# Material y métodos general

Los resultados de este trabajo se presentan en seis capítulos escritos en inglés en formato de artículos científicos (*Capítulos 4-9*). En la Figura 3.1 se muestra un organigrama general del diseño experimental y de los capítulos que incluyen los resultados obtenidos. En cada uno de dichos capítulos se incluye un apartado 'Material y métodos' específico.

En el presente apartado se presentan detalles de los materiales y métodos comunes a todos los capítulos que, por motivos de brevedad y claridad de exposición, no aparecen en los artículos científicos en los que se basan.

## 3.1. Captura

La captura física con redes verticales fue el método utilizado para obtener todos los corzos incluidos en este estudio. La red (Ziboni Ornitecnica, Bérgamo, Italia) estaba dividida en tramos de 50 m de longitud y 2 m de altura, con una malla de 10 x 10 cm. Los tramos de red se unían hasta conseguir una longitud total variable (entre 200 y 1750 m). Las capturas de los corzos salvajes se llevaron a cabo en bosques subalpinos rodeados de prados (1500-1800 m de altitud), en colinas con bosques caducifolios (Italia) y en llanuras boscosas (Francia). Las batidas fueron realizadas por guardas de las Reservas de Caza del Alt Pallars-Aran y del Alt Urgell-Cerdanya, por guardas del Parque Natural del Cadí-Moixeró, por miembros de las sociedades de cazadores (Zona de Caza Controlada del Valle de Aran [Cataluña] y Reserva Nacional de Caza y de Fauna de Chizé [Francia]) y por personal voluntario de distinta índole. La duración de las batidas fue variable, con una media (± desviación típica) de 47 ± 15 minutos/batida (intervalo entre 25 y 75 minutos/batida). Por otro lado, los corzos cautivos se capturaron extendiendo un tramo de 50 m de red en el interior del cercado donde se encontraban. El tiempo necesario para capturar cada uno de los animales varió entre 10 y 25 minutos, desde el momento en que se accedió al cercado.

## 3.2. Registro de la frecuencia cardiaca

La frecuencia cardiaca se registró mediante monitores cardiacos diseñados para deportistas humanos (Polar Vantage NV®, Polar Electro Oy, Kempele, Finlandia). Éstos constan de un transmisor, un receptor y una cinta elástica. El transmisor está

formado por dos electrodos, que se colocan sobre las áreas torácica derecha y precordial izquierda, previamente depiladas, y se sujeta a la caja torácica del animal mediante la cinta elástica. Para mejorar la transmisión y el contacto entre los electrodos y la piel, se aplicó gel electrocardiográfico (Lessa, Barberà del Vallès, España). El receptor debe permanecer a menos de un metro del transmisor para poder recibir la señal, por lo que se colocó alrededor del cuello del animal unido a un collar de plástico. Los monitores cardiacos se colocaban una vez finalizada la batida y después de trasladar al animal hasta el lugar donde se encontraba el material.

Estos monitores cardiacos calculan la frecuencia cardiaca mediante un algoritmo que hace la media del tiempo transcurrido entre latido y latido (Seaward *et al.*, 1990). Los datos almacenados en el receptor se transfirieron al ordenador mediante un programa informático (Polar HR Analysis Software, Polar Electro Oy, Kempele, Finlandia).

## 3.3. Registro de la temperatura rectal

El registro de la temperatura rectal se realizó mediante sondas Mätman datalogger® (Chipsobits Eltex AB, Suecia). Las sondas de temperatura se colocaron siempre una vez instalados los monitores cardiacos. Los datos obtenidos se transfirieron al ordenador mediante el Mätman XL® (Chipsobits Eltex AB, Suecia) para Microsoft Windows®.

## 3.4. Hemograma

El analizador hematológico semiautomático utilizado en este estudio (Sysmex F-800®, Toa Medical Electronics Co. Ltd., Japón) está adaptado para trabajar con sangre de animales y funciona mediante el sistema de impedancia eléctrica. A partir de 0.02 mL de sangre, el hemodiluidor semiautomático Sysmex AD-260® (Toa Medical Electronics Co. Ltd., Japón) realiza una dilución 1:500 para el recuento de leucocitos y la determinación de la concentración de hemoglobina. A partir de esta primera dilución se hace una segunda a razón de 1:100 para conseguir una dilución final 1:50.000 para el recuento de eritrocitos y de plaquetas. A continuación, se añade un hemolizante (Quicklyser II, Sysmex Corporation, Japón) en el recipiente para el recuento de leucocitos y se homogeneiza, para luego colocarlo junto con el recipiente

para el recuento de eritrocitos en los transductores del analizador y proceder a los recuentos.
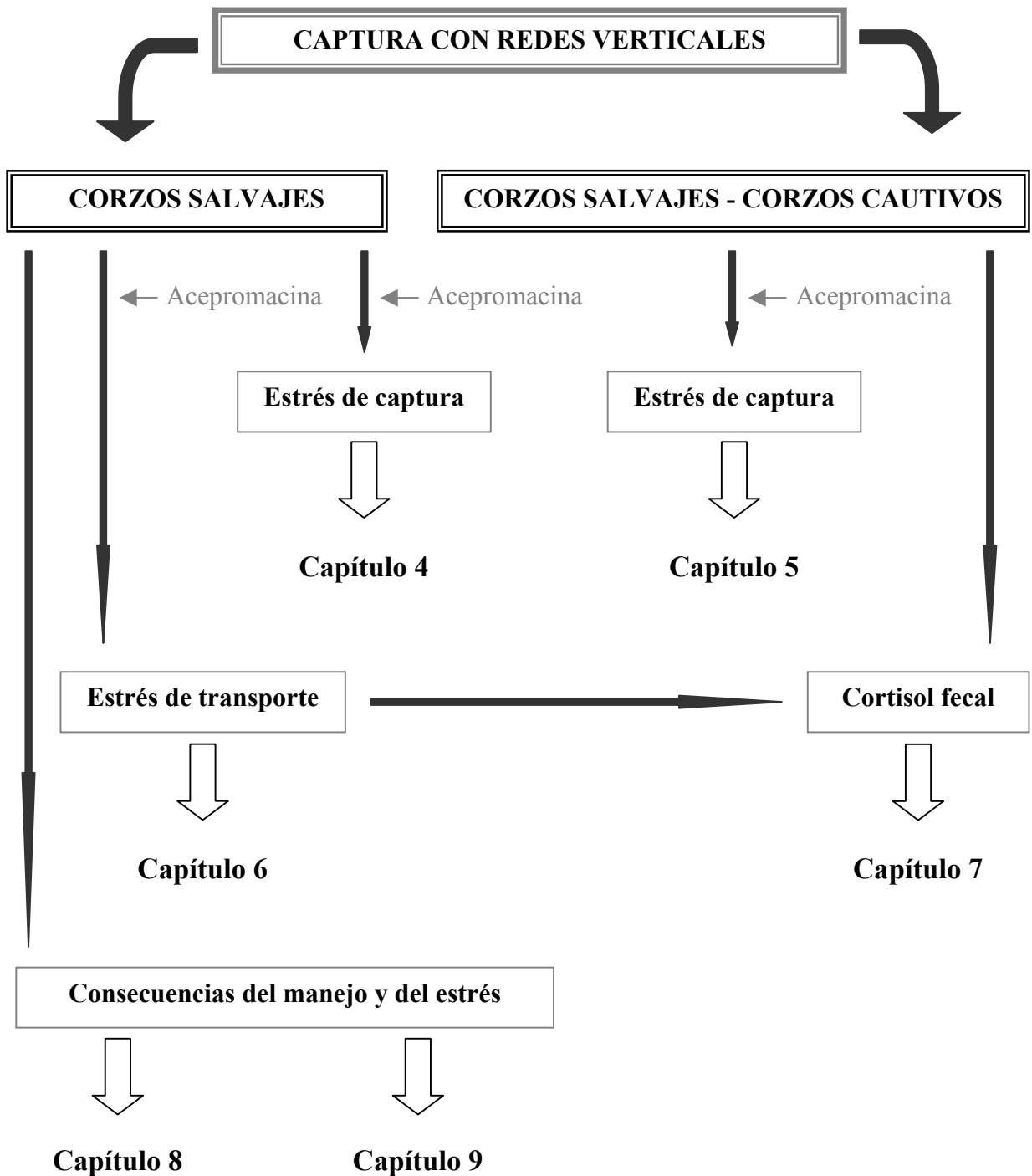


**Figura 3.1.** Organigrama general del diseño experimental y de los capítulos que incluyen los resultados obtenidos.

El valor hematocrito se determinó mediante el método manual, utilizando una centrífuga de microhematocrito (Hawksley, Lancing, Reino Unido) El volumen corpuscular medio (VCM) y la concentración corpuscular media de hemoglobina (CCMH) se calcularon a partir del valor hematocrito, la concentración de hemoglobina y el recuento de eritrocitos. El recuento diferencial de leucocitos se realizó con un microscopio óptico a 1000 aumentos, a partir de extensiones sanguíneas teñidas con una tinción panóptica rápida (Química Aplicada S.A., Amposta, España), sobre un total de 100 leucocitos.

**Tabla 3.1.** Referencias de los juegos de reactivos ('kits') comerciales utilizados para las determinaciones bioquímicas realizadas con el autoanalizador COBAS MIRA®.

| *Parámetro* | *Referencia del 'kit' comercial* |
|---|---|
| Aspartato aminotransferasa (AST) | 1 087 541 [1] |
| Alanina aminotransferasa (ALT) | 1 087 568 [1] |
| Creatina cinasa (CK) | 1 087 533 [1] |
| Lactato deshidrogenasa (LDH) | 1 087 592 [1] |
| Fosfatasa alcalina (ALP) | 1 087 517 [1] |
| Urea | 1 360 604 [1] |
| Creatinina | 124 192 [1] |
| Bilirrubina total | 123 919 [1] |
| Lactato | 124 842 [1] |
| Triglicéridos | 701 882 [1] |
| Colesterol | 1 442 341 [1] |
| Glucosa | 1 442 449 [1] |
| Cloruros | GN 1300 [2] |

[1] Boerhinger Manheim, S.A. (Manheim, Alemania).
[2] Gernon (Lindau, Alemania).

## 3.5. Parámetros bioquímicos

La determinación de los parámetros bioquímicos (enzimas, metabolitos, lípidos, proteínas totales, glucosa y cloruros) se realizó mediante un autoanalizador COBAS MIRA® (Roche, Nutley, NJ, EE.UU.) en el Servicio de Bioquímica Clínica de la Facultad de Veterinaria de la Universidad Autónoma de Barcelona. Este aparato es un espectrofotómetro de flujo discontinuo que trata las muestras de forma individual y que utiliza un sistema de centrifugación para mezclar la muestra con el reactivo. Las

determinaciones se realizaron con juegos de reactivos comerciales, cuyas referencias aparecen en la Tabla 3.1. La actividad sérica de las enzimas se determinó a 37ºC siguiendo los métodos recomendados por la IFCC (International Federation of Clinical Chemistry and Laboratory Medicine).

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

## Abstract

*The aim of this study was to evaluate the effect of a short-acting neuroleptic (acepromazine) on the stress response to capture and restraint in roe deer (*Capreolus capreolus*). A total of 16 roe deer were captured by means of drive-nets. The animals were divided into two groups: animals in the treatment group received an intramuscular injection of acepromazine (0.093 ± 0.003 mg/kg in 0.5 mL; n = 8) whereas the animals in the control group (n = 8) received the same volume of saline. Heart rate and body temperature as well as haematological and biochemical indicators of stress were used to evaluate the effect of the neuroleptic over a three-hour period. Heart rate decreased over time after capture in both groups, but stabilised sooner in the acepromazine-treated roe deer (75 minutes after capture) than in the controls (105 minutes after capture). Body temperature decreased over 45 minutes and then stabilised in both groups. Comparison of blood parameters revealed significantly lower red blood cell (RBC) counts, haemoglobin concentrations, packed cell volume (PCV), and serum CK, AST, ALT and LDH activities in tranquillised animals compared with controls, whereas lymphocyte numbers were significantly lower in the control group. A reduction in PCV and lymphocyte numbers, and an increase in serum creatinine levels were recorded over time in control animals. A reduction in RBC count and haemoglobin concentration, and an increase in serum urea concentrations over time were observed in the treated group. Finally, a decrease in serum lactate and potassium levels, and an increase in serum CK, AST and LDH activities were recorded over time in both groups. The results obtained show the suitability of using acepromazine in capture operations in order to reduce the stress response and prevent capture myopathy in roe deer.*

**Keywords:** *Acepromazine,* Capreolus capreolus*, capture, neuroleptic, roe deer, stress.*

# Introduction

Capture and handling is one of the most stressful events that can happen to wild ungulates and is sometimes associated with considerable mortality. During the capture and restraint of free-ranging wildlife, a variety of negative stimuli can lead to prolonged exertion, exhaustion, stress and hyperthermia. Collectively these factors can cause physiological disorders including lactic acidosis and initiate the development of myositis, muscle necrosis, depressed immune function and possibly death (Spraker, 1982). As a result of the increased concern for the conservation of wildlife species and the increase in game translocation operations, numerous studies have been conducted to measure the stress responses of a variety of species, particularly in relation to their capture and confinement (Franzmann *et al.*, 1975; Seal and Hoskinson, 1978; Kock *et al.*, 1987a,b; Hattingh *et al.*, 1988).

Haematological and biochemical measurements have revealed significant differences in certain variables in relation to the methods used for capturing and handling the animals (Kock *et al.*, 1987b) and it has been suggested that these changes may vary with the species, the type of stress and the individual's previous experience (Price, 1985). The ability to measure stress accurately is particularly important for determining the least stressful method for capturing and handling wildlife species in order to reduce mortality and improve the well-being of the animals (Morton *et al.*, 1995).

An important consideration is that physical restraint in excitable wild ungulates can result in capture myopathy. Capture myopathy, also called exertional rhabdomyolysis, is one of the main adverse consequences of stress in wild animals. Capture myopathy is a syndrome that occurs in wild (free-ranging and captive) mammals and birds. It is associated with the stress of capture, restraint and transportation. In ungulates the syndrome is characterised clinically by depression, muscular stiffness, lack of coordination, paralysis, metabolic acidosis and death. Pathologically, capture myopathy resembles the myodegenerative disorders of domestic cattle, sheep, horse and swine (Chalmers and Barrett, 1982), and it is mainly characterised by muscular and renal lesions (Spraker, 1993).

Roe deer (*Capreolus capreolus*) are valuable wild ungulates because of their ecological function as undergrowth cleaners, and because of the economic importance of the game activity that they generate. The species is undergoing expansion, and is increasingly involved in reintroduction and restocking operations (García-Ferré *et al.*, 1995; Wemmer, 1998) that require its capture and handling. There are few studies on capture stress in roe deer (Meneguz *et al.*, 1996) and none dealing with the use of neuroleptics to reduce stress and its adverse effects in this species. However, there are several studies in farmed red deer (*Cervus elaphus*) dealing with the effect of long-acting neuroleptics over management practices (Diverio *et al.*, 1993; 1996a, b). Otherwise, it is widely believed that short-acting tranquillisers are ineffective when animals are highly stressed at the point of administration, particularly in free-ranging animals, although this needs to be further explored (Diverio *et al.*, 1996b).

Acepromazine is a phenothiazine short-acting neuroleptic agent. While 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 assist in the control of body temperature, basal metabolic rate, emesis, vasomotor tone, hormonal balance, and alertness. Additionally, phenothiazines have varying degrees of anticholinergic, antihistaminic, antispasmodic, and α-adrenergic-blocking effects. The onset of action of acepromazine is fairly slow, requiring up to 15 minutes following intravenous administration (Plumb, 1995). The recommended dose for deer species is 0.05-0.1 mg/kg (Arnemo *et al.*, 1993).

In this study we evaluate the acute stress response caused by physical capture and restraint in roe deer, and the effect of acepromazine on this response by using clinical, haematological and biochemical parameters.

**Material and methods**

Sixteen roe deer, four males (three adults and one fawn [less than one year old]) and 12 females (11 adults and one fawn) captured by means of drive-nets in the National Game Reserve of Alt Pallars-Aran (47º22' N 3º48' E, north-eastern Spain), the

Controlled Hunting Area of Val d'Aran (47º35' N 3º15' E, north-eastern Spain) and a Private Hunting Area (44º40' N 8º30' E, north-western Italy) were used in this study. Eight randomly selected animals, two adult males and six adult females received 2.5 mg (0.093 mg/kg ± 0.003 SEM) of acepromazine (Calmo Neosan® 5 mg/mL, Smithkline Beecham, Madrid) in a volume of 0.5 mL intramuscularly, whereas eight animals, two males (one adult and one fawn) and six females (five adults and one fawn) acted as controls receiving the same volume of saline. The mean liveweight of animals was 24.31 ± 0.77 kg (range 20-26.5).

A total of twenty capture operations carried out in the winters of 1998, 1999 and 2001 were necessary to obtain the sixteen roe deer. Drive-trapping was conducted by a line of beaters, each one within sight of the next, and went on for approximately 45 minutes. Once in the net, the animals were initially restrained by using the net to wrap them in, blindfolded, their legs restrained, and finally introduced in a transport net sack (Ziboni Ornitecnica, Bergamo, Italy), where they were maintained for three hours. Acepromazine or saline were administered after blindfolding the roe deer. At the end of the capture operation, the right thoracic and the left precordial areas of animals were clipped in order to install the heart-rate recording equipment, and also the body-temperature recording device was introduced in the rectum. The roe deer were released in the same place three hours after capture.

The roe deer were fitted with a telemetric heart-rate recording equipment (Polar Vantage NV®, Polar Electro Oy, Kempele, Finland). Adequate records for analysis were obtained from 14 roe deer (seven per group). Heart rate was measured at 60-second intervals for two hours. The arithmetic mean of heart rate values was calculated for every five-minute period for statistical analyses. The roe deer were also fitted with a telemetric body-temperature recording device (Mätman datalogger®, Chipsobits Eltex AB, Sweden), but adequate records were only obtained from 11 animals (five in the treated group and six in the control group) for one hour. Rectal temperature was measured at 60-second intervals. The arithmetic mean of rectal temperature values was calculated for every 15-minute period for statistical analyses. Ambient temperature during capture operations was 0-12º C.

Four blood samples were taken, one at capture (time 0) and one each hour thereafter for three hours (time 1, 2 and 3 respectively). Blood samples (10 mL) were collected from the jugular vein using disposable syringes and 0.8 x 25 mm needles. A 2.5 mL subsample was placed in a commercial tube with anticoagulant (EDTA $K_3$) 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 -18ºC until analyses were completed. Haematological examinations (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 by means of a semiautomatic 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 with blood smears stained with commercial Diff-Quick® type stain (Química Aplicada S.A., Amposta, Spain).

Biochemical analyses were performed by means of an automated analyser (COBAS MIRA®, Roche, Nutley, NJ, USA) except for sodium and potassium concentrations, which were measured by 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).

Statistical analyses were performed by means of repeated measures ANOVAs 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. Also sex, age and the interactions among factors were included in the statistic model. Type 1 autoregression (AR[1]) structure for the covariance matrix of the repeated measures was used. When statistical differences between treatment groups at time 0 were obtained, values were expressed as a time 0

ratio in order to evaluate the effect of acepromazine regardless of initial values. Least square means (LS MEANS) were used because the distribution of animals among groups was unbalanced**.** In all cases, the accepted significance level was *P* < 0.05.
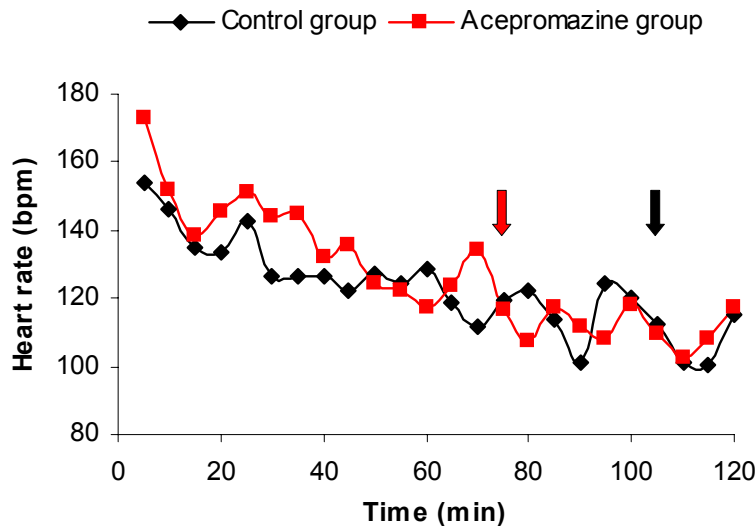


**Figure 4.1.** Mean heart rate of control (*n = 7*) and treated (*n = 7*) roe deer over a two-hour period after capture. Heart rate decreased significantly over time (*P* < 0.05). Arrows indicate when heart rate stabilised in each group (i.e., no statistical differences were found with the following values).

## Results

Once captured and after fitting the heart rate recording devices, the roe deer heart rate (Figure 4.1) decreased over time in both groups (*P* < 0.05). However, it stabilised earlier in the treated roe deer (75 minutes after capture) than in the controls (105 minutes after capture). Body temperature (Figure 4.2) decreased over time in both groups (*P* < 0.05) and it stabilised 45 minutes after capture, as no statistical differences were found between temperature values at minute 45 and those at minute 60.
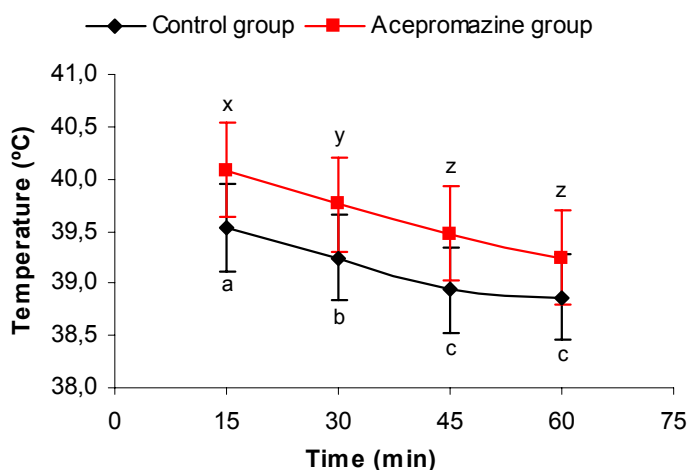


**Figure 4.2.** Body temperature (mean ± SEM) of control (*n = 6*) and treated (*n = 5*) roe deer over one hour after capture. [a, b, c] Values with different superscripts are significantly different from each other in the control group (at least *P* < 0.05). [x, y, z] Values with different superscripts are significantly different from each other in the treatment group (at least *P* < 0.05).

RBC count (Figure 4.3.a) and haemoglobin concentration (Figure 4.3.b) in samples taken from treated animals decreased over time and were significantly lower than those obtained from the control group (at least $P < 0.05$). PCV also was significantly lower in the treated group (Figure 4.3.c), but differences over time were found in the control group (at least $P < 0.05$). Lymphocyte count (expressed as a time 0 ratio) decreased over time in the control group (at least $P < 0.05$), whereas it did not change in the treated animals (Figure 4.3.d). The control group also showed lower lymphocyte numbers than the treated group at time 3 ($P < 0.01$).
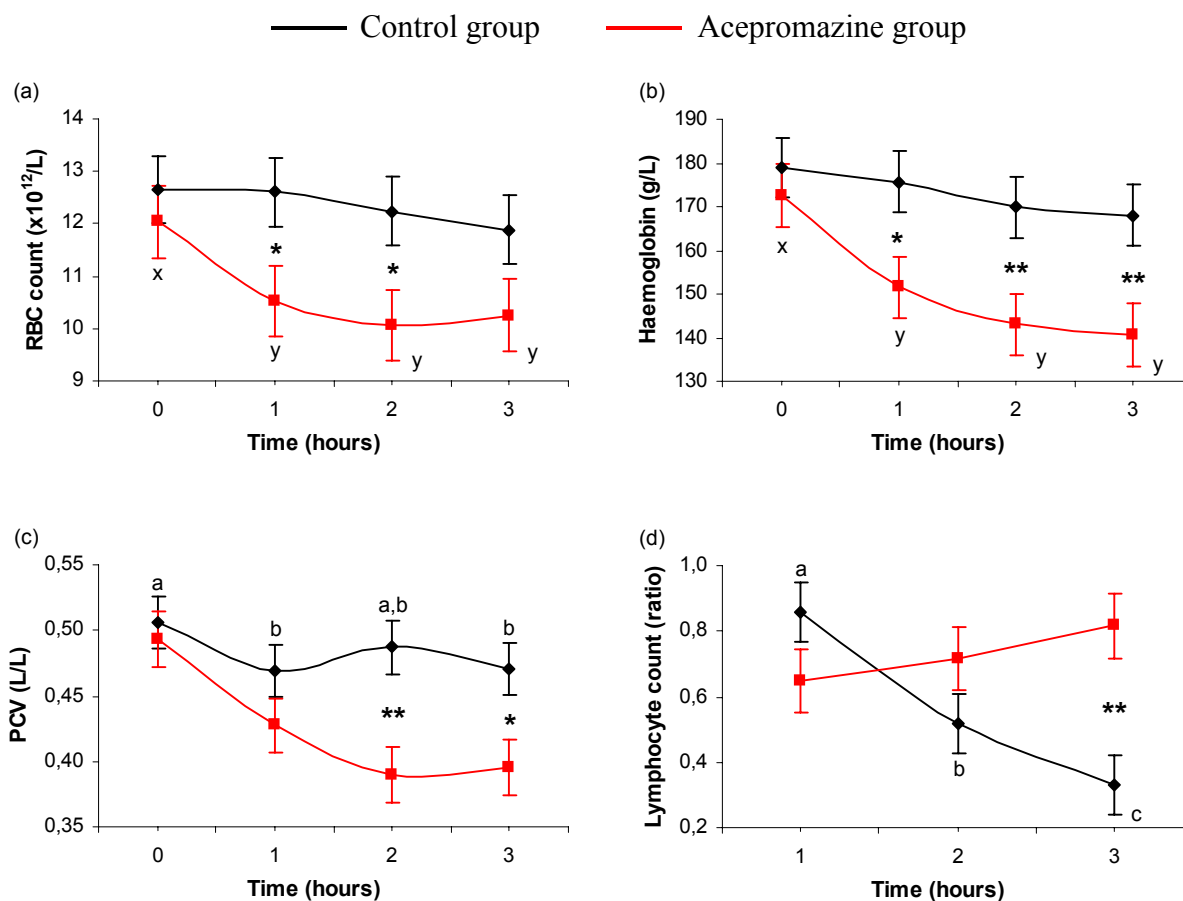


**Figure 4.3.** (a) Red blood cell (RBC) count, (b) haemoglobin concentration, (c) packed cell volume (PCV) (mean ± SEM), and (d) lymphocyte count (expressed as a time 0 ratio ± SEM) of control and treated roe deer over a three-hour period after capture. **\*** Values are significantly different between treatment groups ($P < 0.05$; **\*\*** $P < 0.01$). [a, b ,c] Values with different superscripts are significantly different from each other in the control group (at least $P < 0.05$). [x, y] Values with different superscripts are significantly different from each other in the treated group (at least $P < 0.05$).

Serum creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities increased over time in both groups (at least $P < 0.05$) due to physical stress caused by exercise and restraint. However, they were significantly lower in the treated group compared with control animals (at least $P < 0.05$) from time 2 onwards (CK and AST) and at time 3 (LDH) (Figures 4.4.a, b and d, respectively). Serum alanine aminotransferase (ALT) activity only increased over time in the control group, and was significantly lower in the treated group one and three hours after capture (Figure 4.4.c).
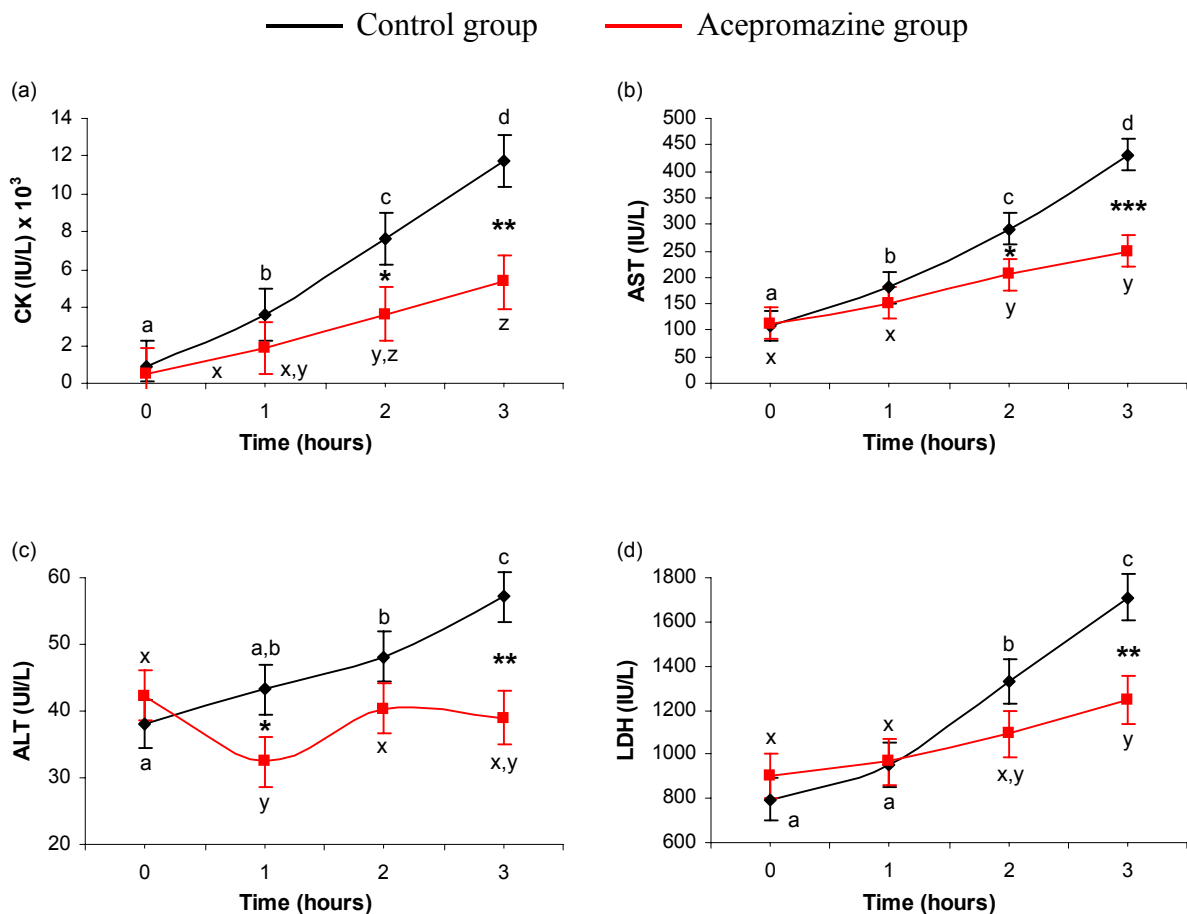


**Figure 4.4.** (a) Serum creatine kinase (CK), (b) serum aspartate aminotransferase (AST), (c) serum alanine aminotransferase (ALT), and (d) serum lactate dehydrogenase (LDH) activities (mean ± SEM) of control and treated roe deer over a three-hour period after capture. **\*** Values are significantly different between treatment groups ($P < 0.05$; **\*\*** $P < 0.01$; **\*\*\*** $P = 0.0001$). [a, b, c, d] Values with different superscripts are significantly different from each other in the control group (at least $P < 0.05$). [x, y, z] Values with different superscripts are significantly different from each other in the treated group (at least $P < 0.05$).

Serum creatinine concentrations increased significantly over time ($P < 0.05$) in the control group (Figure 4.5.a). Serum urea concentrations increased at time 3 ($P < 0.05$) in the treated roe deer but no changes were observed in the control animals (Figure 4.5.b). Serum lactate levels decreased faster (at least $P < 0.05$) in the treated roe deer than in controls (Figure 4.5.c), although when expressed as a time 0 ratio lactate levels did not statistically differ between treatment groups (Figure 4.5.d). Serum potassium levels decreased over time in both groups (at least $P < 0.05$), but the decrease was faster in the control roe deer (Figure 4.6.c). However, no differences were found between treatment groups when expressed as a time 0 ratio (Figure 4.6.d). Finally, serum cortisol concentrations reached the maximum level at time 1 in control roe deer ($P < 0.05$), whereas no differences were observed in the treated deer, nor between treatment groups (Figures 4.7.a, b).

## 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). Measurement of heart rate can be a useful measure of the emotional response of an individual to short-term problems, provided that a distinction is made between the metabolic and emotional effects, and that the measurement itself does not cause too much disturbance (Broom and Johnson, 1993).

In our study, the lack of differences in absolute values of heart rate between the 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). Kock *et al.* (1987a) did not find significant differences in heart rate between normal bighorn sheep (*Ovis canadensis*) and those considered stressed. Read *et al.* (2000) did not find statistical differences in heart rate between wapiti (*Cervus elaphus*) treated with a neuroleptic (zuclopenthixol acetate) and controls. Moreover, Diverio *et al.* (1996b) found a greater increase in heart rates 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. The earlier stabilisation of heart rate recorded in the treated roe deer in comparison with the controls was also observed in the previously cited work. This difference could be explained by the tranquillising effect of the drug.

Porges (1985) proposed that, instead of the basic heart rate, the 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: $23.23 \pm 2.38\%$; treated group: $25.95 \pm 2.67\%$ [Mean $\pm$ SEM]).
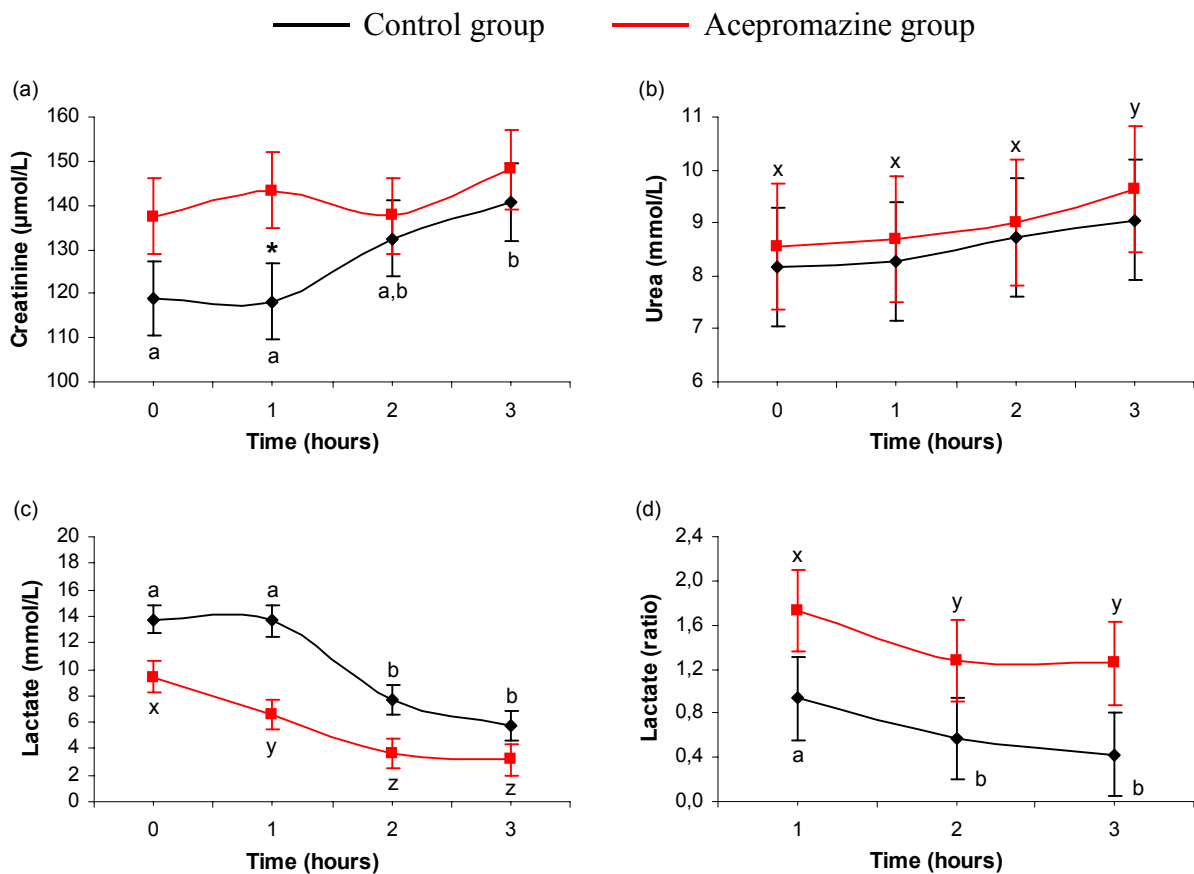


**Figure 4.5.** (a) Serum creatinine concentrations, (b) serum urea concentrations (c) serum lactate concentrations (mean $\pm$ SEM) and (d) serum lactate concentrations expressed as a time 0 ratio ($\pm$ SEM) of control and treated roe deer over a three-hour period after capture. * Values are significantly different between treatment groups ($P < 0.05$). [a, b] Values with different superscripts are significantly different from each other in the control group (at least $P < 0.05$). [x, y, z] Values with different superscripts are significantly different from each other in the treated group (at least $P < 0.05$).

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 results from 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 (Le May *et al.*, 1990; Kent *et al.*, 1993). Correlations have been found among 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). It has been suggested that SIH may be elicited in response to the anticipation of a known or unknown unpleasant event, indicating that SIH may reflect a state of anticipatory anxiety (Lecci *et al.*, 1990). Zethof *et al.* (1994) stated that in mice SIH is time dependent, that it takes 10 minutes to reach a (stable) high level, which is 1 to 1.5ºC higher than the baseline, and that it takes 60 minutes to return to baseline. Moe (1996) found that SIH in farmed silver foxes (*Vulpes vulpes*) lasts 60-90 minutes after a short stressor presentation. Therefore, the changes in body temperature observed in our study resemble those of a SIH response.

Acepromazine did not have any effect on body temperature, although hypothermia is a well described non-desired effect of phenotiazines (Plumb, 1995). Olivier and Miczek (1998) indicate that stress-induced hyperthermia can be suppressed by administering benzodiazepines and serotoninergic agonists but not with phenothiazines. However, the SIH can not be easily prevented in physical capture operations, where the anticipatory anxiety response is elicited before any drug can be administered.

The first step in 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 $\alpha$-adrenergic receptors located in the splenic capsule (Ganong, 1990), and partly to a reduction in plasma volume (Wesson *et al.*, 1979; Cross *et al.*, 1988). In our study, the lower values in RBC count and haemoglobin concentration found in treated animals can be explained by acepromazine's $\alpha$-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 (Figures 4.6.a and 4.6.c).

Total and differential leukocyte counts may be altered by stressful events such as capture and handling. Epinephrine-induced physiologic leukocytosis in ruminants is characterised by neutrophilic leukocytosis with increases in lymphocytes and monocytes and with a mild eosinopenia. Corticosteroid-induced changes include a mature neutrophilia, lymphopenia, eosinopenia and monocytosis (Taylor, 2000). In domestic animals, the neutrophilia and lymphopenia peaks appear after 4-8 hours of exposure to stress (Jain, 1993; Duncan *et al.*, 1994). In our study, the lymphopenia associated with stress leukograms was not observed during the study period in the animals that received acepromazine. Thus, the stress-induced lymphopenia could have been delayed or suppressed by acepromazine.

Muscle enzyme activity increase during capture and handling operations due to increased muscle cell permeability or muscle cell damage (Duncan and Prasse, 1986). These enzymes (ALT, AST, CK and LDH) appear elevated in many stressed wild ungulates and in those suffering from capture myopathy (Kock *et al.*, 1987a; Vassart *et al.*, 1992), although some authors find 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 immobilized by physical or chemical restraint. 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 muscles. This leads to poor tissue perfusion, decreased heat dissipation, and hypoxia (Spraker, 1993). Acepromazine may cause vasodilation in striated muscle arterioles by blocking the $\alpha$-adrenergic receptors or by stimulating the $\beta_2$-adrenergic receptors and, thus, increase muscle blood flow (Booth, 1982; Guyton and Hall, 1996). The results obtained in our study indicate that acepromazine exerts a protective effect against muscle damage, probably due to its vasodilative effect, and suggest that acepromazine

can be used as a preventive treatment of rhabdomyolysis (capture myopathy), as previously indicated for horses (Beech, 1994).

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 produced by catecholamines, have been described (Harthoorn, 1976). Epinephrine (40%) and norepinephrine (20%) cause a decrease in renal blood flow, thus predisposing to renal hypoxia (Guyton and Hall, 1996). Ischemic renal failure has been produced by injecting epinephrine in laboratory animals (Finco, 1997). The differences observed in our study in serum creatinine concentrations between treatment groups 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; Baldessarini, 1996). Moreover, this implies that oxygen supply to kidneys was not impaired, thus reducing the risk of renal hypoxia and consequent renal ischemic necrosis.

Processes which increase protein catabolism will tend to result in increased levels of serum urea (Knowles and Warris, 2000). It has been described that the stress response to capture causes an increase in serum urea concentrations (Gibert, 1991). This increase may be due to physical exercise, diminished renal perfusion and to the effect of glucocorticoids over protein catabolism (Finco, 1997). In our study, however, serum urea only increased in the treated group, which is not correlated with the increase in serum creatinine levels. Urea excretion in ruminants is governed by nitrogen intake. Animals that are on a nitrogen deficient diet excrete almost all blood urea via the gastrointestinal tract and very little via the kidneys (Duncan *et al.*, 1994), which probably happens in free-ranging roe deer during winter. Therefore, this fact could account for the differences in the trend of serum urea and creatinine concentrations.

Hattingh *et al.* (1988) found an increase in lactate levels due to capture and handling operations in wild impala (*Aepyceros melampus melampus*) compared with control values from impalas shot in the brain in the early morning. Our results showed a decrease in serum lactate concentrations, which probably indicates that lactate levels

were returning to baseline. However, this decrease was faster in the treated roe deer (one hour after capture) than in the controls (two hours after capture). In horses, the intravenous administration of acepromazine 20 minutes before exercise resulted in lower serum lactate concentrations after exercise than when horses did not receive the drug. This was related to a protective effect of acepromazine because of its vasodilative action (Freestone *et al.*, 1991; Beech, 1994).
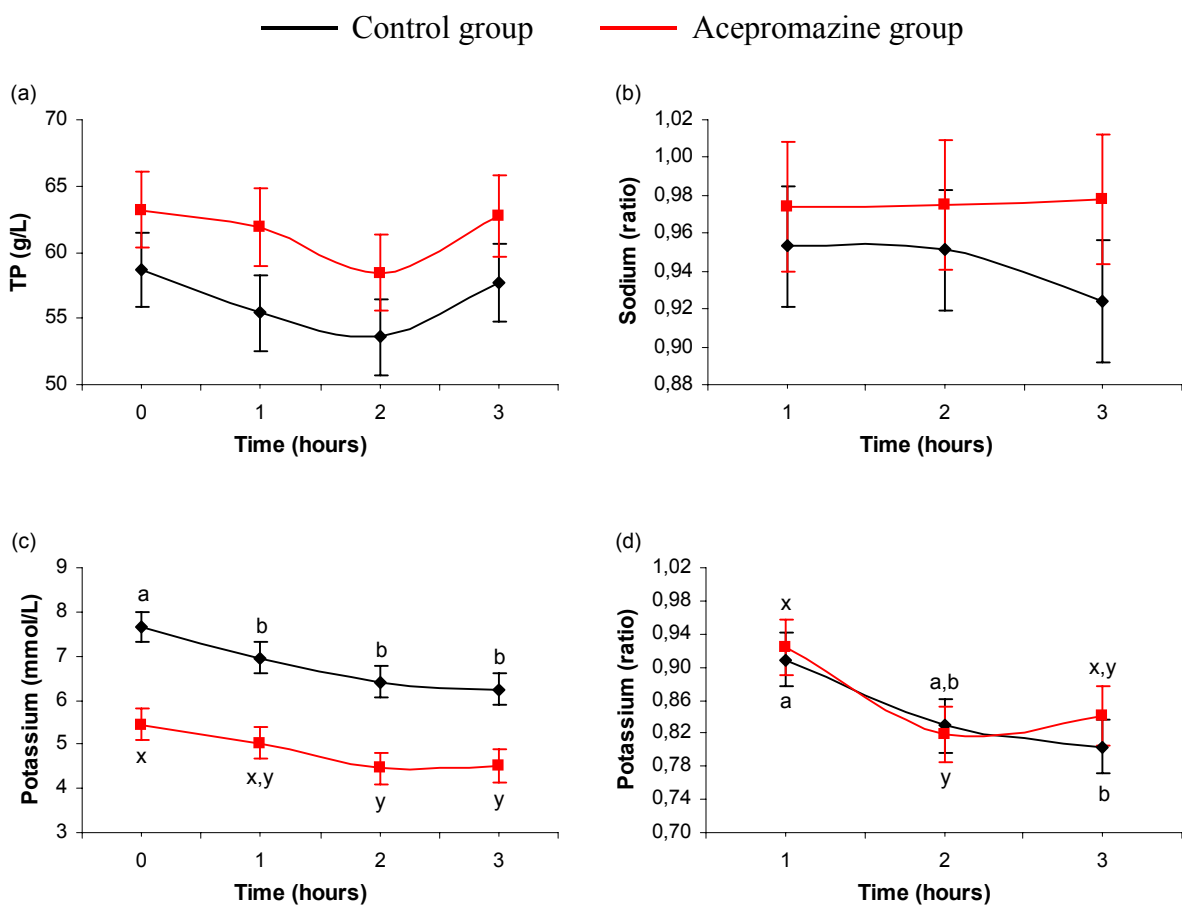


**Figure 4.6.** (a) Serum total protein (TP) concentrations (mean ± SEM), (b) serum sodium concentrations (expressed as a time 0 ratio ± SEM), (c) serum potassium concentrations (mean ± SEM), and (d) serum potassium concentrations expressed as a time 0 ratio (± SEM) of control and treated roe deer over a three-hour period after capture. **\*** Values are significantly different between treatment groups ($P < 0.05$; **\*\*** $P < 0.01$). [a, b] Values with different superscripts are significantly different from each other in the control group (at least $P < 0.05$). [x, y] Values with different superscripts are significantly different from each other in the treated group (at least $P < 0.05$).

The capture stress response causes a hyperkalemia (Gibert, 1991). 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, however, serum potassium levels decreased faster in control animals compared to treated ones. 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 the adrenal glands in response to an extremely wide range of stressors, play a major role in mediating the physiological response to stress, but because of the role of the brain in the release of glucocorticoids, they are widely interpreted as a measure of an animal's psychological perception of a situation, in addition to the extent of its physiological reaction. Many authors have used plasma cortisol as an indicator of stress associated with capture and handling in wildlife species (Franzmann *et al.*, 1975; Seal and Bush 1987; DelGiudice *et al.*, 1990a; Hastings *et al.*, 1992; Morton *et al.*, 1995).

In our study, serum cortisol reached the maximum level one hour after capture in the control roe deer. However, no changes were observed in treated animals. Hartmann (1988) found a peak in cortisol concentrations 30 minutes after an intravenous injection of ACTH in calves. Therefore, the blood withdrawal timing might have influenced the results obtained. Moreover, the lack of differences between groups in serum cortisol levels could be due to the fact that sedative effects do not have a consistent effect on 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 enhancing effect. It has been suggested that chlorpromazine causes systemic release of epinephrine, which may result in an increase in

adrenocorticotropic 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 found in stress-induced plasma cortisol concentrations (Moberg, 1985), together with the low number of individuals per group available in this study, the existence of ultradian, circadian and seasonal rhythms in cortisol secretion (Ingram *et al.*, 1999), and the disturbance caused by the sampling method itself.
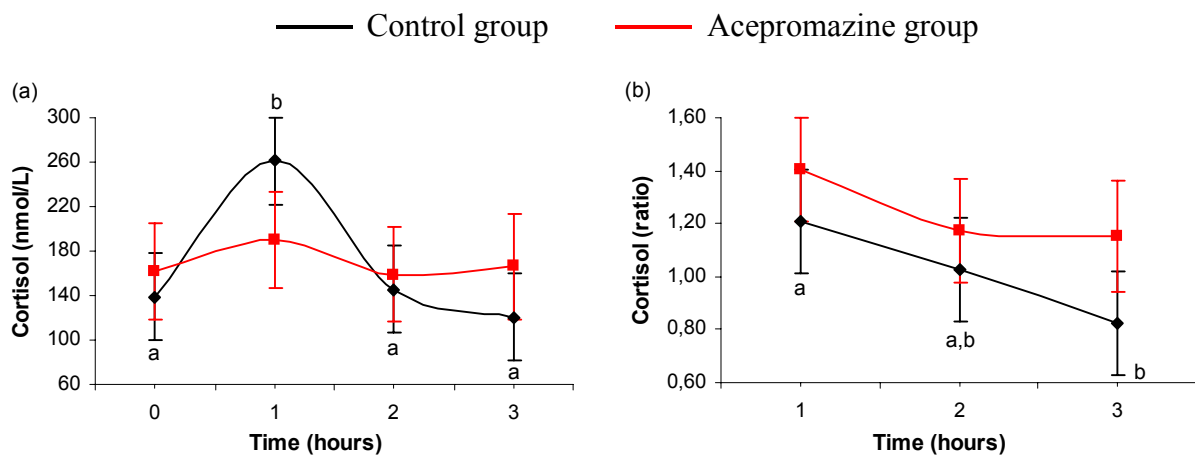


**Figure 4.7.** (a) Serum cortisol concentrations (mean ± SEM) and (b) serum cortisol concentrations expressed as a time 0 ratio (± SEM) of control and treated animals over a three-hour period after capture. [a, b] Values with different superscripts are significantly different from each other in the control group (at least $P < 0.05$).

The results obtained show the suitability of using acepromazine in capture operations of roe deer to reduce the stress response and prevent its adverse effects. The beneficial effect is not only due to the sedative effect of acepromazine, but also to peripheral vasodilation. This vasodilation has a protective effect against the muscular and renal damage that can arise from stress episodes in wild animals, which is directly involved in the pathogenesis of capture myopathy. Thus, animal welfare is partly improved by preventing and reducing adverse effects of stress, although acepromazine had no effect on stress-induced hyperthermia.