BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES TO MANAGEMENT PRACTICES IN RED DEER STAGS

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INTRODUCTION

There is increasing evidence that some farming practices, such as transport and weaning, are stressful and can have adverse effects on deer health and productivity (Griffin et al 1988) Comparatively little is known of the effects of other routine management procedures like yarding, restraint and velvet removal on the welfare of deer Further, it is not yet clear which techniques provide the best measures of stress in deer

Indices of stress are often derived from measures of behaviour, physiology, and biochemistry (Broom 1988) Flight, fight or avoidance behaviour, and interruptions to normal maintenance activities such as resting and food consumption are often indicators that an animal is under some stress

Physiological changes associated with flight or fight responses have been used to measure stress in a wide variety of animals Some common indicators are changes in heart rate and other features of the electrocardiogram (Ehsani et al 1984), and plasma cortisol Van Mourik et al (1985) noted that cortisol levels in Rusa deer following yarding were elevated and similar to values recorded after the administration of a naturally occurring stress hormone (ACTH)

Mackintosh et al (pers comm) reported that plasma cortisol increased in velvetted animals but a similar rise was seen in restrained stags This suggests that velvet removal may be no more stressful than restraint

Progesterone, like cortisol is released from the adrenal glands in times of stress (Plotka et al 1983) Studies by Asher et al (1989) with fallow deer and Jopson et al (1990) with red deer indicated that plasma progesterone may be a more useful measure of severe stress than cortisol, since the peak levels of cortisol release seemed to occur at lower levels of stress than for progesterone

Increases in the concentrations of many other blood constituents, including plasma glucose and lactate, have often been used as indicators of stress (Hattingh et al 1988)

The present study evaluated the changes in a number of behavioural and physiological parameters in red deer during velvetting and associated handling procedures The overall aim was to develop objective measures for assessing the relative stressfulness of routine management practices

METHODS

Animals

Eighteen red deer stags aged 2 to 4 years with liveweights ranging from 87 to 150 kg were used Animals were randomly assigned to three groups (two experimental and one control) on the basis of liveweight and age

Animals were drafted off from the main group of 20 breeding stags in groups of five or six Drafting was undertaken 2 to 18 hours prior to the experiment All animals were pasture fed in a 0 5 ha paddock at the Ruakura Deer Unit and subject to normal management except for the imposed experimental procedures

Procedure

The two experimental groups were subjected to three handling-drug treatments at weekly intervals Handling consisted of a standard procedure (gathering from pasture, yarding, drafting, blood sampling) together with one of two drug treatments administration of a local anaesthetic (lignocaine) with or without chemical restraint with xylazine During the second and third treatments one velvet antler was removed each time according to the regime shown in Table 1 Animals not administered xylazine were restrained in a crush

The control group was subjected to the standard procedure at weekly intervals for three weeks during the velvetting season, and a further condition in February consisting of the standard procedure together with removal of both antlers in the hard stage, while restrained in a crush (Table 1)

A second control group (II) comprising 12 to 15 stags was not handled but observed at pasture

Table 1 Sequence of experimental conditions

n	Handling Treatments I mid Nov to Dec	II late Nov to Dec	III December	4 late Feb
1	l	l + V_1	g + V_2	
1	l	l + V_1	gl + V_2	
1	l	g + V_1	l + V_2	
1	l	gl + V_1	l + V_2	
1	g	g + V_1	l + V_2	
1	g	g + V_1	gl + V_2	
1	g	l + V_1	g + V_2	
1	g	gl + V_1	g + V_2	
1	gl	gl + V_1	g + V_2	
1	gl	gl + V_1	l + V_2	
1	gl	l + V_1	gl + V_2	
1	gl	g + V_1	gl + V_2	
6	C	C	C	C + HV

l = local anaesthetic (lignocaine hydrochloride)
g = chemical restraint (xylazine 0 9 mg/kg IM)
V_n = removal of antlers 1 and 2
HV = removal of both antlers in the hard state
C = restraint but no velvetting or anaesthetic administration

Blood sampling

Indwelling cannulae were placed in all handled animals prior to each weekly treatment and were removed about 24 hr later Animals were restrained in a pneumatic crush during cannulation and for all other blood samplings except when immobilised with xylazine A blood sample was taken by jugular venepuncture immediately prior to cannulation Subsequent samples were obtained via the catheters immediately following cannulation, at minute intervals while restrained for ten minutes (treatment period), and at 30, 120, 180 min and 24 hr after the nominal time of velvet removal Cannulae were removed after the 24 hr sampling

Two 10 ml samples (1 x Heparin, 1 x Fluoride oxalate) were collected on to ice at each sampling interval, and centrifuged within half an hour at 4 C The plasma from each heparinised sample was divided into four aliquots and frozen at -20 C for later progesterone, cortisol and catecholamine assays The plasma from the oxalated blood was divided into two aliquots and analysed immediately for glucose and lactate

Velvet removal

Velvetting (if undertaken) occurred immediately following the 7th blood sample in the 10 min restraint period Local anaesthetic was injected subcutaneously (SC) to block the infratrochlear and zygomaticotemporal nerves 4 min prior to the nominal time of velvetting Xylazine was injected intramuscularly (IM) 30 to 70 min prior to the time of velvet removal or equivalent control period Yohimbine hydrochloride (0 25 mg/kg) was given intravenously (IV) to sedated deer after the 120 min blood sampling

Tourniquets were applied to the antler pedicle in the minute prior to velvetting and removed at the 30 min blood sampling

Behavioural observations

(a) Indoors

The behaviour of individual animals was scored each time they were brought to the crush (Table 2)

Table 2 The range of behaviour scores for each handling operation

Location	Behaviour score
During crush entry	1 = without any handler assistance 6 = requiring heavy pushing
On crush	1 = no movement 6 = prolonged struggling
Exit from crush	1 = stationary 5 = spring forward

(b) At pasture

The behaviour of individuals was scored as walking, standing, grazing or lying (with or without cudding) each minute while at pasture between the 120 min and 180 min blood sampling (about 12 pm to 1 pm), following the 180 min sampling (about 1 pm to 6 pm) and for 1 hr prior to the 24 hr sampling (6 30 am to 7 30 am)

The frequencies of these activities (except for cudding) were recorded simultaneously in a neighbouring group of undisturbed stags (Control Group II)

Electrocardiogram (ECG) recordings

ECG recordings were made on seven of the stags that were immobilised with Rompun and velvetted (Table 1) Suction cup electrodes were applied to shaved areas around the left side of the sternum All animals were subjected to the standard velvetting process The electrodes were connected to a monitor and tape recorder for later analysis on a digitising oscilloscope and paper chart Various aspects of the ECG were measured the P-P (heart rate), QRS, P-QRS, P-T and QRS-T intervals

Recordings were made before, during and after the 10 min blood sampling and velvetting procedures and at intermittent intervals up to the time of yohimbine administration

In addition, ECGs were taken from two of the stags while exposed to various other procedures similar to those occurring during velvetting (gentle shaking of the head while holding the antler, pin pricks on the velvet, pressure on the velvet from a blunt probe, sawing of wood close to the ears, and a loud noise) prior to velvet removal

Analyses

Glucose was assayed automatically using the glucose oxidase peroxidase (GODPOD) method on an Hitachi 717 Analyzer Lactate was measured enzymatically using an automated procedure on the same Analyzer Progesterone was assayed by radioimmunoassay after an indirect extraction procedure

The statistical significance of the data were tested by ANOVA.

RESULTS

Behaviour

Indoors

There were differences in the behaviour of individual animals in the yards with the 4 yr old animals being easier to handle than the younger ones Most animals required some assistance to push them along the final 1-2 m of the race into the crush Four animals usually walked on without assistance and one was consistently difficult to move, frequently showing aggressive behaviours (teeth grinding, strong eye contact)

There was no significant tendency for animals to become more or less difficult to guide onto the crush over the successive 18 crush entries for each individual ($p > 0.05$) The entry score averaged over all animals was 2 4 on the first handling treatment and 2 7 on the third Further, there were no consistent differences between velvetted and non-velvetted deer,

On most occasions the stags remained stationary or flinched up to three times while in the crush for blood sampling or velvetting This pattern was consistent throughout the experiment with the average score in the crush being 2 3 on the first treatment and 1 9 on the last ($p>0.05$) Again, there was no difference between velvetted and control animals

Most animals exited from the crush at a walk or fast walk and this did not vary during the experiment or between handling treatments ($p>0.05$)

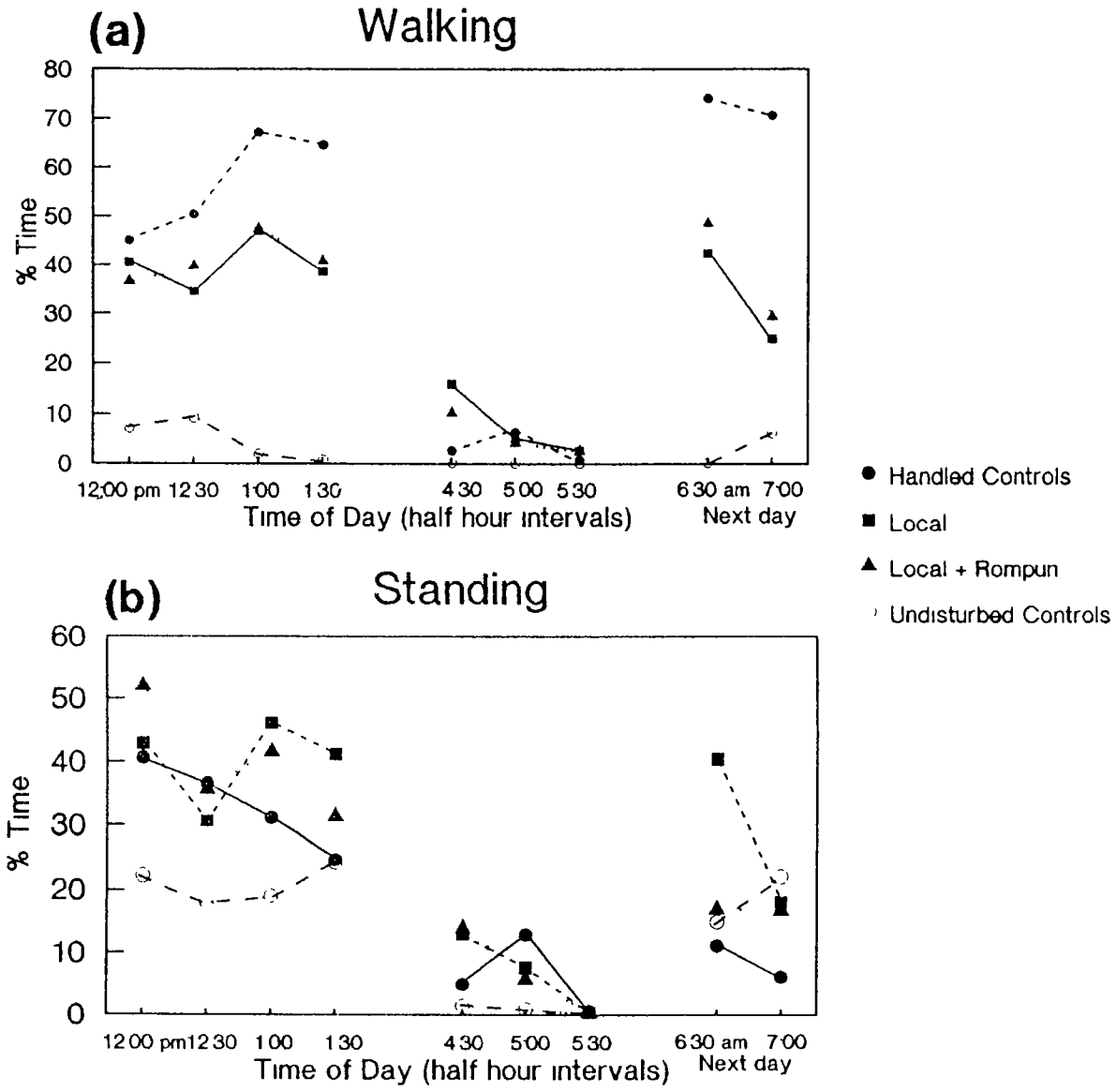

Fig 1 Percent of time spent walking (a) and standing (b) by each group after release to pasture The handling treatments were administered approximately 2 h prior to release

At Pasture

The proportion of time spent in each of the various classes of activities (walking, standing, grazing, lying and cudding) was averaged across individuals within treatment groups and summed over half hour periods

Walking and Standing

Figure 1 shows that walking and standing were the predominant activities for the first 4 half-hour periods upon release to pasture (about 2 hr after administration of the handling treatments) Walking usually involved pacing backwards and forwards along the fencelines interspersed by short periods standing motionless There were no differences between the treatment groups ($p>0.05$) in the frequencies of these activities However, the undisturbed control group spent significantly less time walking or standing ($p<0.05$) than all other groups

Between 4 30 pm and 6 00 pm (6 to 7 hr after handling) the walking and standing activities of all groups were similar ($p>0.05$), but by the following morning handled groups spent more time walking again than the undisturbed controls

Grazing

All handled groups spent about 10 percent of the time grazing throughout the afternoon The time spent grazing was consistently less than for the undisturbed group ($p<0.05$) The grazing activity of the handled stags was much less intense than that of the undisturbed animals and was often interspersed between long periods pacing the fenceline The following morning the handled stags again grazed less than the non handled animals

Lying

The handled animals spent virtually no time lying down in the first two hours after release to pasture While these animals usually spent about 30 percent of the time resting in the late afternoon (also cudding), they always spent less time lying than the undisturbed stags $p<0.05$) Further, this difference was still evident the following morning

Blood Parameters

Glucose

For animals within a particular drug treatment, there were no significant differences in plasma glucose levels between animals that were de-antlered and those that were left intact Therefore, the data for antler removal and non-removal were combined and are shown in Figure 2(a)

Two distinct patterns of variation in plasma glucose were seen (Figure 2a) For animals not given xylazine, the glucose levels increased by about 5% during cannulation, rose a further 20% between cannulation and the beginning of the 10 min sampling period, and peaked 2-3 hr after a handling treatment before returning to baseline at the 24 hr sampling There were no consistent differences in the glucose profiles for animals given local anaesthetic and the non-anaesthetised controls

The second pattern was shown by the immobilised stags and was characterised by a 100% increase in glucose levels during the period of chemical restraint and for at least one hour afterwards followed by a return to baseline at 24 hr

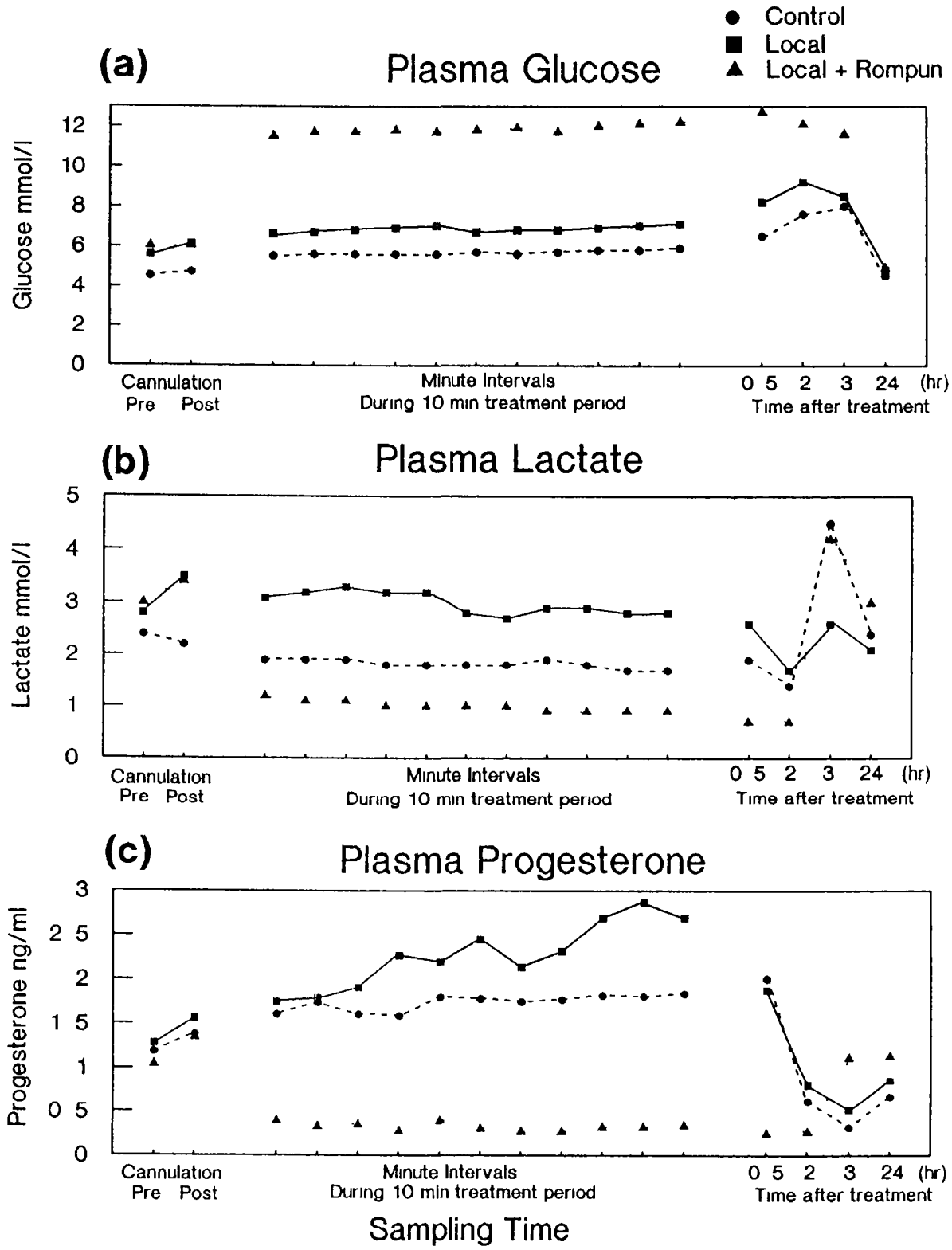

Fig.2 Plasma glucose (a), lactate (b) and progesterone (c) levels during each phase of the blood sampling and handling treatments.

There was no tendency for the plasma glucose levels at the time of the pre-cannulation blood sampling to increase or decrease over the successive handling treatments

Lactate

For animals within a particular anaesthetic treatment, there were no significant differences in plasma lactate levels between animals that were de-antlered and those that were left intact Therefore, as with the glucose measures, the data for antler removal and non-removal were combined and are shown in Figure 2(b)

A trend for lactate levels to increase during cannulation for the animals on the drug treatments and to decrease for the control animals can be seen in Figure 2(b) These changes were not significant

For animals restrained in the crush during treatment, lactate levels declined by 10% following cannulation, declined a further 10% while in the crush for 10 min, and showed a variable pattern thereafter before returning to baseline at the 24 hr sampling There were no consistent differences in the lactate profiles for animals given local anaesthetic and the non-anaesthetised controls

The pattern for chemically-immobilised stags differed in that the post-cannulation decrease in lactate levels was much larger (40%) The plasma lactate concentrations continued to decline while the animals were sedated, but increased to 140% of baseline after administration of yohimbine hydrochloride before returning to baseline 24 hr later

There was a tendency for the plasma lactate levels at the time of the pre-cannulation blood sampling to decrease over the successive handling treatments

Progesterone

For animals within a particular drug treatment, there were no significant differences in plasma progesterone levels between animals that were de-antlered and those that were left intact Therefore, the data for antler removal and non-removal were combined and are shown in Figure 2(c)

The trend for progesterone levels to increase during cannulation was significant ($p<0.05$) For the animals restrained in the crush, the progesterone levels continued to rise (by about 15% over post cannulation values) during the 10 min sampling period The plasma progesterone levels declined rapidly to about one-third of baseline values 2-3 hr after the time (nominal) of velvet removal and were still only two-thirds of baseline at the 24 hr sampling There were no consistent differences in the progesterone profiles for animals given local anaesthetic and the controls (no drug treatment)

The progesterone levels of the chemically-restrained animals fell to 30% of post-cannulation values and remained low while immobilised Within one hour of yohimbine administration the plasma progesterone values had returned to pre-cannulation levels

There was a trend ($p<0.05$) for the plasma progesterone levels in the first blood sample (pre-cannulation) to decrease over successive handling treatments

Electrocardiogram (ECG) Characteristics

Electrocardiograms were obtained from chemically-restrained animals only All animals showed a marked bradycardia following xylazine administration but this decreased linearly with time Averaged across all animals, heart rate increased from 33 beats per minute (bpm) 10 min after injection to 66 bpm 90 min later

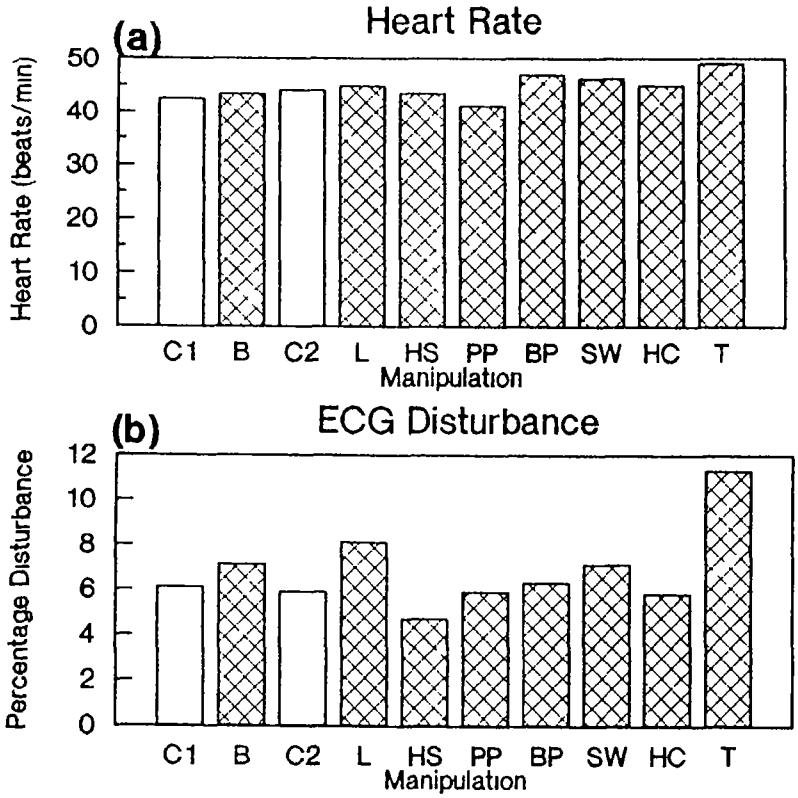

Fig. 3 Heart rate (a) and percentage disturbance of the ECG (b) during procedures similar to some aspects of velvetting and during blood sampling

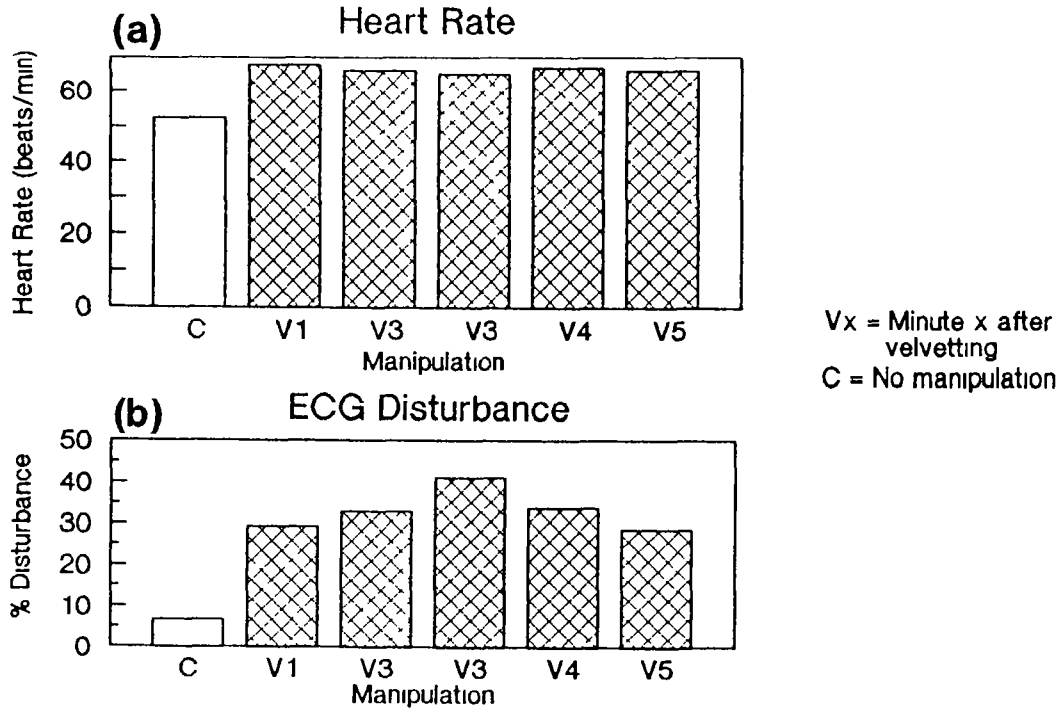

Fig 4 Heart rate (a) and percentage disturbance of the ECG (b) during a control period and after velvetting

The effects of the various manipulations on ECG parameters was assessed by comparing measures taken during the manipulations (3 to 5 min of recordings) with measures taken in the preceding 3 min Where appropriate, the results were averaged across animals

Blood sampling, administration of local anaesthetic, tourniquet application, and all other components of the velvetting procedure except the sawing of the antler had no discernible effect on heart rate or other characteristics of the cardiac cycle (Figure 3)

In two stags velvet removal had no effect upon the cardiac parameters measured, but in the remaining five animals heart rate was elevated ($p < 0.05$) for at least five minutes after removal (Figure 4) In addition, the percentage of disturbed ECG measures (depression of the ST junction, changes in ST shape and changes in the T wave pattern) increased (Figure 4)

The manipulations on the two animals that showed no change in cardiac parameters during velvetting were performed within 45 min of xylazine injection The interval from sedation to velvetting in the remaining animals was between 50 and 70 min

DISCUSSION

The unusually high levels of walking and standing, and low levels of grazing and resting, shown by all stags at pasture after handling strongly suggests that some aspect(s) of the manipulations disturbed the animals Rushen (1984) has shown that disruption of normal maintenance activities and frequent repetition of one or two behavioural characteristics are often observed in farm animals that are kept in apparently stressful environments It would seem that velvet removal under local anaesthetic with or without chemical immobilisation did not contribute significantly to the stress of handling in the present study since there were no consistent differences in the behavioural responses at pasture between velvetted and non-velvetted animals

Further, the similarity of the plasma chemical profiles, and of the indoor behaviour scores, between velvetted and non-velvetted animals in each treatment group (chemically and non-chemically restrained) supports the notion that velvet removal did not increase the stress of handling or cause the disturbed behaviour at pasture These results correspond with those of Mackintosh et al (pers comm) who found that cortisol levels increased by similar amounts in velvetted and non-velvetted stags

Further, as all handled animals behaved similarly, it seems that the practices common to all groups (yarding, restraint, blood sampling) and not velvet removal led to the changes in blood constituents and behaviour at pasture

It would be premature, however, to suggest that velvet removal does not cause any stress Increases in heart rate and ECG disturbance, two characteristics often associated with a stress response (Ehsani et al 1984), were observed in stags that were velvetted 45 min or longer after sedation with xylazine Animals velvetted less than 45 min after immobilisation did not show these reactions It is not clear if the local anaesthetic was ineffective in those deer showing the cardiac cycle changes, or if the limited analgesic properties of xylazine diminished rapidly after about three-quarters of an hour Further research on chemically and non-chemically immobilised animals is required before drawing firm conclusions about the effect of velvetting on the cardiac cycle

The elevations in plasma glucose and progesterone seen during the intensive blood sampling routines in all non-chemically immobilised stags implicates stress in the handling regime Increases in the plasma levels of these compounds have been reported in other studies where deer and cattle have been exposed to stressors such as herding, restraint, blood sampling or injection of ACTH (Hattingh et al 1988, Jopson et al 1990, Plotka et al 1983)

Rushen (1986) has shown that sheep become increasingly reluctant to re-enter raceways in which aversive treatments (electro-immobilisation, restraint, shearing) have been applied to the animals In the present study there was no consistent tendency for the stags to become more reluctant to enter the

crush where cannulation, restraint, blood sampling and velvetting had occurred This suggests that these handling routines were not particularly stressful Further, the trend for lactate and progesterone levels to decrease over successive weekly treatments suggests that the procedures may have become even less aversive with repeated handling

The most likely source of stress seems to have arisen from the practices of herding, yarding, drafting and restraining the animals Animals which were difficult to move or flighty in the yards prior to restraint in the crush showed the greatest changes in the levels of plasma constituents Further, the levels in these animals were higher than for most other animals at the time of the first blood sampling and this disparity was maintained during subsequent sampling

It is possible that the disturbance created by the initial handling and restraint masked the effects of subsequent manipulations such as velvet removal Additional work is required to determine the separate effects of the various components of the handling routines, and the peak stress responses that are elicited by these procedures

Lactate levels of non-sedated animals in the present experiment tended to decline over the course of a treatment This contrasts with the results of other studies where lactate levels have increased during herding and transport (Hattingh et al 1988, Tollersrud et al 1971) This discrepancy may be attributable to a difference in the amount of exercise performed by the animals Blood lactates increase during exercise (Cooper et al 1989) and the animals in the present experiment were usually held firmly in a crush while those in other studies have been free moving Plasma lactates increased markedly in some animals in the current study, especially following long periods of activity at pasture This suggests that lactates may be a better index of physical stress than psychological stress

The known pharmacological effects of xylazine present difficulties in interpreting the effects of handling routines alone in the sedated animals Hyperglycaemia was observed in xylazine-treated animals and similar xylazine-induced changes have been reported elsewhere (Meyer-Jones et al 1977) The low plasma levels of progesterone may be due to sedation per se or to a direct pharmacological influence of xylazine It seems probable that the low levels of lactate observed in sedated animals is attributable to inactivity

Not all measured parameters had returned to baseline (pre-cannulation) levels 24 hrs after initiation of a particular handling treatment In all treatment groups some disruption to behaviour at pasture remained However, plasma levels of lactate and glucose were similar to those at the first sampling Recovery of plasma progesterone differed according to treatment Xylazine-treated animals returned to first sampling values and non-sedated stags remained below initial observed levels These results suggest that routine handling practices may have a protracted influence on some aspects of behaviour and physiology

This study highlights the importance of combining behavioural and physiological measures in the objective assessment of the stressfulness of routine management procedures

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