ± 6	37 ± 7	38 ± 6
Grazing	13 ± 3	16 ± 2	6 ± 2	8 ± 2
Walking	2 ± 1	2 ± 0	2 ± 1	2 ± 1
Fence Walking	1 ± 1	1 ± 0	5 ± 3	1 ± 0

Although the proportions of time both treatment groups spent in each activity were broadly similar on a particular Day, there were differences between Days. For example, the amount of time spent grazing was lower on Day 2 for both treatments. The performance of behaviours are also presented according to their occurrence in am or pm observation periods (Table 4).

Table 4. Proportions (%) of time spent in each activity for each treatment group in the paddock (am and pm) on Days 1 and 2 of the experiment. Percentages are to nearest integer, sem is shown (n=9 for each group).

	Day 1				Day 2			
	Controls		Velvets		Controls		Velvets	
Activity	am	pm	am	pm	am	pm	am	pm
Lying	40±14	42±10	39±12	51±7	53±10	51±6	62±8	45±7
Standing	46±12	41±9	46±9	30±7	42±10	33±5	32±7	42±8
Grazing	11±5	14±4	14±5	17±2	3±1	7±2	5±2	10±3
Walking	2±1	2±1	1±1	2±1	2±1	2±0	1±1	2±1
Fence Walking	1±1	1±1	0	1+1	0±0	7±5	0±0	1±1

The proportion of time each treatment group spent in each activity often differed between the am and pm periods; however the magnitudes of the variations were small or modest. The increases and decreases of these changing patterns of activity from am to pm were similar across most treatment groups (eg. all groups spent more time grazing in the pm than in the am). The Day 2 data for velveted animals differ from the trends observed in the other groups. Post-velveting, the proportion of time spent lying was less, and the time spent standing and grazing was greater, than in the am observation period.

Discussion

The results of this study as presented here are preliminary as several other metabolic, endocrine and behavioural measures have yet to be analyzed.

Plasma cortisol level is a widely used indicator of stress. The levels of cortisol measured both at pasture and in the yards/crush are similar to those previously reported (paddock: Ingram *et al.*, 1994; crush: Matthews and Cook, 1991). Cortisol levels at pasture were higher on the second day of the present study than on the first day. This is believed to be a chronic stress effect attributable to either a second day of handling and/or a fourth day of wearing a backpack. There were no differences in cortisol levels between controls and velvets on Day 2, suggesting that velveting was not acting as an additional stressor to that of yarding and associated manipulations. These findings and this conclusion are similar to previous studies when no differences in cortisol levels were observed in control and velveted animals (eg. Matthews and Cook, 1991); however, in the present study we have eliminated many of confounding effects of repeated handling inherent in the experimental design of the earlier studies.

No previous study has determined the HR response to velveting for more than a few minutes after velvet removal; in the present study HR was recorded for at least 3.5 hours following the procedure. No gross differences in HR due to velveting were evident (Table 2), suggesting that velveting does not affect this measure of the cardiovascular system. On the other hand, HR and other features of the ECG response were disturbed during and

shortly after velveting (up to 5 min; Matthews and Cook, 1991). The HR data from the present study are currently being analyzed further to determine whether these same short term effects were present.

There were two particular times when mean HRs for the 2 treatment groups diverged (Figs. 2a and b). These changes were found to be associated with changes in general activity. Between 1:00 - 1:30pm on Day 1 animals in both treatment groups that were standing began grazing. However a higher proportion of animals in the velvet group changed their activities. Similarly, at 6pm on Day 2, some velvets that had been lying stood and grazed, whilst the control animals did not alter their activity pattern. It has been shown previously that HR varies systematically according to the activity performed at the time (Baldock *et al.*, 1988). In the present study it was found that changing from either lying to standing, or from standing to grazing, raised HR by approximately 10 bpm. Thus, the HR differences observed were probably caused by changes in the activities of animals. It is not clear if the differences in activity were attributable to velvet removal (see below). The HR data are currently being analyzed with respect to each activity to determine if there is any effect of velveting on this important measure of welfare.

Appreciable variation in activity patterns between individuals within a treatment group was apparent, ie. values for lying and standing ranged from 0 - 80%, and 10 - 90%, respectively. Much of this variation was due to a few animals which stood for most of an observation period probably because of slipped or restrictive backpacks. A similar number of animals were affected in both treatments. Whenever this was observed the backpack was adjusted when the animal was next yarded to relieve the problem, hence Day 1 and am variations are greater than at other times (Table 4).

As mentioned in the discussion of HR changes, on Day 2 velveted animals spent increased amounts of time standing and grazing after 6pm compared to the previous hour (data not shown). This observation may suggest that the activity changes were responses to increasing levels of pain as the effectiveness of the local anaesthetic declined. However, it must be stated that there were several other times during the study (before velveting) when similar differences in the performance of activities between treatment groups were recorded. Thus, these behavioural changes could be chance events and not necessarily a consequence of stress or pain induced behaviour as previously reported (eg. variously increased levels of walking, grazing, lying, and/or standing behaviours; Matthews and Cook, 1991; Matthews *et al.*, 1990; Pollard *et al.*, 1991, 1992, 1993). The performance of other behaviours, eg. head shaking, ear flicks, grooming, aggression, and jumping, has also been reported to increase after velveting (Pollard *et al.*, 1991, 1992, 1993). In the present study a few head shaking events were observed while the animals were at pasture, however it was noted during the experiment that head shakes occurred in all observation periods and individuals from both treatment groups were involved. The video tapes taken of animals in the yards and paddock will allow these data to be quantified but they have yet to be analyzed. Thus, based on behavioural measures, the velveted animals were no more stressed than non-velveted controls.

Preliminary analyses of HR and plasma cortisol data suggest that velvet removal under mechanical restraint and with local analgesia resulted in a similar level of physiological stress to that measured in non-velveted animals subjected to the same handling procedure.

As reported in previous years, the elevated levels of stress parameters observed appear to be attributable to the preparation of animals for velveting (yarding, drafting, crushing and administration of local anaesthetic) (Matthews *et al.*, 1990; Matthews and Cook, 1991). In the present study, however, the possible confounding effects of stress due to repeated handling and restraint has been reduced by the use of recently developed remote blood sampler and HR recording equipment. Future applications of these devices will allow determination of the separate contributions of the yarding, drafting, and restraint procedures to the observed stress response. One of the major procedural advances made in the present study has been the ability to monitor behaviour and obtain physiological measures from animals in their normal environment without disturbance. In this respect we have been able to show that velveted animals have similar levels of some physiological stress parameters to control animals and resume normal activities soon after return to the paddock.

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