Physiological quantification of pre-slaughter handling stress in red deer

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Abstract

To assess the impact of pre-slaughter handling on the welfare of red deer, eleven biochemical variables were measured in the blood of commercially slaughtered deer (DSP treatment. n=8) and compared with values from deer from the same source but shot at pasture under minimal stress (PAD treatment· n=8) Blood collected during exsanguination was assayed to determine levels of cortisol, progesterone, glucose, lactate, protein, albumin, creatine kinase, lactate dehydrogenase, aspartate aminotransferase, packed cell volume, and creatinine Elevated levels of all variables except creatinine, glucose and protein were observed in the DSP deer compared with the PAD deer ($P<0.05$) A review of the literature indicated that for *post-mortem* evaluation of pre-slaughter stress, plasma cortisol and serum enzymes were the most reliable indicators of psychological stress and muscular damage/exertion respectively. According to these indicators the PAD deer were not psychologically stressed at the time of death, but the DSP stags were under a moderate degree of stress, and exhibited high levels of muscular damage/exertion, probably as a consequence of fighting during lairage.

Introduction

Current commercial pre-slaughter handling of deer commonly lasts at least one day and involves a range of potential stressors including close confinement with other deer, human presence, unfamiliar environments and vehicular motion. Even minor handling results in increases in physiological stress indicators (Carragher *et al* , 1997, Ingram *et al* , 1997) Therefore the protracted period of handling prior to slaughter would be expected to have a major physiological, and welfare, impact To assess the extent and range of variables affected, blood from paddock-shot deer was compared with blood from deer slaughtered commercially The variables measured were intended to provide reference values for future studies aimed at improving pre-slaughter management practices

Methods

Full details of the experimental protocol are provided in Pollard *et al.* (in prep) Briefly,

58 stags aged 15 months were allocated to two groups and held in separate 0.5 ha paddocks near a set of deer yards, and in close proximity to a deer slaughter plant (DSP) starting five weeks before the experiment. During the five weeks the deer were fed 100g barley /head twice daily, from the back of a moving flat-deck truck

Within each group, deer were allocated to either paddock shooting (PAD treatment, n=8 deer), slaughter at the nearby plant (DSP treatment, n=8 deer) or no treatment Over four successive days, groups of two DSP stags plus four spare animals were transported for three hours, held in lairage at a DSP overnight, then shot with a captive bolt pistol and exsanguinated. One deer from each paddock group was head-shot with a 223-calibre rifle during morning feeding, exsanguinated and transported to the DSP for further processing, on the same days and at approximately the same times as the two DSP deer were killed Stags from both treatments were electrically stimulated during exsanguination (60 s at pulse width eight milliseconds, pulse frequency 69 msecs).

Behaviour of the DSP deer during overnight lairage was recorded during eight hours (2000 hr - 0400 hr), using an infrared video camera and light source For 30 minutes at 90-minute intervals the number of antagonistic encounters and the specific activities (biting, kicking with the forelegs or butting) which comprised each encounter were recorded from the videotapes

Blood samples were collected at exsanguination. For determination of plasma progesterone, cortisol, packed cell volume (PCV), lactate, and lactate dehydrogenase (LDH), samples were collected in heparinised tubes, and for plasma glucose determination blood was collected in tubes containing sodium fluoride The blood was stored at 4 ^{0}C then plasma was removed and stored frozen at -20 ^{0}C Progesterone and cortisol levels were measured in duplicate samples using direct double antibody radioimmunoassays (RIA) PCV was measured using a Hermle Z 230H centrifuge. Plasma lactate and LDH were measured using Roche Diagnostics kits (numbers 139084 and 1489518, respectively) Glucose was measured using a glucose oxidase method (Trindler, 1969) For determination of serum CK, creatinine, AST, total protein and albumin, samples were collected in blood tubes and stored at 0 ^{0}C. Serum was drawn off and analysed in accordance with the Beohringer Mannheim/Hitachi System Manufacturer's methods

All data were analysed by analysis of variance, with animal within group within day as the block structure and slaughter treatment as the treatment structure

Results

Nearly all of the plasma and serum variables measured showed considerable differences between DSP and PAD treatments (Table 1) The exceptions were plasma glucose, and serum creatinine and total protein (Table 1)

Table 1. Means (and ranges in brackets) of physiological variables for DSP and PAD treatments, plus SED and significance level of differences between treatments (ns not significant, * P<0.05, ** P<0.01, *** P<0.001).

	Treatment			
	DSP	*PAD*	*SED*	*Sig*
PCV (%)	49 (45-52)	46 (41-48)	0.98	*
AST (iu/l)	183 (82-348)	67 (44-89)	33 5	*
Albumin (g/l)	28 (26-31)	26 (23-28)	0.56	**
Creatine kinase (iu/l)	2441 (596-5340)	260 (142-619)	224	***
Creatinine (umol/l)	115 (89-129)	107 (88-127)	6.9	ns
Protein (g/l)	70 (64-74)	64.6 (58-72)	2.56	ns
Cortisol (ng/ml)	28 (17-36)	5.6 (0-13)	3.53	***
Progesterone (ng/ml)	0 7 (0 2-2.1)	0 2 (0-0 4)	0.19	*
LDH (iu/l)	1269 (862-1659)	717 (508-809)	99	***
Lactate (mmol/l)	8.8 (6.4-13 1)	3 5 (2 0-5 7)	0 95	***
Glucose (mmol/l)	7 2 (6 8-7 4)	7 0 (6.6-7.4)	0 130	ns

During overnight lairage there was a considerable amount of antagonistic behaviour, with a mean of 23 (SED 4 13) encounters per hour, but no obvious pattern in activity over the observation period. Butting other deer with the head was the most frequent type of antagonistic activity (88% of encounters) Biting was seen in just over half of the encounters, and kicking (with the forelimbs) occurred in a small proportion of observations (8%).

Discussion

As expected, pre-slaughter handling was associated with elevations in blood metabolites indicative of stress and muscle injury The following considers each of the variables measured, using comparisons with previous studies of red deer, to gain some idea of the levels of stress imposed by the two treatments and the usefulness of the variables in evaluating pre-slaughter stress.

Cortisol

Plasma cortisol has been extensively measured and was concluded to be a reliable and sensitive indicator of pre-slaughter handling stress in livestock (Shaw & Tume, 1992). However Diverio *et al.* (1996) observed fluctuations and large individual differences in baseline concentrations of cortisol in red deer hinds, and Goddard *et al* (1994) highlighted the influence of animal type and management

on cortisol responses to handling. Values differed between stags and hinds in one study (Smith & Dobson, 1990) but not in another (Grigor *et al.*, 1998b)

Remote sampling has been used to monitor the cortisol response to handling and recovery. Levels rose within minutes of handling disturbance (Carragher *et al* , 1997) and the estimated recovery time was 3 75 hr after the end of handling (Ingram *et al* , 1994)

In the present study, the deer shot at pasture had a mean plasma cortisol value of 5 6 ng/ml, which was similar to the low values obtained from wild stags which were shot after stalking (Bradshaw & Bateson, 2000) and farmed stags which were shot at pasture (Smith & Dobson, 1990) or sampled (remotely) whilst grazing (Carragher *et al* , 1997, Ingram *et al.*, 1997)

The mean cortisol value for the DSP deer was substantially higher, at 27.5 ng/ml, than that for the PAD deer In comparison with other studies this was a moderate level of elevation. Other stags subjected to transport and a short period of handling/lairage prior to slaughter showed similar values (Smith & Dobson, 1990) but hinds treated similarly yielded values of 40-60 ng/ml (Grigor *et al.*, 1997a, Jago *et al.*, 1997) Relatively high values, in the 50-70 ng/ml range, have been obtained from farmed stags subjected to handling (mechanical restraint and various other treatments) in the normal farm environment (Matthews & Cook, 1991, Matthews *et al* , 1994, Ingram *et al.*, 1994, 1997) In wild deer, a mean plasma cortisol level of 73 4 ng/ml was recorded in stags which had been hunted with hounds (Bradshaw & Bateson, 2000) and a mean of 82.6 ng/ml was observed in wild hinds subjected to several handling treatments (Goddard *et al* , 1994) The moderate levels seen in the DSP deer could reflect that they were only moderately, not highly, stressed at the time of slaughter. It is unlikely that the moderate levels resulted from depletion as plasma cortisol was maintained at high levels for several days during artificial stimulation with ACTH (Asher *et al* , in press)

Progesterone

Plasma progesterone varies with the reproductive cycle in red deer hinds, but also increases as a result of adrenal stimulation (Jopson *et al* , 1990). Following artificial stimulation with ACTH, peak plasma progesterone levels were reached within 15-30 minutes and generally returned to original levels within two hours (Jopson *et al.*, 1990)

Different studies have yielded a wide range in progesterone levels in restrained/handled deer, from 0 2 to 8 ng/ml (Jopson *et al* , 1990, Matthews *et al* , 1990; Matthews & Cook, 1991; Ferre *et al* , 1998), and wide variation between individual levels during handling were observed (Jopson *et al* , 1990) In the present study, the mean level seen in the PAD deer (0.15 ng/ml) was possibly a baseline level, and was significantly lower than that of the DSP deer (0 73 ng/ml)

Glucose

Plasma glucose is an indirect indicator of stress, mediated via the catecholamines and/or glucocorticoids, but also varies with the nutritional state of the animal (with food deprivation leading to reduced glucose levels, Shaw & Tume, 2000). In response to yarding and restraint in a deer crush, levels rose to become significantly different from baseline within an hour of disturbance (Carragher *et al* , 1997) and it was observed that a return to pre-treatment levels of around 4 mmol/l occurred within 5 3 hr (Ingram *et al* , 1994)

In the present study respective means for PAD and DSP deer were 7.0 and 7 2 (SED 0.13) mmol/l. These means were not significantly different and comparable to levels observed in transported, yarded or handled deer (5-9 mmol/l Jago *et al* , 1997, Ingram *et al* , 1994, Carragher *et al* , 1997) and were within the normal range for undisturbed deer (Webster *et al.*, 1996). It is possible that despite any initial effects of handling, mechanisms that maintain normal blood glucose levels had restored normal levels in the DSP deer during the protracted period of handling and lairage before slaughter

Lactate

Elevated blood lactate levels result from breakdown of muscle glycogen as a result of extreme muscular exertion, and from catecholamine release leading to rapid glycogenesis (Shaw and Tume, 1992) Catecholamine release results from fear or excitement but large increases were also seen

following captive bolt stunning, leading to the suggestion that lactate levels are of limited use in evaluating pre-slaughter stress (Shaw & Tume, 1992)

An apparent baseline level of lactate, recorded in stags at pasture, was 0 94 mmol/l (Ingram *et al.*, 1994) Levels rose soon after the onset of handling (Carragher *et al* , 1997) and the estimated recovery time was 2.7 hr after handled stags were released back to pasture (Ingram *et al* , 1994)

In the present study, lactate levels were relatively high (8.8 mmol/l) in the DSP deer, in comparison with the PAD deer (3 5 mmol/l), and also with hinds slaughtered after transport and a short lairage (4.0 mmol/l, Jago *et al* , 1997), handled stags (1-5 1 mmol/l, Carragher *et al.*, 1997, Waas *et al.*, 1997) While an elevation in the DSP stags was consistent with a response to handling, the shooting techniques differed between PAD and DSP deer therefore this comparison may not be valid.

Protein and albumin

Consistent with some other studies on handling and pre-slaughter treatments in farmed red deer (Grigor *et al* , 1997b, 1998a), no difference in plasma total protein was observed between PAD and DSP treatments In the review by Shaw and Tume (1992) it was considered that protein levels may be of little use in assessing pre-slaughter stress, but could be of value in identifying pathological lesions in the carcass Serum protein levels may also reflect nutritional status (Wiklund, 1996), muscular activity (Bradshaw & Bateson 2000), and also dehydration, although Grigor *et al* (1997a) considered that the rumen provided a sufficient reservoir of food and water for deer to be able to withstand transport and lairage

Other previous studies which have investigated effects of pre-slaughter treatments on protein levels include the study of wild deer by Bradshaw and Bateson (2000), in which protein levels were higher in those which were killed by hunting, rather than injured (by poor shooting and other causes). Increased protein levels were observed in deer recovering from transport (Grigor *et al.*, 1997b) and evidence of an interaction between nutritional background and the response to pre-slaughter handling on protein levels in reindeer was observed by Wiklund *et al* (1996).

Albumin levels have not been commonly measured in studies of stress in red deer, but were higher in the DSP deer than in the PAD deer Bradshaw and Bateson (2000) found elevated levels in hunted deer compared with injured deer, and attributed this difference to the greater muscular activity of the hunted deer

Enzymes

CK, LDH and AST are cellular enzymes which leak into the blood as a result of damage or muscular exertion (Boyd, 1988) DSP deer showed elevated levels of all three enzymes measured in comparison with PAD deer.

Rises in LDH, attributed largely due to the isoenzyme LDH-5 from skeletal muscle, occurred during pre-slaughter handling (Goddard *et al.*, 1997) It was thought that plasma LDH took 1-2 hours to increase in response to stress, and that it had a relatively long half-life in plasma because no decrease occurred during 18 hours of rest in lairage (Goddard *et al* , 1997).

Mean levels of LDH in the PAD deer (717 iu/l) were higher than those observed previously in handled stags (with means of 400-600 iu/l; Abeyesing *et al.*, 1997) The mean of 1269 iu/l seen in DSP deer was greater than LDH levels observed in slaughtered hinds which had been transported and held for a short time in lairage (Jago *et al* , 1997), or wild hinds which were captured and restrained (Marco & Lavin, 1999) but lower than those observed in hunted deer (Bradshaw & Bateson, 2000).

AST values for PAD deer were between those found previously in stalked stags (Kent *et al* , 1980, Bradshaw & Bateson 2000), while the DSP deer had relatively high AST levels compared with the slaughtered hinds observed by Jago *et al* (1997) and also compared with hunted or captured wild stags (Marco & Lavin, 1999, Bradshaw & Bateson, 2000)

CK has been measured in many studies of handled deer Baseline levels may be around 260 iu/l as seen in stalked stags (Bradshaw and Bateson, 2000) and in the present study, although lower values (mean=50 iu/l) were observed in the stags shot by Kent *et al* (1980) Levels of CK were sensitive to

transport, with increases related to transport distance (Jago *et al*, 1997) and winding roads (Grigor *et al*, 1998a) Serum CK has a half-life of a few hours (Boyd, 1988) A rest period of 2-3 hrs was insufficient to clear CK from the blood (Grigor *et al*, 1997a, 1988a, b) but over 18 hours of lairage, a decrease from a mean of 997 iu/l post-transport to 271 iu/l was observed (Grigor *et al*, 1997a).

In the present study CK levels in the DSP deer were particularly high, compared with all studies on slaughtered or handled deer, except the hunted deer in Bradshaw and Bateson's (2000) study. Given that they had been held in lairage for 18 hours, as in the study by Grigor *et al.* (1997a), effects of handling and transport the previous day should have diminished. Therefore the high CK levels at slaughter may have reflected muscular exertion or activity during lairage or immediately pre-slaughter Behavioural records support the possibility of exertion and damage during lairage as a mean of 23 antagonistic encounters per hour was observed The deer studied by Grigor *et al* (1997a) fought far less, with a mean of less than two antagonistic encounters per hour.

The frequency of antagonistic encounters in the DSP deer in the present study was higher than the average of 14/hr (range 3-34/hr) observed over one year at the same slaughter premises (Pollard *et al.*, 1999) The relatively high frequency occurred possibly because the study was carried out at the onset of the mating season in early autumn, when inter-stag aggression begins to increase (Lincoln, 1992) Nevertheless, a year's average of 14 antagonistic encounters per hour represents a high level of aggression The costs, benefits and methods of lairage need to be scrutinised. It has been suggested that to minimise stress, deer should be slaughtered as soon as possible after arriving at the plant (Kay *et al*, 1991, Grigor *et al*, 1997a)

PCV

PCV in deer increases rapidly in response to splenic contraction due to sympathetic nervous system activity (Cross *et al*, 1988) Using remote sampling, the recovery time for PCV levels was estimated to be 0 5 hr (Ingram *et al*, 1994, Carragher *et al*, 1997)

Mean PVC values of 46 and 49 % for PAD and DSP deer respectively were similar to values obtained from other slaughtered deer (Grigor *et al*, 1997a) and deer restrained manually for blood sampling (Ingram *et al.*, 1994, Ferre *et al.*, 1998; Grigor *et al.*, 1998a). Although the mean value was significantly lower in the PAD deer, it was higher than values obtained using remote sampling (values of around 30% or less, Ingram *et al*, 1994; Webster *et al.*, 1996, Waas *et al.*, 1997; Ferre *et al.*, 1998). Therefore it is likely that the physiological response to slaughter included splenic contraction, elevating PCV values in the PAD deer, but why a difference between treatments was found is not known

Creatinine

No differences in serum creatinine levels were observed between PAD and DSP treatments. Previously, elevated levels were found in hunted compared with stalked deer, and hunted compared with injured deer, and these effects were attributed to greater muscular activity in the hunted deer (Bradshaw and Bateson, 2000). Elevated values were also attributed to protein catabolism during starvation (Nieminen, 1980, cited in Wiklund *et al*, 1996) In reindeer in poor condition, lower levels were seen in deer which were subjected to transport and lairage prior to slaughter, compared with those which were slaughtered directly (Wiklund *et al*, 1996)

Conclusions

Compared with paddock shooting, pre-slaughter handling resulted in increased levels of 8 of 11 biochemical variables studied At-slaughter interpretation of the likely welfare cost to the animals of pre-slaughter handling could most reliably be achieved through the measures of plasma cortisol and serum enzymes, in particular CK which has been extensively studied and has a relatively short half-life Other variables have previously shown confounding effects of nutrition (glucose, protein, creatinine), been studied infrequently (albumin) and shown high variation between results from different studies or with reproductive state (progesterone), or were probably influenced by killing method (PCV, lactate)

On the basis of cortisol and serum enzymes, the paddock-shot deer were not physically or psychologically stressed prior to slaughter, but the commercially-slaughtered deer were at least moderately psychologically stressed and exhibited high levels of muscle damage and/or exertion, probably as a consequence of fighting during 18 hours of lairage

The present research has provided baseline levels and evaluation techniques for future assessments of pre-slaughter handling regimes It would be worthwhile applying these to determine optimal regimes that ensure both good animal welfare and product quality

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