
Capítulo 6

Transport stress in roe deer (*Capreolus capreolus*): effect of a short-acting neuroleptic

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Abstract

The aim of this study was to evaluate the effect of a short-acting neuroleptic (acepromazine) on the stress response to transport in roe deer (Capreolus capreolus). Twenty-one roe deer were submitted to a nine-hour road journey to reintroduce and restock this species into Catalonia (north-eastern Spain). The animals were divided into two groups: animals in the treatment group received an intramuscular injection of acepromazine (0.13 ± 0.013 mg/kg in 0.5 mL; $n = 9$) while animals in the control group received the same volume of saline ($n = 12$). Clinical (heart rate and body temperature, measured during transport using remote devices), haematological and biochemical indicators of stress were used to evaluate the effect of the neuroleptic. Heart rate increased during transportation, but no differences were found between groups. Body temperature decreased during transportation in both groups, but this reduction was faster in acepromazine-treated animals. Comparison of blood parameters before and after transport revealed: significantly lower RBC counts and haemoglobin concentrations after transport in treated animals compared with control animals; a reduction in lymphocyte count, eosinophil count and serum creatinine levels over transport in treated animals; a decrease in serum potassium levels over transport in the control group; an increase in serum creatine kinase (CK), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities over transport in control animals; an increase in serum urea and chloride concentrations over transport in both groups; and finally, a decrease in serum glucose concentrations in both groups. Results obtained show the suitability of using acepromazine in transport operations in order to reduce the stress response and prevent its adverse effects in roe deer.

Keywords: *Animal welfare, Capreolus capreolus, neuroleptic, road transport, roe deer, stress.*

Introduction

The regular translocation of wildlife has become a common management practice. Transportation of animals is often essential for wildlife managers, zoo keepers and farmers, and in order to minimise welfare problems it is necessary that the best transport conditions are used.

There are a number of reviews in the scientific literature concerning the road transport of cattle (Tarrant, 1990; Trunkfield and Broom, 1990; Warriss, 1990; Knowles, 1995; Knowles, 1999), sheep (Knowles, 1998), goats (Sanhoury *et al.*, 1989) and pigs (Warriss, 1987; 1998a,b; Tarrant, 1989). However, only limited information exists on the effects of transport in wild and semi-wild animals, which may respond to handling in different ways to the more docile, domesticated species (Brelurut, 1991).

Stress is usually caused by excessive muscular exertion or fear during capture, loading, or transportation. The many unfamiliar events that occur during transportation lead to both psychological stress as well as the accompanying physical stress of muscular exertion. This excessive muscular exertion may result in damage to locomotor, respiratory, or heart muscles, resulting in capture myopathy (or exertional rhabdomyolysis). Capture myopathy is a syndrome that occurs in wild (free-ranging and captive) mammals and birds. In ungulates the syndrome is characterised clinically by depression, muscular stiffness, lack of coordination, paralysis, metabolic acidosis and death (Spraker, 1993). Pathologically, capture myopathy resembles the myodegenerative disorders of domestic cattle, sheep, horses and swine (Chalmers and Barrett, 1982), and it is mainly characterised by muscular and renal lesions (Spraker, 1993).

Wild animals transported after capture are generally in a state of variable stress brought about by the effects of chasing, capturing and handling. These effects may be suppressed by tranquillisers administered after mechanical capture. Acepromazine is a member of the phenothiazine group of short-acting neuroleptic agents. Although the exact mechanisms of action are not fully understood, the phenothiazines block post-synaptic dopamine receptors in the central nervous system and may also inhibit the

release of, and increase the turnover rate of dopamine. They are thought to depress portions of the reticular activating system which is important for the control of body temperature, basal metabolic rate, emesis, vasomotor tone, hormonal balance, and alertness. In addition to their dopamine-blocking actions, phenothiazines have varying degrees of anticholinergic, antihistaminic, antispasmodic, and α -adrenergic-blocking effects (Plumb, 1995). Cowan *et al.* (1962) studied the use of acepromazine for tranquillising deer of the genus *Odocoileus*. The recommended dose for deer species is 0.05-0.1 mg/kg (Arnemo *et al.*, 1993). Our main reason for choosing acepromazine for this investigation was the widespread use of this drug in veterinary practice. Another possible choice would be one of the butyrophenones, but these present an increased risk of extrapyramidal side-effects (Poling *et al.*, 1990). Hofmeyr (1981) described extrapyramidal syndrome in several wild ungulate species treated with haloperidol.

The roe deer (*Capreolus capreolus*) is undergoing expansion, and is increasingly submitted to reintroduction and restocking operations (García-Ferré *et al.*, 1995) that require transportation. Several studies have been carried out into the behavioural and physiological responses of farmed red deer (*Cervus elaphus*) to road transport (Waas *et al.*, 1997; Grigor *et al.*, 1998; Waas *et al.*, 1999), but no studies have been carried out on roe deer. Moreover, it is widely believed that short-acting tranquillisers are ineffective when the animals concerned are highly stressed at the moment of administration, particularly in free-ranging animals, but this needs to be further explored (Diverio *et al.*, 1996b).

The aim of this study was to evaluate the stress response to transport in roe deer and the effect of acepromazine on this response by using clinical (heart rate and body temperature), haematological and biochemical parameters.

Materials and Methods

Animals

Twenty-one roe deer were used in this study: 10 males (six adults, four fawns [less than one year old]) and 11 females (six adults, five fawns) captured by means of drive-

nets in the National Game Reserve of Chizé in mid-western France. The mean liveweight of the subjects was 19.73 ± 1.3 kg (range 12.3-26.7 kg; $n = 21$).

Treatment groups

Nine randomly selected animals, four males (one adult, three fawns) and five females (three adults, two fawns) received 2.5 mg ($0.13 \text{ mg/kg} \pm 0.013 \text{ SEM}$) of acepromazine (Calmo Neosan[®] 5 mg/mL, Smithkline Beecham, Madrid, Spain) in a volume of 0.5 mL intramuscularly. Twelve control animals, six males (five adults, one fawn) and six females (two adults, four fawns) received 0.5 mL of saline intramuscularly (Fisiológico Braun, Braun Medical S.A., Rubí, Barcelona, Spain).

Procedure

Animals were captured by means of drive-nets and were immediately placed in individual transport boxes designed for roe deer. They were then transported for 5 minutes in a van to the control centre of the Reserve. Once there, acepromazine or saline were administered, blood samples withdrawn, and the animals marked with ear tags, weighed and aged and reintroduced into their transport boxes. In addition, the right thoracic and left precordial areas of certain animals were clipped in order to install the heart-rate recording equipment, and the body-temperature recording device was introduced into the rectum. Twelve of the 21 roe deer had been captured approximately 48 hours before the transport day and maintained in captivity during that time, whereas the rest of the transported deer were captured on the transport day. The transport boxes were placed in the van, where animals remained until the onset of road transport ('pre-transport period' = 25 minutes). Transportation lasted nine hours and was mainly on the motorway. A second blood sample was taken upon arrival and the recording equipment removed.

This procedure was repeated twice, the overall study being carried out in two parts. The first part took place in January 1999 with ten animals being transported, and the second part took place in March 2001 using 11 animals.

Equipment

Ten roe deer (five per group) were fitted with a telemetric heart rate recording device (Polar Vantage NV[®], Polar Electro Oy, Finland). The device comprised a transmitter and a receiver. The transmitter was attached to a girth belt supplied by the manufacturer for use by athletes. The two electrodes forming the transmitter were covered with electrode coupling gel to maintain a good electrical contact. The heart-rate signal was telemetrically transmitted within a range of 1 m to the receiver, which was placed around the neck of the animal. Heart rate was measured at 60-second intervals throughout the journey. Using the manufacturer's software program, the data were transferred later for further analysis. The arithmetic mean of heart rate values was calculated for every five-minute period for statistical analyses.

Thirteen roe deer (seven in the treated group, six in the control group) were fitted with a telemetric body-temperature recording device (Mätman datalogger[®], Chipsobits Eltex AB, Sweden). Rectal temperature was measured at 90-second intervals and the total recording period lasted for 5.5 hours along the journey. Using the manufacturer's software program, the data were transferred later for further analysis. The arithmetic mean of rectal temperature values was calculated for every 15-minute period for statistical analyses. Ambient temperature during capture and transport was 6-12°C.

Blood collection and analyses

Two blood samples (10 mL) were taken, one before and one after transportation. Blood samples were obtained using disposable syringes and 0.8 x 25 mm needles. Blood collected from the jugular vein was placed in a tube with EDTA K₃ as an anticoagulant, and used for haematological analyses. The remainder of the blood was placed in a serum collection tube, allowed to clot at room temperature, centrifuged (3,000 rpm for 10 minutes), and resultant serum used for biochemical analyses. Serum was kept at -18°C until analyses were completed. All serum samples were analysed at the same time after a three-month period. Samples from the control and treated groups were treated equally. Haematological examinations of red blood cell (RBC) count, haemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and

white blood cell (WBC) count were performed using a semi-automatic analyser (Sysmex F-800[®], Toa Medical Electronics Co. Ltd., Japan). Packed cell volume (PCV) was measured by the standard microhaematocrit method with a haematocrit centrifuge (Micro-Haematocrit Centrifuge, Hawksley, Lancing, UK) at 14,000 rpm for 5 minutes to adjust the values obtained with the analyser. Differential leukocyte counts were performed using blood smears stained with commercial Diff-Quick[®] type stain (Química Aplicada S.A., Amposta, Spain). Biochemical analyses were performed using an automated analyser (COBAS MIRA[®], Roche, Nutley, NJ, USA) except for sodium and potassium concentrations, which were measured using flame photometry (Corning 410C[®], Corning Medical, Medfield, USA), and serum cortisol, which was determined using an ELISA commercial kit (DRG Cortisol EIA-1887, DRG Diagnostics, Germany).

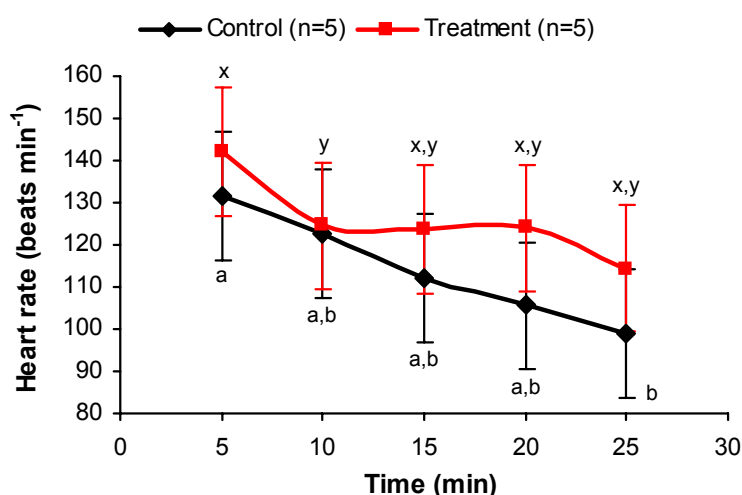


Figure 6.1. Heart rate (mean \pm SEM) during pre-transport period for control and treated roe deer

a, b Values with different superscripts are significantly different from each other ($P < 0.05$) in the control group.
 x, y Values with different superscripts are significantly different from each other ($P < 0.05$) in the treated group.

Statistical analyses

Repeated measures ANOVAs were carried out using the PROC MIXED procedure of the SAS[®] statistics software package (SAS Institute Inc., Cary, NC, USA). The main factor was treatment (acepromazine or saline) and the repeated factor was time. The animal's sex, age, capture day (two days before transport or the transport day), and transport group (first or second transport procedure), and their interactions, were included in the statistical model. A type 1 autoregression (AR[1]) structure for the covariance matrix of the repeated measures was used. Least square means (LS

MEANS) were used because the distribution of animals between groups was unbalanced. In all cases, the accepted significance level was $P < 0.05$.

Results

Heart rate did not differ significantly between groups. It decreased significantly ($P < 0.05$) during the pre-transport period (Figure 6.1) and increased during the transport period (Figure 6.2) in both groups. Body temperature (Figure 6.3) decreased over time in both groups, but it reached baseline levels 2 hours earlier ($P < 0.05$) in those animals that received acepromazine than in the controls.

Haematological parameters are shown in Table 6.1. RBC count and haemoglobin concentration in samples taken from treated animals after transportation were significantly lower ($P < 0.05$) than those obtained from control animals. Lymphocyte and eosinophil counts decreased over transport ($P < 0.05$) in the roe deer treated with acepromazine.

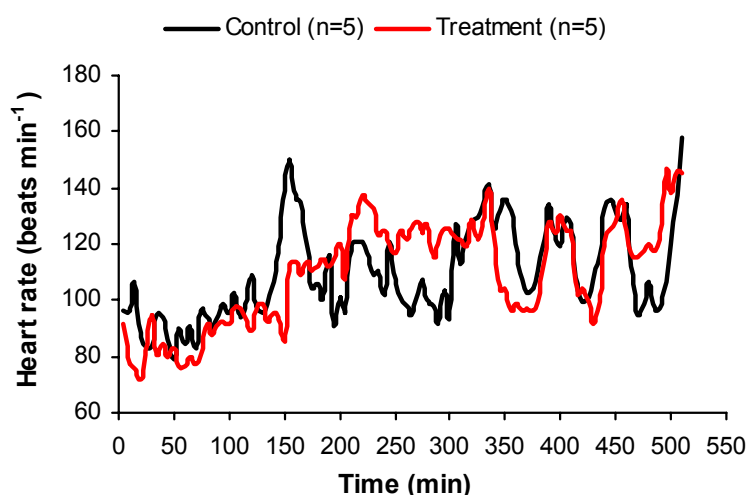


Figure 6.2. Mean heart rate during transport for control and treated roe deer. Heart rate increased significantly over time in both groups ($P < 0.05$).

Serum biochemical parameters are shown in Table 6.2. Serum CK, AST ($P < 0.05$) and ALT ($P < 0.001$) activities increased over transport in the control group, whereas in the treated group they did not change. Serum lactate dehydrogenase (LDH) activity did not show statistical differences. Urea concentrations increased over transportation ($P < 0.05$) in both groups of roe deer. Serum creatinine concentrations decreased

significantly over transportation ($P < 0.05$) only in the treated group. Serum glucose levels declined in both groups during transportation. Serum chloride concentrations increased significantly ($P < 0.05$) during transportation in both groups. Serum potassium levels decreased over transportation ($P < 0.05$) only in the control group. No significant differences between groups were observed in serum cortisol levels.

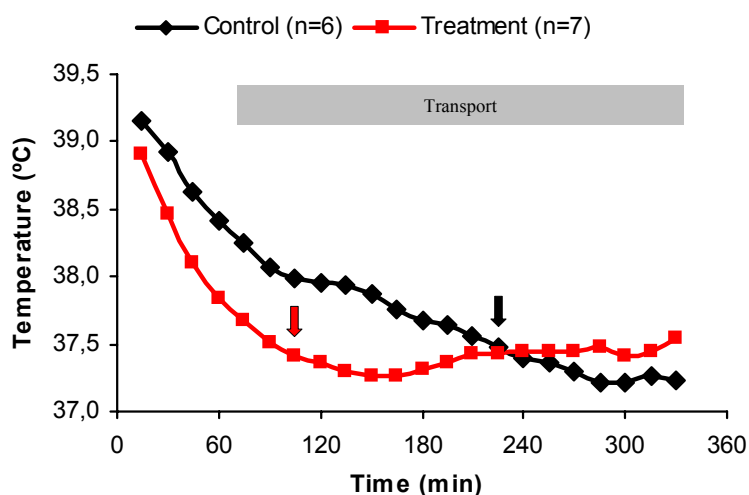


Figure 6.3. Mean body temperature over transport from control and treated roe deer. Body temperature decreased significantly in both groups ($P < 0.05$). Arrows indicate stabilisation of body temperature (i.e. no statistical differences were found between that measurement and those registered later).

Discussion

Heart rate is one of the most widely used acute stress indicators (Broom and Johnson, 1993) and is considered to be an objective way of assessing the autonomic nervous system's response to psychological stressors (Hopster and Blockhuis, 1994). In our study, the lack of differences in heart rate between treatment groups could be due to reflex tachycardia secondary to hypotension caused by acepromazine (Plumb, 1995) or to the high inter-individual variability normally found in this parameter (Hopster, 1998). Diverio *et al.* (1996b) found a greater increase in heart rate in red deer treated with a long-acting phenothiazine than in untreated deer during the 30 minutes immediately following stressor application, which was attributed to reflex tachycardia. Kock *et al.* (1987a) did not find significant differences in heart rate between normal bighorn sheep (*Ovis canadensis*) and those considered stressed.

The observed increases in heart rate during transportation are in agreement with results obtained by Horalek and Jones (1993) in red deer, who attributed such increases to the movement of the vehicle rather than noise or confinement. On the other hand, some researchers have reported decreases in red deer heart rates during transportation (Waas *et al.*, 1997; Grigor *et al.*, 1998), which were associated with their ability to adapt to the movement of the vehicle over time. This disparity could result from different road transport conditions (mainly road-type differences). Our results were obtained from transport conducted along a motorway, which can be considered the best road type in welfare terms, and, in spite of this, roe deer's heart rate increased during transport. In sheep, it has been described an increase in heart rate after loading, staying high during the first 9 hours of travelling (Parrot *et al.*, 1998).

Porges (1985) proposed that, instead of the basic heart rate, the physiologic variability in heart rate may be a better indicator of both the status of the individual's nervous system and its capacity to respond to environmental demands. The coefficient of variation of heart rate, used as a measure of heart-rate variability (Hopster and Blokhuis, 1994), was not significantly altered by acepromazine (control group: $36.70\% \pm 6.18\%$; treated group: $27.11\% \pm 8.17\%$ [mean \pm SEM]).

Increases in body temperature in certain stressful situations can be explained not only by physical activity, but also by stress-induced hyperthermia (SIH) (Moe and Bakken, 1997; Bakken *et al.*, 1999). SIH is a regulated shift of the thermoregulatory set-point (Kluger *et al.*, 1987; Briese and Cabanac, 1990) mediated by prostaglandin E and interleukins 1 and 6 (LeMay *et al.*, 1990; Kent *et al.*, 1993). Correlations have been found between SIH, the sympathoadrenal medullary system and the hypothalamic-pituitary-adrenal axis, which agrees with the proposal that SIH is a stress-mediated response (Groenink *et al.*, 1994). Zethof *et al.* (1994) stated that in mice SIH is time-dependent, taking 10 minutes to reach a (stable) high level, which is $1.0\text{-}1.5^{\circ}\text{C}$ higher than the baseline, and then taking 60 minutes to return to baseline. Moe (1996) found that SIH in farmed silver foxes (*Vulpes vulpes*) lasts 60-90 minutes after presentation of a short stressor. It has been suggested that SIH may be elicited in response of the anticipation of a known or unknown unpleasant event, indicating that SIH may reflect a state of anticipatory anxiety (Lecci *et al.*, 1990).

Animals which have substantial adrenal cortex responses during handling and transport show increased body temperature. The increase is usually of the order of 1°C, but the actual value at the end of a journey depend upon the extent to which any adaptation of the initial response has occurred (Broom, 2000). Although acepromazine can cause an alteration in thermoregulatory mechanisms (Plumb, 1995), this cannot explain the differences between groups, as body temperature followed the same progression in both groups and then stabilised at the same baseline level. Therefore, it can be inferred that acepromazine does not suppress stress-induced hyperthermia, but improves adaptation to the new situation by accelerating temperature reduction. Taking into account that hyperthermia plays a very important role in the pathophysiology of capture myopathy (Chalmers and Barrett, 1982), it also can be suggested that acepromazine reduces the tissular necrosis risk induced by a prolonged augmentation of body temperature.

The first stage of the stress response is the activation of the sympathetic nervous system, stimulating the adrenal medulla and releasing catecholamines. Increases in RBC count, haemoglobin concentration, and PCV are associated with splenic contraction caused by the action of catecholamines on α -adrenergic receptors located in the splenic capsule (Ganong, 1990), and are also partly attributable to a reduction in plasma volume (Wesson *et al.*, 1979; Cross *et al.*, 1988). In our study, changes in RBC count and haemoglobin concentration can be explained by acepromazine's α -adrenergic-blocking effect. This provokes relaxation of the spleen and the consequent splenic sequestration of erythrocytes (Turner and Hodgetts, 1960; Jain, 1993). Haemodilution caused by acepromazine due to lowering of blood pressure can be ruled out because, if this was the case, total protein and sodium concentrations would also have decreased (Table 6.2). Although it has been reported that acepromazine decreases PCV (Bush, 1993), no differences were found between groups in our study; this parameter did, however, show a decreasing trend similar to that shown by the RBC count and haemoglobin concentration (Table 6.1). The lack of significant effect could be attributable to the greater variability associated with PCV measurement using the microhaematocrit method, for which the coefficient of variation is 2% (Wintrobe, 1974).

Total and differential leukocyte counts respond to a variety of stimuli, including capture and transport. Catecholamines released during the alarm phase may be responsible for the initial neutrophilia and lymphocytosis. Corticosteroids released during the resistance phase contribute further to neutrophilia, but may cause a decrease in lymphocyte counts. In domestic animals, the neutrophilia and lymphopenia peaks appear after 4-8 hours exposure to stress (Duncan and Prasse, 1986; Jain, 1993). In red deer sedated with xylazine, Cross *et al.* (1988) attributed the decrease in lymphocyte counts to the splenic sequestration of lymphocytes, but in a later work (Cross *et al.*, 1989) they observed the same lymphopenia in splenectomised animals. The common mechanism of lymphopenia after corticosteroid exposure is redistribution of lymphocytes to bone marrow or body compartments. Long-term corticosteroid exposure may cause lympholysis (Schultze, 2000). A possible explanation for the differences observed in lymphocyte count in our study is a delay caused by acepromazine in the stress-induced lymphopenia. The mechanism of the decrease in eosinophil numbers is uncertain but is believed caused by intravascular lysis (steroids induce apoptosis of eosinophils), decreased release from bone marrow, sequestration in organs such as the spleen and liver, and increased tissue migration (Jain, 1993; Young, 2000). Therefore, the decrease in eosinophil count over transport observed in the treated animals is difficult to explain, although an alteration in eosinophil distribution could have happened because of acepromazine. The great variability obtained in the eosinophil count could also be responsible for these results.

Muscle enzyme activity (ALT, AST, CK, LDH) increases during capture and handling operations due to increased muscle cell permeability or muscle cell damage (Duncan and Prasse, 1986). These enzymes appear elevated in many stressed wild ungulates and in those suffering from capture myopathy (Kock *et al.*, 1987a; Vassart *et al.*, 1992). Some authors have found that CK and AST levels are the most sensitive indicators of muscular disorders (Chapple *et al.*, 1991). When muscle activity begins, blood flow increases but is intermittent. Blood flow decreases as muscle contracts because of the compression of vessels, and increases during relaxation -a process called the 'muscle pump' (Guyton and Hall, 1996). The muscle pump is active when the animal is running but it is inactive when it is immobilised by physical or chemical restraint or is standing in a crate. In most situations, the muscles of frightened animals

that are not running are in a relatively isometric state of contraction, which hinders blood flow into the muscles. This leads to poor tissue perfusion, decreased heat dissipation, and hypoxia (Spraker, 1993). Results obtained from this study indicate that acepromazine exerts a protective effect against muscle damage due to its vasodilative effect and demonstrate its importance as a preventive treatment of rhabdomyolysis (capture myopathy), as previously indicated for horses (Beech, 1994).

Table 6.1. Haematological values (mean \pm SEM) of control and treated roe deer before and after transportation.

Parameters	Before transport		After transport	
	Control (n=12)	Treated (n=9)	Control (n=12)	Treated (n=9)
Red blood cells ($\times 10^{12} \text{ L}^{-1}$)	12.42 \pm 0.35 ^a	11.37 \pm 0.40 ^{a,b}	12.40 \pm 0.35 ^a	10.91 \pm 0.40 ^b
Packed cell volume (L L^{-1})	0.44 \pm 0.013	0.45 \pm 0.015	0.46 \pm 0.013	0.43 \pm 0.015
Haemoglobin (g L^{-1})	166.91 \pm 4.12 ^a	163.56 \pm 4.72 ^{a,b}	169.98 \pm 4.12 ^a	156.56 \pm 4.72 ^b
MCV (fl)	35.94 \pm 0.91 ^a	40.07 \pm 1.05 ^b	37.15 \pm 0.91 ^{a,b}	39.42 \pm 1.05 ^b
MCHC (g dL^{-1})	37.62 \pm 0.84	36.15 \pm 0.96	36.98 \pm 0.84	36.64 \pm 0.96
MCH (pg)	13.45 \pm 0.21 ^a	14.43 \pm 0.25 ^b	13.52 \pm 0.21 ^a	14.38 \pm 0.25 ^b
White blood cells ($\times 10^9 \text{ L}^{-1}$)	4.817 \pm 0.403	4.900 \pm 0.465	4.533 \pm 0.403	4.955 \pm 0.465
Differential leukocyte count				
Lymphocytes ($\times 10^9 \text{ L}^{-1}$)	1.959 \pm 0.168 ^a	2.037 \pm 0.193 ^a	1.612 \pm 0.168 ^{a,b}	1.407 \pm 0.198 ^b
Monocytes ($\times 10^9 \text{ L}^{-1}$)	0.083 \pm 0.021	0.097 \pm 0.025	0.084 \pm 0.021	0.113 \pm 0.025
Neutrophils ($\times 10^9 \text{ L}^{-1}$)	2.659 \pm 0.371	2.609 \pm 0.429	2.757 \pm 0.371	3.431 \pm 0.429
Eosinophils ($\times 10^9 \text{ L}^{-1}$)	0.159 \pm 0.042 ^a	0.178 \pm 0.048 ^a	0.045 \pm 0.042 ^{a,b}	0.016 \pm 0.048 ^b
Basophils ($\times 10^9 \text{ L}^{-1}$)	0.004 \pm 0.003	0.00 \pm 0.004	0.00 \pm 0.003	0.005 \pm 0.004

^{a, b} Values with different superscripts are significantly different from each other ($P < 0.05$); values without superscripts are not significantly different from each other. SEM, standard error of the mean.

Any process which increases protein catabolism will tend to result in increased levels of serum urea (Knowles and Warris, 2000). The increase of urea concentrations in our study was probably due to physical exercise, to the effects of glucocorticoids on protein catabolism, and also to food deprivation (Finco, 1997).

Serum creatinine levels can be used to assess renal function. In some ungulates, however, increased creatinine concentrations, resulting from muscular activity and decreased renal excretion because of vasospasm in the kidney caused by catecholamines, have been described (Harthoorn, 1976). The decrease in serum creatinine concentrations during transportation in the treated group can be explained by the α -adrenergic-blocking effect of acepromazine over renal arterioles, where it promotes vasodilation and thus allows the continued filtration and excretion of creatinine (Jarvik, 1970). Moreover, this implies that oxygen supply to kidneys was not impaired, thus reducing the risk of renal hypoxia and consequent renal ischemic necrosis.

Both increases and decreases in lactate concentrations have been reported as a result of transport. Because lactate concentrations vary greatly during the course of the transport period, measured values will depend on the actual timing of the blood sampling (Waas *et al.*, 1997). Our results did not show statistical differences in serum lactate levels between the treated and control groups, nor over time. This lack of differences may be due to the time elapsed between the first and the second blood samples. Nonetheless, acepromazine has been suggested to decrease the production and/or increase the clearance of lactate because of its vasodilative action (Freestone *et al.*, 1991; Beech, 1994).

The decrease in serum glucose levels over transport in both groups may be due to food deprivation during transport (Knowles and Warris, 2000), which would correspond with the increased serum urea concentrations. The stress-induced hyperglucemia also could be responsible for the higher glucose values found before transport. The absence of group differences in serum glucose levels is in agreement with the results obtained by Brearley *et al.* (1990) comparing cattle treated with either saline or acepromazine submitted to 5 minutes of transport.

Serum sodium concentrations did not change during transport (Table 6.2). This corresponds with results obtained by Grigor *et al.* (1998) in red deer transported for six hours. Changes in sodium levels use to be associated with variations in plasma volume, and given that in this study total protein concentrations did not change either,

it can be stated that there were no signs of dehydration. On the other hand, changes in chloride concentrations which are not associated with a similar change in sodium levels are usually associated with acid-base imbalances. A disproportionate increase in chloride, as was seen in our study, is most commonly associated with normal to low hyperchloremic metabolic acidosis, and may be seen as a compensatory response to a primary respiratory alkalosis (Carlson, 1997).

Table 6.2. Blood biochemistry (mean \pm SEM) of control and treated roe deer before and after transportation.

Parameters	Before transport				After transport			
	Control	<i>n</i>	Treated	<i>n</i>	Control	<i>n</i>	Treated	<i>n</i>
ALT (IU L ⁻¹)	122.75 \pm 33.89 ^{a*}	12	89.07 \pm 39.45 ^a	8	173.42 \pm 33.89 ^{b*}	12	108.89 \pm 39.14 ^{a,b}	9
AST (IU L ⁻¹)	1288 \pm 1003 ^a	12	2563 \pm 1177 ^a	8	2104 \pm 1003 ^b	12	2317 \pm 1165 ^{a,b}	9
CK (IU L ⁻¹)	15 084 \pm 4981 ^a	12	5738 \pm 5938 ^a	8	22 108 \pm 4981 ^b	12	8403 \pm 5784 ^{a,b}	9
LDH (IU L ⁻¹)	1675 \pm 477	12	2012 \pm 593	8	2872 \pm 477	12	1872 \pm 553	9
ALP (IU L ⁻¹)	68.91 \pm 15.84	11	92.40 \pm 18.61	8	83.32 \pm 15.49	12	92.59 \pm 18.22	9
Urea (mmol L ⁻¹)	10.25 \pm 1.60 ^a	12	10.98 \pm 1.99 ^{a,c}	8	15.11 \pm 1.60 ^{b,c}	12	16.81 \pm 1.91 ^b	9
Creatinine (μ mol L ⁻¹)	121.11 \pm 5.75 ^{a,b}	11	126.5 \pm 6.74 ^a	8	124.64 \pm 5.75 ^{a,b}	12	118.37 \pm 6.74 ^b	8
Lactate (mmol L ⁻¹)	2.97 \pm 0.71	11	3.67 \pm 0.83	8	5.39 \pm 0.69	12	4.15 \pm 0.83	8
Total bilirubin (μ mol L ⁻¹)	4.87 \pm 1.44	12	3.57 \pm 1.44	8	6.58 \pm 1.44	12	6.53 \pm 1.44	8
Glucose (mmol L ⁻¹)	10.03 \pm 0.95 ^a	12	11.75 \pm 0.95 ^a	8	7.23 \pm 0.95 ^b	12	6.81 \pm 0.95 ^b	8
Total proteins (g L ⁻¹)	72.80 \pm 2.74	11	71.81 \pm 2.74	8	75.06 \pm 2.84	10	74.21 \pm 2.84	9
Cholesterol (mmol L ⁻¹)	1.20 \pm 0.08	12	1.16 \pm 0.08	8	1.28 \pm 0.08	12	1.10 \pm 0.08	8
Triglycerides (mmol L ⁻¹)	0.37 \pm 0.06 ^a	11	0.59 \pm 0.06 ^b	8	0.28 \pm 0.06 ^a	11	0.30 \pm 0.06 ^a	8
Sodium (mmol L ⁻¹)	148 \pm 2.73	10	146 \pm 2.73	8	145.8 \pm 3.61	6	146.5 \pm 3.61	9
Chloride (mmol L ⁻¹)	104.37 \pm 1.12 ^{a,c}	11	102.55 \pm 1.12 ^a	8	107.74 \pm 1.16 ^b	10	106.46 \pm 1.16 ^{b,c}	8
Potassium (mmol L ⁻¹)	6.27 \pm 0.25 ^a	11	5.55 \pm 0.25 ^{a,b}	8	5.41 \pm 0.27 ^b	10	5.26 \pm 0.27 ^b	9
Cortisol log (ng mL ⁻¹)	1.71 \pm 0.12	11	1.89 \pm 0.12	8	1.62 \pm 0.12	10	1.77 \pm 0.12	9

^{a, b, c} Values with different superscripts are significantly different from each other ($P < 0.05$; $*P < 0.001$); values without superscripts are not significantly different from each other. SEM, standard error of the mean. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase.

During exercise, potassium is released from working skeletal muscles (Van Beaumont *et al.*, 1973). Following the completion of exercise, potassium quickly declines controlled by extrarenal factors including insulin, catecholamines, glucocorticoids and acid-base balance (Bia and DeFronzo, 1981). In our study, serum potassium levels decreased over transportation in the control group whereas no change was observed in the treated group. It has been suggested that by influencing electrolyte movements, acepromazine may alter neuromuscular excitability and impair the development of exertional rhabdomyolysis (Freestone *et al.*, 1991).

Glucocorticoid levels rise in response to many short-term challenges in life and their measurement gives valuable information about the welfare of animals (Broom and Johnson, 1993). Glucocorticoid hormones, produced in and released from the cortex of adrenal glands in response to an extremely wide range of stressors, play a major role in mediating the physiological response of stress and are widely interpreted as a measure of an animal's psychological perception of a situation. Many authors have reported significant increases in cortisol associated with transport (red deer [Smith and Dobson, 1990; Brelurut, 1991; Waas *et al.*, 1997; Grigor *et al.*, 1998]; cows [Kenny and Tarrant, 1987]). However, our results did not reveal any difference between the samples collected before and after transport. It has been reported that plasma glucocorticoid levels give information about treatments lasting up to two hours, but are less useful for journeys lasting longer than this (Broom, 2000), as happened in our study. On the other hand, the lack of differences between treatment groups in serum cortisol levels could be due to the fact that sedative effects are unrelated to plasma cortisol concentrations. Brearley *et al.* (1990) found that at a similar depth of sedation, xylazine suppressed the cortisol response to stress whereas acepromazine had a slight potentiating effect. It has been suggested that chlorpromazine causes systemic release of epinephrine, which may result in an increase in adrenocorticotrophic hormone (ACTH) release and hence cortisol release (Bruss, 1980). Other reasons for the lack of differences between groups could be the great inter-individual differences in stress-induced plasma cortisol concentrations (Moberg, 1985) and the low number of individuals per group available in this study.

Animal Welfare Implications

Although this is a small study, the results show that during transport of roe deer, acepromazine is suitable for reducing the stress response and preventing its adverse effects. These benefits arise not only because of the sedative effect of acepromazine, but also because it causes peripheral vasodilation. This vasodilation has a protective effect against the muscular and renal damage which can arise from stress episodes in wild animals and which is directly involved in the pathogenesis of capture myopathy. Thus, animal welfare is improved by preventing and reducing adverse effects of stress and by accelerating adaptation to new situations.

Capítulo 7

Non-invasive evaluation of the effect of captivity and transport in roe deer (*Capreolus capreolus*)

Abstract

Faecal cortisol metabolites (11,17-dioxoandrostanes: 11,17-DOA) were measured in 13 free-ranging and 14 captive roe deer (Capreolus capreolus) in order to evaluate the effect of captivity on this parameter and its usefulness as an indicator of chronic stress in this species. Furthermore, faecal 11,17-DOA were analysed before and after a nine-hour transport in 20 roe deer to evaluate the stress response to transport and the effect of acepromazine on this response. The captive roe deer had higher faecal 11,17-DOA concentrations than the free-ranging ones ($P = 0.005$). No statistical differences were found between the roe deer treated with acepromazine and the control group, nor between the samples collected before and after transport. It is suggested that the higher 11,17-DOA concentrations found in captive roe deer reflect a higher chronic stress status that could explain exaggerated acute stress responses to novel experiences in these animals.

Key words: *Animal welfare, cortisol, faeces, metabolites, roe deer, stress.*

Introduction

Traditionally, glucocorticoids have been measured in blood plasma. However, blood sampling of wild animals requires restraint and/or anaesthesia, and this approach can itself elicit an ‘adrenal’ stress response (Monfort *et al.*, 1998). Many authors have used plasma cortisol as an indicator of stress associated with capture and handling in wildlife species (Franzmann *et al.*, 1975; Hastings *et al.*, 1992; Morton *et al.*, 1995), but studying the role of stress in welfare of animals is made difficult by the need to sample an animal’s response to environmental stressors without imposing a confounding stress by the act of sampling itself. Furthermore, as plasma glucocorticoids can vary seasonally, circadianly and also ultracircadianly (Turner, 1984; Nilssen *et al.*, 1985; Ingram *et al.*, 1999), a blood sample represents plasma glucocorticoid concentrations within a narrow time frame. Therefore non-invasive methods, such as measuring glucocorticoid metabolites in faeces, are desirable because animals need not be captured and restrained and, although Sousa and Ziegler (1998) found diurnal fluctuations in faecal glucocorticoid levels in common marmosets (*Callithrix jacchus*), it is likely that fluctuations due to secretory patterns are attenuated in faeces (Goymann *et al.*, 1999) because they are dampened by the ‘pooling’ of corticosteroids in faeces (Monfort *et al.*, 1998).

The measurement of faecal 11,17-dioxoandrostanes (11,17-DOA), a group of cortisol metabolites, has proved to be an appropriate technique for monitoring adrenocortical activity in roe deer (*Capreolus capreolus*) (Dehnhard *et al.*, 2001). These metabolites have also been measured for evaluating adrenocortical activity in a variety of species (primates: Wallner *et al.*, 1999; Bahr *et al.*, 2000; domestic ruminants: Palme and Möstl, 1997; Palme *et al.*, 2000; horses and pigs: Möstl *et al.*, 1999; hares [*Lepus europaeus*]: Teskey-Gerstl *et al.*, 2000; cats and dogs: Schatz and Palme, 2001; spotted hyenas [*Crocuta crocuta*]: Goymann *et al.*, 1999; various: Wasser *et al.*, 2000).

Roe deer are increasingly submitted to reintroduction and restocking operations (García-Ferré *et al.*, 1995; Wemmer, 1998) that involve their capture, handling and

transportation and thus, a subsequent stress response. This makes it necessary to investigate the effect of stress on animal welfare and to reduce it by means of better management, pharmacological treatments and/or improvement of captive settings.

The objectives of this study were to evaluate the differences in the chronic stress status between captive and free-ranging roe deer, evaluate the stress response to transport and test the effect of acepromazine on this response by measuring faecal cortisol metabolite concentrations.

Materials and Methods

Animals and collection of samples

Faecal samples were obtained from the rectum of 13 free-ranging roe deer (2 adult males, 1 young [less than one year old] male, 9 adult females, 1 young female) captured by means of drive-nets in the winters of 1998, 1999 and 2001 in the National Game Reserve of Alt-Pallars Aran (47°22' N 3°48' E, north-eastern Spain), the Controlled Hunting Area of Val d'Aran (47°35' N 3°15' E, north-eastern Spain) and a Private Hunting Area (44°40' N 8°30' E, north-western Italy) and 14 captive roe deer (3 adult males, 3 young males, 2 adult females and 6 young females) also captured by means of drive-nets in the enclosures where they were maintained (four different enclosures with similar features) in December 2000.

Faecal samples were also collected from 20 roe deer captured by means of drive-nets in the National Game Reserve of Chizé (mid-western France) before and after being submitted to a nine-hour transport in January 1999 (first transport) and March 2001 (second transport). These animals were divided into two groups: nine randomly selected animals, four males (one adult and three fawns [less than one year old]) and five females (three adults and two fawns) received 2.5 mg ($0.13 \text{ mg/kg} \pm 0.013 \text{ SEM}$) of acepromazine (Calmo Neosan[®] 5 mg/mL, Smithkline Beecham, Madrid, Spain) in a volume of 0.5 mL intramuscularly, whereas 11 animals, six males (five adults and one fawn) and five females (one adult and four fawns), received 0.5 mL of saline intramuscularly (Fisiológico Braun, Braun Medical S.A., Rubí, Barcelona). The mean

sample collection interval was 13.9 hours (range: 9.3 - 22 hours). In all cases faecal samples were frozen and stored at -20°C until analysis.

Measurement of faecal cortisol metabolites

Frozen faeces were first lyophilised using a dry freezer (Cryodos, Telstar S.A., Terrassa, Spain) for 96 h, pulverised and maintained in hermetically capped plastic tubes until analysis. Subsamples of 0.1 g of lyophilised faeces were weighed and placed into glass test tubes. The subsamples were rehydrated with 0.4 mL of bidistilled water. Then, the subsamples were extracted with bidistilled water-methanol 80% (1 mL + 4 mL respectively) and after shaking for 30 minutes, the mixture was centrifuged (2500 g; 15 min.), diluted (1:10 with assay buffer) and portions of 50 μL (in duplicate) were subjected to the 11-oxoaetiocholanolone-EIA (measuring 11,17-DOA). This EIA (enzyme-immunoassay) was described by Palme and Möstl (1997) and validated for use in roe deer by Dehnhard *et al.* (2001).

Statistics

The 11,17-DOA concentrations were subjected to a logarithmic transformation in order to normalise the data and reduce the heterogeneity of variance. The comparison between captive and free-ranging animals was performed by means of an ANOVA using the PROC GLM procedure of the SAS[®] statistics software package (SAS Institute Inc., Cary, NC, USA), considering sex, age and all interactions as additional factors. The effects of transport and acepromazine were analysed by repeated measures ANOVAs using the PROC MIXED procedure of the same statistics package, with the main factor being treatment (acepromazine or saline) and the repeated factor being time. Also sex, age, transport group (first or second transport) and interactions among factors were included in the statistical model. Type 1 autoregression (AR[1]) structure for the covariance matrix of the repeated measures was used. In all cases, accepted significance level was at least $P < 0.05$.

Results

No statistical differences were found between the treated and the control group after a nine-hour road transport, nor between the samples collected before and after

transportation. Captive roe deer had higher faecal 11,17-DOA concentrations ($P = 0.005$) than the free-ranging ones (Figure 7.1). Furthermore, 11 of the 20 transported roe deer had statistically higher values of faecal 11,17-DOA before and after transportation than the rest of the transported deer (Figure 7.2). These 11 animals had been captured approximately 48 hours prior to the onset of road transport and maintained in captivity during that time.

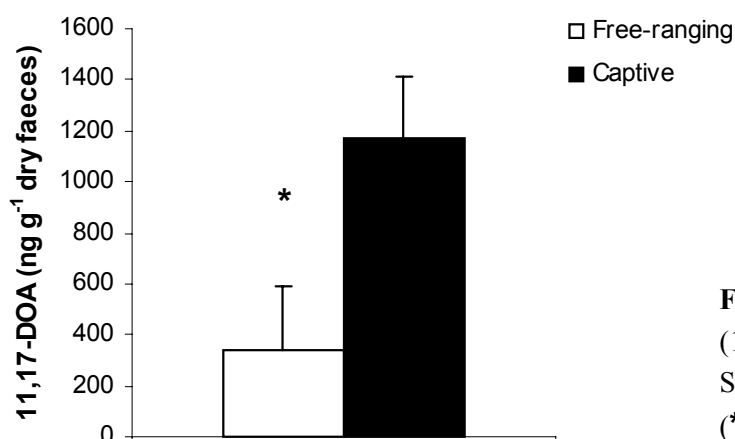


Figure 7.1. Faecal 11,17-dioxoandrostan (11,17-DOA) concentrations (mean \pm SEM) of captive and free-ranging roe deer (* $P = 0.005$).

Discussion

The regular translocation of wildlife has become a common management practice. Transportation of animals may be essential for wildlife managers, zoo keepers and farmers. Because transport is usually unavoidable, it is necessary to find the best transport conditions to minimise welfare problems, and consequently it is necessary to measure the welfare status of the animals. Measurement of faecal cortisol metabolite concentrations has already been used for assessing transport stress in domestic (Palme *et al.*, 2000) and captive wild animals (Goymann *et al.*, 1999; Dehnhard *et al.*, 2001) and seems well suited to further elucidate the reactions that occur in such stressful events.

In our study, the lack of differences between treatment groups submitted to the transport operation can be explained by the high variability in the delay between adrenocorticotrophic hormone (ACTH) secretion and peak concentrations of faecal

cortisol metabolites found in roe deer (range: 6.1-22.6 hours; mean: 11.8 hours), which roughly corresponds to the food transit time from the duodenum to the rectum (Dehnhard *et al.*, 2001). Therefore, the 11,17-DOA levels measured in the samples 'after transport' still represented pre-transport concentrations. It is also possible that the cortisol metabolites secreted during the capture event and the 48 hours captivity period in 11 of the 20 roe deer masked the effect of both transport and acepromazine on the 11,17-DOA concentrations.

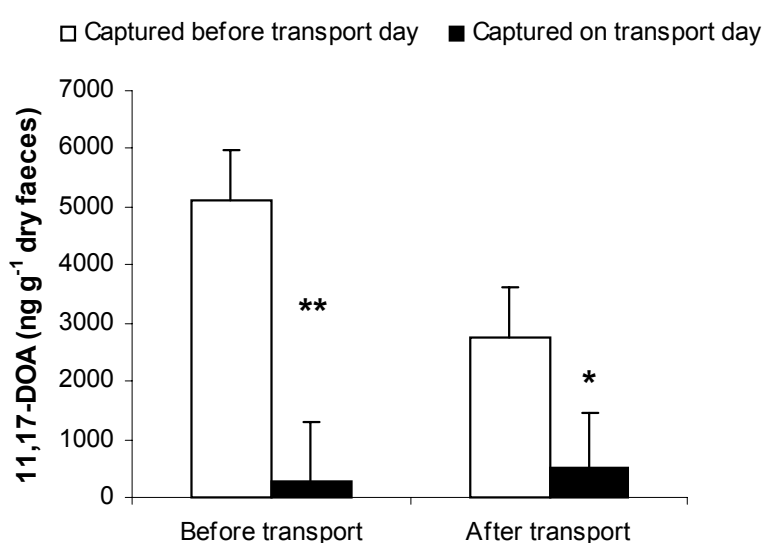


Figure 7.2. Faecal 11,17-DOA concentrations (mean \pm SEM) before and after transportation of roe deer captured 48 hours prior to the transport day and captured the same day (* $P = 0.0059$, ** $P = 0.0005$).

Many important differences in the biological characteristics of wild and captive animals reflect their respective adaptations to often very different environments, e.g. fearfulness toward humans. In nature, wild animals usually exhibit marked avoidance of humans, while in captivity contact with people is often frequent, and tameness or a lack of avoidance behaviour represents an adaptation with important consequences in terms of animal suffering or stress. Captive animals also have to adapt to uniformity of diet, reduced social distances, inability to escape from dominant conspecifics and to limitations in the quantity and quality of space available for perceptual and locomotor stimulation to improve their well-being (Price, 1985).

It has been reported that repeated exposure to different unpleasant stimuli may sensitise the hypothalamic-pituitary-adrenal cortex (HPA) and the sympathetic-

adrenomedullary (SA) axes so that a test with a novel disturbing stimulus elicits a greater response than normally (Broom and Johnson, 1993). The higher 11,17-DOA concentrations found in captive roe deer reflect a higher chronic stress status that could explain exaggerated acute stress responses to novel experiences in these animals, which should be considered in terms of management and welfare. Franceschini *et al.* (1997) found higher faecal cortisol levels in captive woolly monkeys (*Lagothrix lagotricha*) than in wild-living ones. Our results indicate that measurement of cortisol metabolites can be a useful chronic stress indicator whenever we know that no stressful episodes occurred approximately 12 hours before sampling. Thus, this non-invasive method has many potential implications in terms of animal welfare, captive breeding programs and ecosystem health (Franceschini *et al.*, 1997).

Determination of cortisol metabolites in faeces is a simple and non-invasive method for continually assessing the adrenocortical activity of captive animals and thus for evaluating enrichment programs, improved captive settings and the risk of stress-related diseases. It also can be used in wild populations as an indicator of habitat health, so that efforts could be made to improve or restore it.