Capítulo 5

Differences in the capture stress response between captive and free-ranging roe deer (*Capreolus capreolus*) and its modulation by acepromazine

Capítulo basado en: ***Differences in capture stress between captive and free-ranging roe deer: effect of acepromazine.*** Montané, J., Marco, I., López-Olvera, J., Rossi, L., Manteca, X. and Lavín, S. *Journal of Wildlife Management (en evaluación).*

Abstract

*This study examined the differences in the capture stress response between captive and free-ranging roe deer (*Capreolus capreolus*) and its modulation by acepromazine over a three-hour period following the capture operation. We captured 32 roe deer (16 free-ranging and 16 captive) using drive-nets. These two groups were further divided into two sub-groups: animals in the treatment group received an intramuscular injection of acepromazine (n = 8 free-ranging and 8 captives) whereas animals in the control group received the same volume of saline (n = 8 free-ranging and 8 captives). Heart rate and body temperature decreased over time (P < 0.05) after capture in all four groups. However, heart rate stabilised sooner in the treated roe deer than in the controls, whereas body temperature stabilised sooner in the free-ranging roe deer than in the captives. Red blood cell (RBC) count and haemoglobin concentration decreased over time in the treated deer, were significantly lower in the treated group than in the control one in both free-ranging and captive animals, and were also lower in free-ranging animals than in captives (P < 0.01). Packed cell volume (PCV) was lower (P < 0.01) in the treated deer than in the controls in both free-ranging and captive animals. Lymphocyte count decreased over time in all groups except for free-ranging treated animals (P < 0.001). Leukocyte and neutrophil counts did not differ significantly among groups, showing a significant increase over time (P < 0.01). Serum creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) activities, and serum creatinine and urea concentrations increased significantly over time (P < 0.01) in control captive animals. Serum AST activity also increased over time in control free-ranging animals (P < 0.01). Serum CK, AST, ALT and LDH activities, and serum creatinine were significantly higher in captive control animals than in captive treated ones and free-ranging controls. Serum lactate concentrations decreased over time in all four groups (P < 0.001). Just after capture, lactate levels were higher in captive animals than in the free-ranging ones (P = 0.0001). However, one hour after capture lactate levels were lower in treated animals, regardless of whether they were captive or free-ranging (P < 0.01). Cholesterol and glucose concentrations were significantly higher (P < 0.01) in captive animals in comparison with the free-ranging ones. Moreover, serum glucose concentrations began to decrease one hour after capture (P < 0.01) in captive animals in both the treated and the control groups, although they were significantly lower in those that received acepromazine. Serum potassium levels decreased over time only in the control groups (P < 0.01). No differences were observed in serum cortisol levels. These results show the beneficial effect of using acepromazine to prevent capture myopathy in physical capture operations, especially in captive roe deer, and a differential stress response between captive and free-ranging animals, which should be considered when capturing and handling roe deer.*

Key words: *Acepromazine, capture, captivity, free-ranging, 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. Traditionally, increased plasma levels of corticosteroids have been used as a measure of stress. However, the adrenal cortical response does not always occur, suggesting an over-reliance on this parameter as the sole measure of stress. When an animal is confronted with a potentially stressful situation it relies on three biological systems to cope: behaviour, the autonomic nervous system, and the neuroendocrine system (Moberg, 1987). Since multiple organs are affected by the adaptive response to the imposed stressor, measurement of several objective variables will provide a more reliable description of stress and possibly assist in earlier detection of capture myopathy (Kock *et al.*, 1987a; Moberg, 1987; Hattingh, 1988). The ability to measure stress accurately is of particular importance 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).

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 experience of an animal early in life may have important effects on adult behaviour. Animals born and reared in confinement may react more favourably to such conditions than animals born and reared in relatively unrestricted environments and confined later in life. 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. 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). If

captive animals do not adapt to these factors, they may result in chronic stress. Chronic stress may produce chronic elevations of adrenal hormones (Kant *et al.*, 1987) or result in a sensitisation of the hypothalamic-pituitary-adrenal cortex (HPA) (Dallman *et al.*, 1991; Martí *et al.*, 1999) and the sympathetic-adrenal medullary (SA) (McCarty *et al.*, 1988) axes that can lead to exaggerated responses to novel experiences (Broom and Johnson, 1993). Novelty is a very strong stressor (Dantzer and Mormède, 1983; Grandin, 1997), and even tame animals can have an extreme flight reaction when suddenly confronted with novelty that is perceived as a threat. In the wild, novelty and strange sights or sounds are often a sign of danger (Grandin, 2000). We can find in the literature some studies dealing with the variations in haematological and biochemical values in wild ungulates (Franzmann and Thorne, 1970; Franzmann, 1971; DelGiudice *et al.*, 1987; Hattingh *et al.*, 1988, 1990; Sikarskie *et al.*, 1990; Peinado *et al.*, 1995) and carnivores (De Villiers *et al.*, 1995*;* Constable *et al.*, 1998) kept in captivity.

Capture myopathy is a serious and potentially fatal consequence of capturing and handling wild animals. It is a syndrome that occurs in wild (free-ranging and captive) mammals and birds. This syndrome is characterized by varying degrees of homeostatic imbalances resulting from increased muscular activity, autonomic nervous system activity and physical injury. As a result of the increased muscular activity associated with capture and handling, body temperature is elevated, muscle damage may result in leakage of enzymes, and anaerobic activity contributes to lactic acidosis. In ungulates the syndrome is characterized clinically by depression, muscular stiffness, lack of coordination, paralysis, metabolic acidosis and death. Pathologically, capture myopathy is mainly characterized by muscular and renal lesions (Spraker, 1993).

One method of decreasing the incidence of stress, injuries, and mortality in wild animals is the use of neuroleptics. These drugs are used to alleviate anxiety, have a variable duration of action depending on formulation and have few side effects when used appropriately. Beneficial effects in animals include general calming, indifference to new and unnatural surroundings, loss of fear of people, and reduction in aggressive behaviour (Ebedes and Raath, 1999). Neuroleptic agents have been used successfully around the world in the management of many wild herbivores but there are no reports to date in roe deer (*Capreolus capreolus*). Acepromazine is a member of the

phenothiazine group of short-acting neuroleptic agents. This drug is used prophylactically by some equine practitioners in horses prior to exercise to decrease the incidence of exertional rhabdomyolysis (Freestone *et al.*, 1989). The recommended dose for deer is 0.05-0.1 mg/kg (Arnemo *et al.*, 1993).

In this study, we assessed the differences in the capture stress response between captive and free-ranging roe deer, as well as the differences in the effect of acepromazine on this response by using clinical, haematological and biochemical parameters.

Materials and Methods

Animals

Free-ranging group

Sixteen free-ranging roe deer, four males (three adults, one fawn [less than one year old]) and 12 females (11 adults, 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) constituted the free-ranging group 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, Spain) in a volume of 0.5 mL intramuscularly, whereas eight animals, two males (one adult, one fawn) and six females (five adults, one fawn), acted as controls and received the same volume of sterile saline intramuscularly. 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 the body-temperature recording device was introduced in the rectum.

Captive group

Sixteen captive roe deer, seven males (three adults, four fawns) and nine females (three adults, six fawns), captured by means of drive-nets inside the enclosures (erecting a net and chasing them into it) where they were kept in December 2000, constituted the captive group. They had been in captivity since they were young. Eight randomly selected animals, three males (one adult, two fawns) and five females (one adult, four fawns), received 2.5 mg (0.146 mg/kg ± 0.011 SEM) of acepromazine (Calmo Neosan® 5 mg/mL, Smithkline Beecham, Madrid, Spain) in a volume of 0.5 mL intramuscularly, whereas eight animals, four males (two adults, two fawns) and four females (two adults, two fawns), received the same volume of saline. The mean liveweight of animals was 19.31 ± 1.08 kg (range 12.5-26 kg). Drive-trapping lasted for 10-25 minutes. The restraining procedure and the collocation of the recording equipment was the same as described for the free-ranging group. One of the control animals died at the end of the study period. At necropsy this animal showed an haemorrhage in the oropharynx and 1-2 mm, dispersed, solid noduli in both lungs. A histopathological diagnosis of granulomatous pneumonia was made.

Heart rate and body temperature

We fit the roe deer with telemetric heart-rate recording devices (Polar Vantage NV®, Polar Electro Oy, Kempele, Finland). Adequate records for analysis were obtained from 14 free-ranging (seven per group) and 15 captive roe deer (seven in the control group and eight in the treated one). 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. We also fit the roe deer with telemetric body-temperature recording devices (Mätman datalogger®, Chipsobits Eltex AB, Sweden), but adequate records were only obtained from 11 free-ranging (five in the treated group and six in the control group) and 12 captive roe deer (six per 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 0-12^{o}C.

Blood samples and analyses

We took four blood samples, one at capture (time 0) and one each hour thereafter for three hours (time 1, 2 and 3, respectively). Blood samples were obtained using disposable syringes and 0.8 x 25 mm needles. Blood collected (10 mL) from the jugular vein was placed in a tube with EDTA K_3 as anticoagulant and used for haematological analyses (2.5 mL). 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.

We used a semi-automatic analyser (Sysmex F-800®, Toa Medical Electronics Co. Ltd., Japan) for 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). 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 five minutes to adjust the values obtained with the analyser. We performed differential leukocyte counts using blood smears stained with commercial Diff-Quick® type stain (Química Aplicada S.A., Amposta, Spain).

We used an automated analyser (COBAS MIRA®, Roche, Nutley, NJ, USA) for biochemical analyses, except for sodium and potassium concentrations, which were measured by flame photometry (Corning 410C®, Corning Medical, Medfield, USA), and serum cortisol, which was determined by competitive enzyme-immunoassay (DRG Cortisol EIA-1887, DRG Diagnostics, Germany).

Statistics

We performed repeated measures ANOVAs using the PROC MIXED procedure of the SAS® statistics software package (SAS Institute Inc., Cary, NC, USA). The main factors in the statistical model were treatment (acepromazine or not) and free-ranging/captive status, and the repeated factor was time (sampling at 0, 1, 2 and 3 hours). The animal's sex and age, and the interactions among factors were also included in the statistical model. We used a type 1 autoregression (AR[1]) structure for the covariance matrix of the repeated measures. 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. We used least square means (LS MEANS) because the distribution of animals among groups was unbalanced. The accepted significance level was $P < 0.01$ for haematological and biochemical parameters, and $P < 0.05$ for heart rate and body temperature.

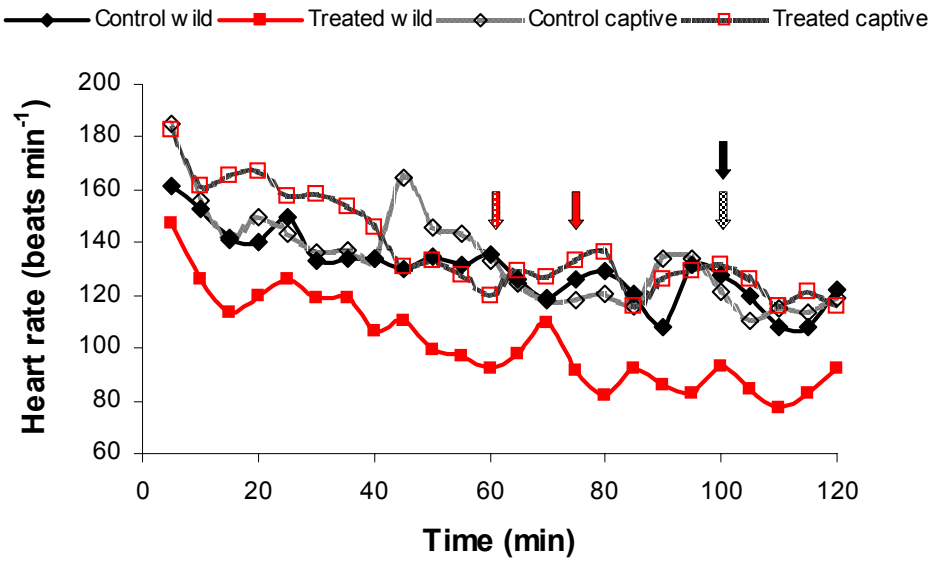

Figure 5.1. Mean heart rate over a two-hour period after capture. Heart rate decreased significantly over time in all groups ($P < 0.05$). Arrows indicate when heart rate stabilised in each group (i.e., no statistical differences were found with the following values). Heart rate was significantly lower ($P < 0.05$) in the treated free-ranging roe deer than in the treated captives.

Results

Heart rate decreased over time ($P < 0.05$) after capture in all four groups (Figure 5.1). However, it stabilised sooner in the treated roe deer (60 minutes after capture in the captive group and 75 minutes after capture in the free-ranging one) than in the controls (100 minutes after capture). Heart rate was significantly lower in the treated free-ranging deer than in the treated captives. Body temperature also showed a decrease over time ($P < 0.05$) in all four groups (Figure 5.2), but it stabilised earlier in the free-ranging animals (30 minutes after capture) than in the captives (45 minutes after capture).

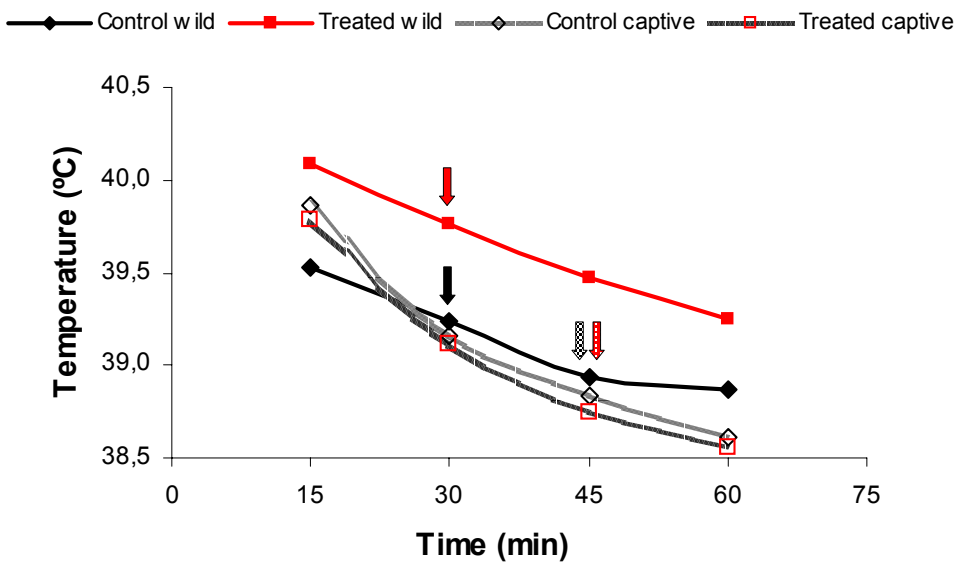

Figure 5.2. Body temperature over a one-hour period after capture. Body temperature decreased significantly over time in all groups ($P < 0.05$). Arrows indicate when body temperature stabilised in each group (i.e., no statistical differences were found with the following values).

RBC count (Figure 5.3.a) and haemoglobin concentration (Figure 5.3.b) decreased over time in the treated groups and also were significantly lower (at least $P < 0.01$) than those obtained from the control group in both free-ranging and captive animals. RBC count was significantly lower in control free-ranging animals than in the captives three hours after capture ($P < 0.01$). Haemoglobin concentration was also significantly lower in free-ranging animals than in the captives from one hour after capture onwards

in treated animals and from two hours after capture onwards in controls ($P < 0.01$). PCV (Figure 5.3.c) was significantly lower in the treated roe deer than in the controls (at least $P < 0.01$). In the treated captive deer, PCV increased between the second and the third hour after capture ($P < 0.01$).

Lymphocyte count (Figure 5.4.b) decreased over time in the free-ranging and captive control groups and also in the captive treated group (at least $P < 0.001$), whereas it did not change in the free-ranging treated ones. Leukocyte and neutrophil counts (Figures 5.4. a and c, respectively) did not differ significantly among groups, showing a significant increase over time (at least $P < 0.01$). Eosinophil count (Figure 5.4.d) decreased over time only in the free-ranging control roe deer (at least $P < 0.01$).

Serum creatine kinase (CK) activity increased significantly over time (at least $P < 0.01$) only in the control captive animals (Figure 5.5.a). Moreover, it was significantly higher in the captive control animals than in the captive treated and free-ranging controls from time 2 onwards (at least $P < 0.01$). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) (Figures 5.5. b, c and d, respectively) activities showed a similar pattern to that of CK, but AST activity also increased over time in the control free-ranging animals ($P < 0.01$) in addition to control captives.

Serum creatinine concentrations (Figure 5.6.a) increased significantly over time only in the captive control group, and they were significantly higher (at least $P < 0.01$) than those of the captive treated group. Serum creatinine concentrations in the control captives were also significantly higher than those in control free-ranging animals (at least $P < 0.01$). Urea concentrations (Figure 5.6.b) increased over time ($P < 0.01$) only in the control captive group. Serum lactate concentrations (Figure 5.6.c) decreased over time in all four groups (at least $P < 0.001$). Just after capture (time 0), lactate levels were higher in the captive deer than in the free-ranging ones ($P = 0.0001$). However, one hour after capture lactate levels were lower in treated animals, regardless of whether they were captive or free-ranging (at least $P < 0.01$). Serum glucose concentrations (Figure 5.6.d) began to decrease one hour after capture (at least $P < 0.01$) in captive animals in both the treated and the control group, although they

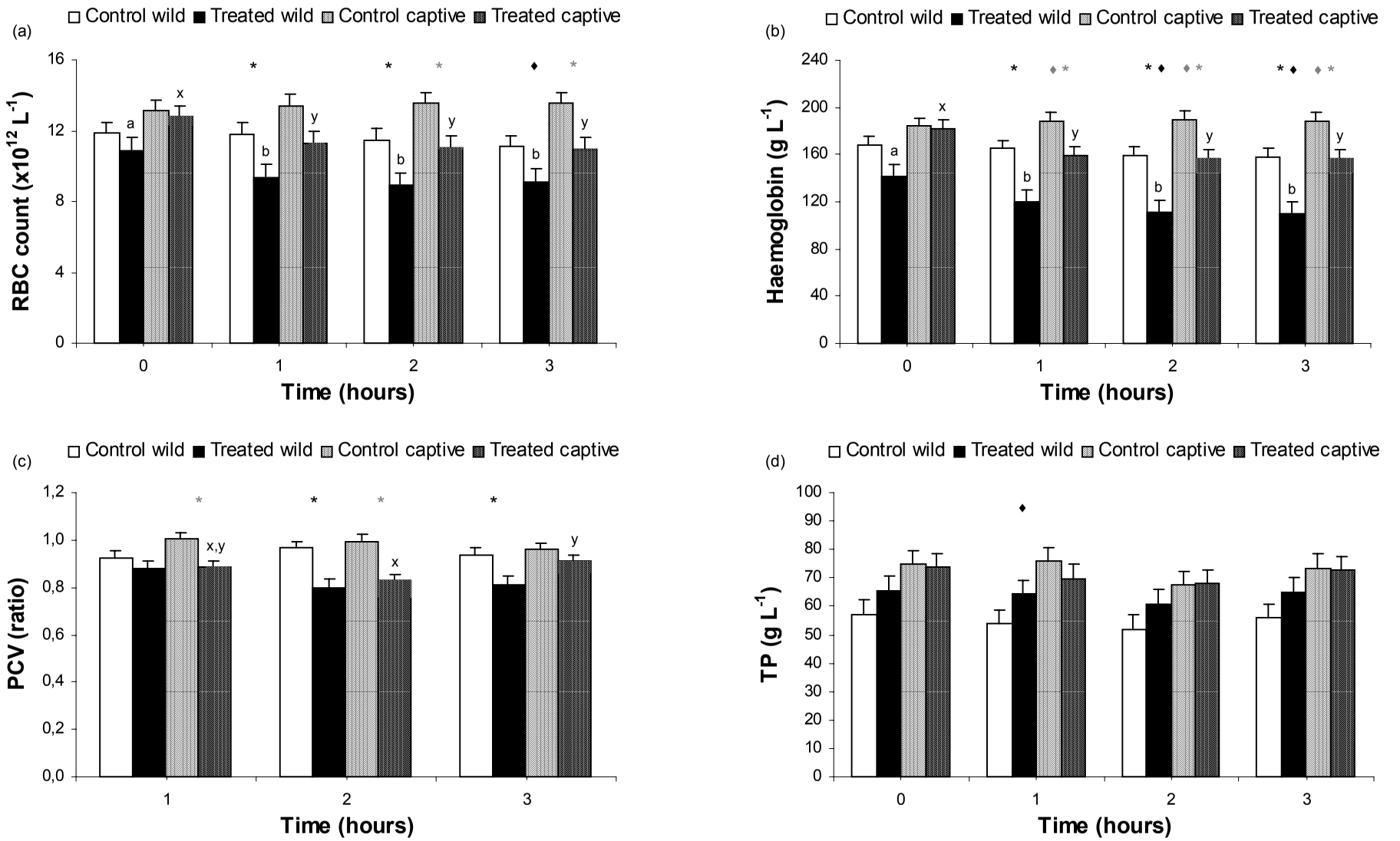

Figure 5.3. (a) Red blood cell (RBC) count, (b) haemoglobin concentration, (c) packed cell volume (expressed as a time 0 ratio ± SEM) (PCV), and (d) total proteins (mean ± SEM) over a three-hour period after capture. * Values are significantly different between control and treated free-ranging animals, * values are significantly different between control and treated captive animals (at least $P < 0.01$). ♦ Values are significantly different between control groups, ♦ values are significantly different between treated groups (at least $P < 0.01$). [a, b] Values with different superscripts are significantly different over time in the free-ranging groups (at least $P < 0.01$). [x, y] Values with different superscripts are significantly different over time in the captive groups (at least $P < 0.01$).

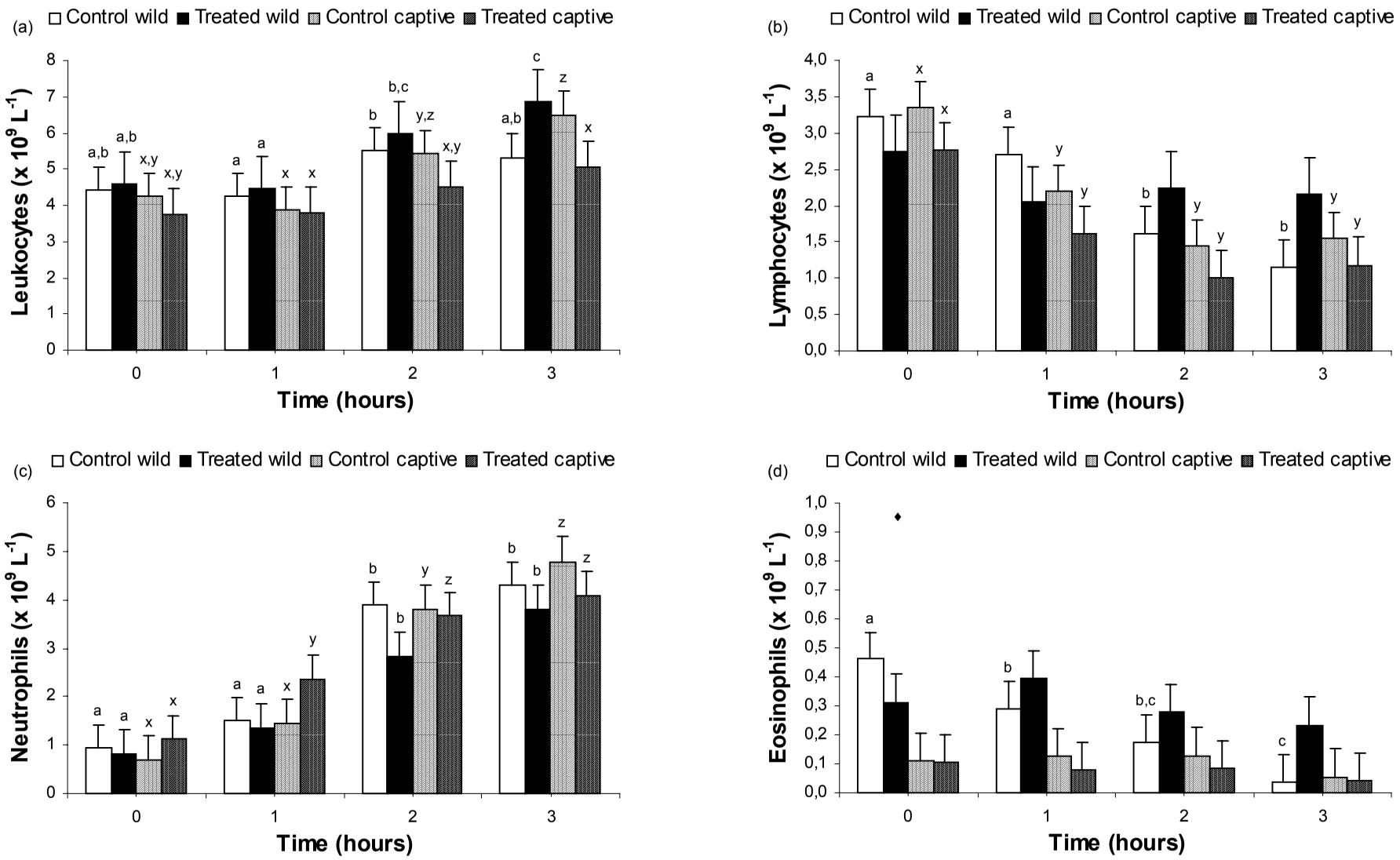

Figure 5.4. (a) Leukocyte count, (b) neutrophil count, (c) lymphocyte count, and eosinophil count (mean ± SEM) over a three-hour period after capture. ♦ Values are significantly different between control groups [a, b, c] Values with different superscripts are significantly different over time in the free-ranging groups (at least $P < 0.01$). [x, y, z] Values with different superscripts are significantly different over time in the captive groups (at least $P < 0.01$).

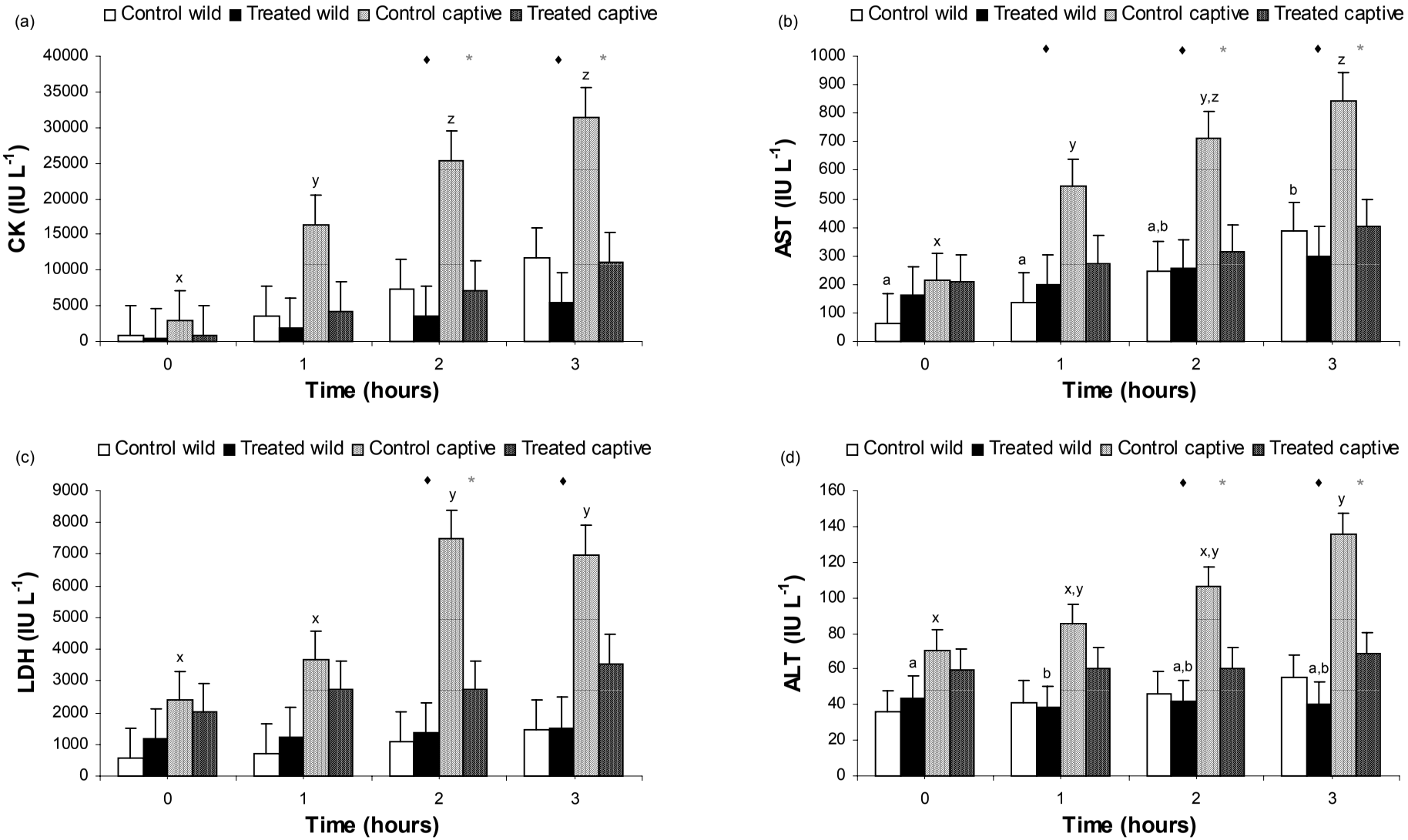

Figure 5.5. (a) Serum creatine kinase (CK), (b) serum aspartate aminotransferase (AST), (c) serum alanine aminotransferase (ALT), and (d) serum lactate dehydrogenase (LDH) activities (mean ± SEM) animals over a three-hour period after capture. * Values are significantly different between control and treated captive animals (at least $P < 0.01$). ♦ Values are significantly different between control groups. [a, b] Values with different superscripts are significantly different along time in the free-ranging groups (at least $P < 0.01$). [x, y, z] Values with different superscripts are significantly different along time in the captive groups (at least $P < 0.01$).

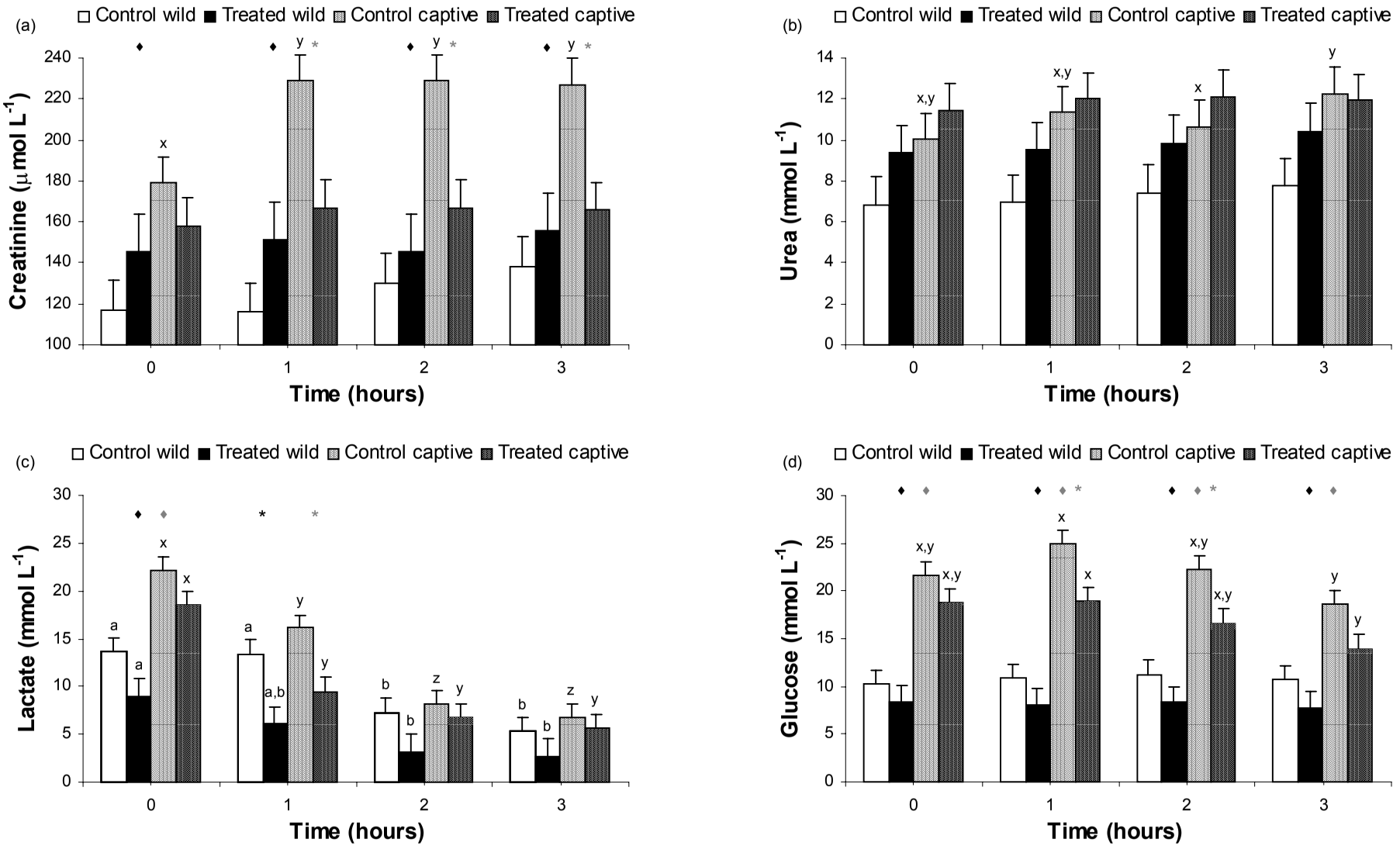

Figure 5.6. (a) Serum creatinine, (b) serum urea, (c) serum lactate, and (d) serum glucose concentrations (mean ± SEM) over a three-hour period after capture. * Values are significantly different between control and treated free-ranging animals, * values are significantly different between control and treated captive animals (at least $P < 0.01$). ♦ Values are significantly different between control groups, ♦ values are significantly different between treated groups (at least $P < 0.01$). [a, b] Values with different superscripts are significantly different over time in the free-ranging groups (at least $P < 0.01$). [x, y, z] Values with different superscripts are significantly different over time in the captive groups (at least $P < 0.01$).

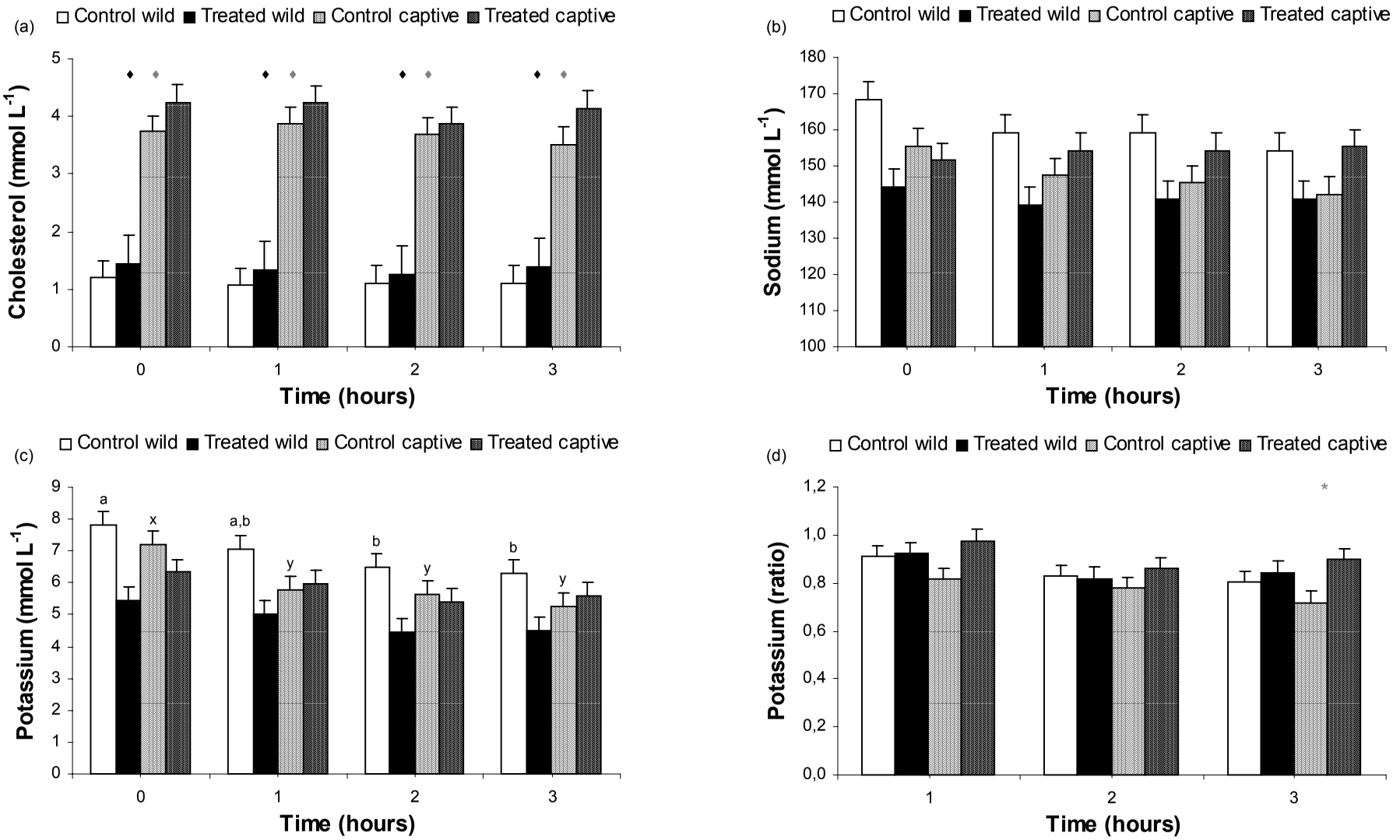

Figure 5.7. (a) Serum cholesterol, (b) serum sodium and (c) serum potassium concentrations (mean ± SEM), and (d) serum potassium expressed as a time 0 ratio (± SEM) over a three-hour period after capture. * Values are significantly different between control and treated captive animals (at least $P < 0.01$). ♦ Values are significantly different between control groups, ♦ values are significantly different between treatment groups (at least $P < 0.01$). [a, b] Values with different superscripts are significantly different along time in the free-ranging groups (at least $P < 0.01$). [x, y, z] Values with different superscripts are significantly different along time in the captive groups (at least $P < 0.01$).

were significantly lower in those that received acepromazine. Moreover, serum glucose levels were consistently higher in captive animals in comparison with the free-ranging ones (at least $P < 0.01$).

Cholesterol concentrations were significantly higher ($P = 0.0001$) in captive animals in both the control and the treated groups in comparison with the free-ranging ones (Figure 5.7.a). Serum potassium levels decreased over time only in the control groups (at least $P < 0.01$) (Figure 5.7.c), whereas when expressed as a time 0 ratio, lower potassium values were observed at time 3 in the control captive group in comparison with the treated group. No difference was observed in serum cortisol levels (Figure 5.8).

Discussion

Effect of acepromazine

Heart rate is considered to be an objective way of assessing the autonomic nervous system's response to psychological stressors (Hopster and Blockhuis, 1994). There must be a maximal level of heart rate, however, so it is not possible to differentiate among responses to stimuli which all elicit the maximal response. In this circumstance, an additional measure has to be used, such as the delay before the high heart rate returns to resting levels (Broom and Johnson, 1993). In our study, the lack of differences in the absolute values of heart rate between treatment groups could be due to reflex tachycardia secondary to hypotension caused by acepromazine (Plumb, 1995) or simply 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 (*Cervus elaphus*) treated with a long-acting neuroleptic than in untreated deer during the 30 minutes immediately following stressor application, which was attributed to reflex tachycardia. The earlier stabilisation of heart rate in treated animals in comparison with controls, as occurred in our study, was also observed in the previously cited work. This difference could be explained by the tranquillising effect of the drug.

During normal activity, about 25% of the total body heat produced is from muscle contraction but, during exercise, the amount of heat generated by muscle contraction can increase 40-60 times (Haskins, 1995). However, increases in body temperature in certain stressful situations can be explained not only by physical activity, but also by another component called stress-induced hyperthermia (SIH) (Moe and Bakken, 1997; Bakken *et al.*, 1999). 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). 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 stress-induced hyperthermia response.

Acepromazine, however, 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 is an anticipatory anxiety response (Lecci *et al.*, 1990) that can not be easily prevented in physical capture operations, where the anticipatory anxiety response is elicited before any drug can be administered.

The general adaptive response to stress includes the release of catecholamines from cerebral adrenergic neurons, sympathetic nerves, and the adrenal medulla (Chrousos and Gold, 1992). Increases in RBC count, haemoglobin concentration and PCV are associated with splenic contraction caused by the action of catecholamines on the 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, the differences observed between treatment groups in RBC count, haemoglobin concentration and PCV 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). In our study, haemodilution caused by acepromazine due to lowering of blood pressure can

be ruled out because total protein (Figure 5.3.d) and sodium concentrations (Figure 5.7.b) would also have been different between treatment groups.

Hormones released in the stress response have also influence on total and differential leukocyte counts. 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 they may cause a decrease in lymphocyte counts. In domestic animals, the neutrophilia and lymphopenia peaks appear after 4-8 hours of exposure to stress (Jain, 1993; Duncan *et al*, 1994). The lymphopenia associated with stress leukograms was not observed during the study period in the free-ranging animals that received acepromazine. This suggests that the stress-induced lymphopenia could be delayed or suppressed by acepromazine in this group. On the other hand, the stress-induced neutrophilia appeared in all four groups.

In cattle, it has been described an increase in muscle enzymes after administration of epinephrine and adrenocorticotropic hormone (ACTH) (Holmes *et al.*, 1973; Sconberg *et al.*, 1993). Activity of muscle enzymes (ALT, AST, CK and 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). Most dramatic rises are seen in generalized muscle disease, but muscular exertion also promotes an elevation of these enzymes (Bartsch *et al.*, 1977). Acepromazine may cause vasodilation in striated muscle arterioles by blocking the α-adrenergic receptors or by stimulating the β_2-adrenergic receptors, and thus increase muscle blood flow (Booth, 1982; Guyton and Hall, 1996). The results obtained in our study indicate that acepromazine exerted a protective effect against muscle damage in captive animals by increasing the muscle blood flow during the stress response, and demonstrate its importance as a preventive treatment of rhabdomyolysis (Beech, 1994).

Increased creatinine concentrations resulting from muscular activity and decreased renal excretion because of vasospasm in the kidney produced by catecholamines have been described in some ungulates (Harthoorn, 1976). The differences between the

captive control and the captive treated groups in serum creatinine may 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). Furthermore, this implies that oxygen supply to kidneys was not hindered, thus preventing renal hypoxia and consequent renal ischemic necrosis. The blockade of the α-adrenergic receptors caused by acepromazine is believed to assist in prevention of renal ischemia and maintenance of adequate kidney function during general anaesthesia (Booth, 1982).

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, the effect of glucocorticoids over protein catabolism and to diminished renal perfusion (Finco, 1997). Urea passively diffuses with water from the tubular lumen back into the blood. The amount of absorption is inversely related to urine flow through the tubules. If urine flow is decreased, urea reabsorption is greater and blood urea concentration increases (Duncan *et al.*, 1994). Therefore, an increase in urea reabsorption together with a diminished glomerular filtration rate, as occurs during the stress response due to contraction of renal arterioles, may explain the increase over time in serum urea levels observed in the control captive roe deer in comparison with the treated ones. However, this effect was not observed in the free-ranging roe deer.

Endogenous lactic acid production increases as tissue perfusion and oxygenation decrease (Duncan *et al.*, 1994). 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 in all four groups after having reached the highest values at capture, which indicates that they were returning to baseline as time passed. However, serum lactate levels were lower one hour after capture in the animals that received acepromazine. In one study of normal Thoroughbreds, 7 mg of acepromazine given intravenously before exercise resulted in lower serum lactate concentrations after exercise than when horses did not receive the drug. This suggests that acepromazine may decrease the production and/or

increase the clearance of lactate because of its vasodilative action (Freestone *et al.*, 1991; Beech, 1994).

Plasma glucose levels are increased following the secretion of adrenal medullary and cortical hormones, but they can also be reduced by vigorous activity (Broom and Johnson, 1993). In our study, glucose levels were lower in the captive animals treated with acepromazine in comparison with the controls, which might be attributed to the tranquillising effect of the drug in this group. Armario *et al.* (1990) reported that, under appropriate conditions, glucose levels can be a good index of the intensity of acute stress experienced by rats.

During high intensity exercise there is a rise in plasma potassium concentrations. Research has demonstrated that the intensity of the exercise and the time taken to collect samples following exercise will influence the level of plasma potassium (Harris and Snow, 1988). Following the completion of exercise, potassium quickly declines controlled by extrarenal factors including insulin, catecholamines, glucocorticoids and acid-base balance (Bia and DeFronzo, 1981). However, in our study, serum potassium levels only decreased over time in control animals. The vasodilative effect of acepromazine could have reduced the degree of muscle cell damage, and thus the release of intracellular potassium. This could have prevented the activation of extrarenal mechanisms that control potassium homeostasis in treated roe deer. It has also been suggested that by influencing electrolyte movements, acepromazine may alter neuromuscular excitability and impair the development of exertional rhabdomyolysis (Freestone *et al.*, 1991). Moreover, a significant difference in serum potassium concentrations was found between treatment groups in the captive roe deer, whereas no differences were recorded in the free-ranging animals. This suggests that captive roe deer could be more sensitive to the effect of acepromazine on serum potassium concentrations.

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, 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; DelGiudice *et al.*, 1990a; Hastings *et al.*, 1992; Morton *et al.*, 1995). In our study, no significant differences between treatment groups were observed in serum cortisol levels. These results could be explained because of sedative effects of acepromazine are unrelated to plasma cortisol concentrations. The measurement of changes in plasma 11-hydroxycorticosteroid (11-OHCS) levels has not been found to be a satisfactory mean of assessing any reduction of stress with some sedative agents (McKenzie and Snow, 1977). Brearley *et al.* (1990) found in cattle 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 ACTH release and hence cortisol release (Bruss, 1980).

Effect of captivity

Repeated exposure to different unpleasant stimuli may sensitise the hypothalamic-pituitary-adrenal cortex (HPA) and the sympathetic-adrenal medullary (SA) axes so that a test with a novel disturbing stimulus elicits a greater response than such a test would normally. The hormonal response to modifying factors is common to a wide spectrum of stimuli, including capture and handling. Capture and restraint results in a rapid increase of glucocorticoids, usually within 5-10 minutes and reaches a maximum within 30-60 minutes. The collection of serial blood samples in free-living birds and captive populations has proved to show a great deal about sensitivity to modifying factors and current conditions. It has been found that although baseline glucocorticosteroid levels may be similar in captive and free-living birds, captives tend to have a greatly enhanced elevation of glucocorticosteroids following restraint (Wingfield *et al.*, 1997). Hattingh *et al.* (1988, 1990) reported higher epinephrine, dopamine and cortisol levels caused by physical capture in boma-kept impala (*Aepycerus melampus melampus*) than in their wild counterparts, which were further responsible for many of the other responses seen in their blood. Therefore, the differences observed between captive and free-ranging animals in our study could be due to a sensitisation caused by captivity-induced chronic stress.

On the other hand, an effect of 'conditioning' also could explain the differences between captive and free-ranging roe deer if we assume that captives are less accustomed to physical exercise than free-ranging ones. Tollersrud *et al.* (1971) observed a differential response to physical stress in lambs which were either used to or unaccustomed to activity. Physical exercise caused larger changes in serum enzymes and other blood constituents in lambs that had been kept and fed indoors for over a month, than lambs that had been at pasture.

Heart rate is a useful measure of welfare in the short term, but of little value when comparing long-term conditions. However, long-term conditions can affect changes in heart rate which occur in test situations (Broom and Johnson, 1993). On the other hand, lower values for heart rate can be found in fit animals during exercise and recovery (Sneddon *et al.*, 1989). In our study, heart rate was significantly lower in the free-ranging treated animals than in the captive treated ones. This difference could be due to their better physical condition and/or to the lack of a chronic stress-induced sensitisation to novel stressors. However, this difference was only observed between treated groups. Therefore, it could be attributed to a more marked effect of acepromazine in the captive roe deer than in the free-ranging ones, which would accentuate the hypotensive effect of the drug and, consequently, the reflex tachycardia.

The coefficient of variation of heart rate has been used as a measure of heart rate variability (Hopster and Blokhuis, 1994) and is suggested that it may be a good indicator of both the status of the nervous system of the individual and its capacity to respond to environmental demands (Porges, 1985). In our study, no differences were recorded between captive and free-ranging roe deer (captive group: 23.18% ± 2.74%; free-ranging group: 24.59% ± 1.76% [mean ± SEM]). However, the captive roe deer that died at the end of the study period, showed a very low coefficient of variation (10.47%).

The body temperature stabilised sooner in the free-ranging animals than in the captives. This could be due to a greater delay between the onset of drive-trapping and the insertion of the rectal probe in the free-ranging animals than in the captive group,

and thus the body temperature curves could have failed to correspond. However, lower rectal temperatures have been reported in fit animals during exercise and recovery (Sneddon *et al.*, 1989; Gatta *et al.*, 1998). Moreover, Trunkfield *et al.* (1991) recorded a significant increase in body temperature in crate-reared calves, which also showed the largest cortisol response to handling and transport, in comparison with those at pasture. Therefore, both conditioning and the free-living condition could explain the differences observed in body temperature between free-ranging and captive roe deer.

The lower RBC count and haemoglobin concentration values in the free-ranging roe deer compared with the captives could be due to an effect of conditioning. There are studies reporting both lower (Sneddon *et al.*, 1989) and higher RBC counts (Escribano *et al.*, 1995) in unfit animals than in fit ones. Also, there are reports of increases and decreases in haemoglobin concentration and PCV values with training (Sneddon *et al.*, 1989, Noel de Burlin *et al.*, 1994; Escribano *et al.*, 1995; Pratt *et al.*, 1996; Robertson *et al.*, 1996; Gatta *et al.*, 1998). As we can see, training gives rise to variable RBC counts, haemoglobin concentrations and PCV values probably caused by the different training schedules applied in these studies. However, the higher values in the captive roe deer could also be attributable to a sensitisation of the SA axis, which is involved in splenic contraction (Ganong, 1990).

An stress-induced lymphopenia was observed in both captive groups, regardless of whether they were treated or not. It has been reported that stress-susceptible pigs have lower lymphocyte counts by 60 or 120 minutes post stress than stress-resistant pigs, suggesting a greater production and more rapid use of corticosteroids by stress-susceptible pigs (Evans, 2000). It is possible that captivity constitutes a sensitising factor causing a similar effect on lymphocyte counts, as acepromazine did not exert the same effect in captive and free-ranging roe deer and lymphocyte counts decreased faster in the captives. Hopster *et al.* (1998) suggested that environmental stress could be the responsible for a sudden fall in lymphocyte numbers in stress-susceptible cows. However, the rapid and large-magnitude reduction in lymphocyte numbers was likely not attributable to increased plasma cortisol concentrations. These authors considered neural catecholamines and endogenous opioids possible candidates for these stress-induced changes in lymphocyte distribution.

The differences between captive and free-ranging control roe deer in serum CK, AST, LDH and ALT activities can be explained by an effect of conditioning, as it has been reported that these parameters are higher in unfit animals after exercise (Chapple *et al.*, 1991; Beech, 1997). Sikarskie *et al.* (1990) reported higher AST activities in ranched American bison (*Bison bison*) than in free-ranging ones as occurred in our study. The LDH_5 isoenzyme (which occurs mainly in striated muscle and liver) has been found to increase in park deer after capture (Jones and Price, 1990). Of particular interest in this study was the fact that increases continued in animals lying quietly with their heads covered. Hence it seems that the release of this isoenzyme into plasma cannot be just a consequence of exercise, but is a response of the animal to a disturbing situation (Broom and Johnson, 1993). Thus, it is possible that the differences observed in our study were not only due to habituation to physical activity but also to sensitisation of captive animals to novel situations.

Factors influencing muscle mass such as disease of muscle and character of muscle as influenced by physical training, may affect the size of the creatine pool and thus the daily production of creatinine (Finco,1997). Querengaesser *et al.* (1994) found that creatinine concetrations decreased after training and increased after bouts of exercise in dogs. It has been described that training improves renal function, specially glomerular filtration rate (Kronfeld *et al.*, 1977). Therefore, the higher values in the captive control roe deer in comparison with the free-ranging ones could be due to an effect of conditioning. However, we can not rule out the sensitisation of the axes involved in the stress response caused by captivity.

Differences in BUN (blood urea nitrogen) concentrations have been observed in different populations of white-tailed deer (*Odocoileus virginianus*) (Seal *et al.*, 1978), pronghorn antelope (*Antilocapra americana*) (Seal and Hoskinson, 1978) and bighorn sheep (*Ovis canadensis*) (Franzmann, 1972) grazing in different habitats. Therefore, the mean BUN concentration of wild animal population may be used as a guide to protein intake (Seal *et al.*, 1972a). In our study, no differences in serum urea concentrations were observed between captive and free-ranging roe deer. However, 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). Thus, if we accept that captive roe deer were better fed, decreased renal perfusion could have had a more marked effect in this group than in free-ranging roe deer. This fact could explain that urea levels did not increase over time in the control free-ranging roe deer as happened in the captives. Otherwise, the differences observed also might be due to differences in the stress response between captive and free-ranging animals.

The initially higher values of serum lactate levels in the captive roe deer were probably due to an effect of conditioning (Sneddon *et al.*, 1989; Geor *et al.*, 1999), as lactate production is lower in trained than in untrained subjects performing the same exercise. They can also result from differences in the intensity of exercise performed before capture, as the rate of lactate production is closely related to it (Cardinet III, 1997), and the roe deer kept in captivity performed a shorter but more intense exercise. Hattingh *et al.* (1988, 1990) also reported higher lactate values in boma-kept impala than in free-ranging ones.

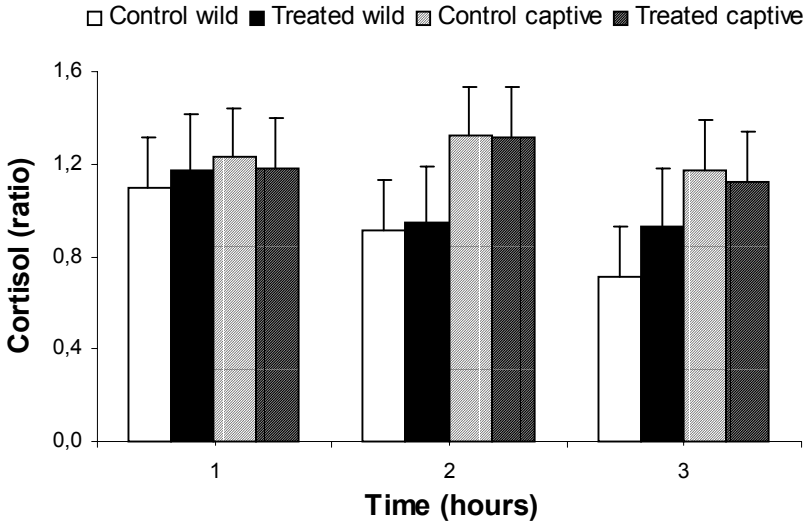

Figure 5.8. Serum cortisol concentrations (expressed as a time 0 ratio ± SEM) over a three-hour period following the capture operation.

Catecholamines and corticosteroids released during the stress response have a hyperglycemic effect (Duncan *et al.*, 1994). The higher serum glucose concentrations observed in the captive roe deer are in agreement with the findings of Hattingh *et al.*, (1988, 1990), who also reported higher values in boma-kept impala than in free-ranging ones. Our results can be explained by differences in the diet (DelGiudice *et*

al., 1987) or to a sensitisation of the hypothalamic-pituitary-adrenal (HPA) (Dallman *et al.*, 1991; Martí *et al.*, 1999) and the sympathetic-adrenal medullary axes (SA) (McCarty *et al.*, 1988) in the captive animals caused by chronic stress.

Serum cholesterol has been measured following exposure to a variety of unpleasant situations, but the responses do not appear to be consistent and are therefore of little value as indicators of welfare (Broom and Johnson, 1993). In our study, the consistently higher cholesterol concentrations observed in captive animals were probably due to diet (Hattingh *et al.*, 1990; Peinado *et al.*, 1995).

Although we would expect to find differences in serum cortisol levels between free-ranging and captive roe deer according to the work of Wingfield *et al.* (1997), there are several factors that can affect the levels recorded and cause difficulty in the interpretation of the results (Rushen, 1991). Among these factors we highlight the great inter-individual differences in stress-induced plasma cortisol concentrations (Moberg, 1985), the existence of ultradian, circadian and seasonal rhythms in cortisol secretion (Turner, 1984; Nilssen *et al.*, 1985; Ingram *et al.*, 1999), the disturbance caused by the sampling method itself (blood sampling was more time-consuming in certain animals), and the low number of individuals per group available in this study.

Management implications

The results obtained support the use of acepromazine in capture operations of roe deer, especially in captive animals, in order 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.

These results also illustrate the potential for difference in stress responses between free-ranging and captive roe deer. Therefore, the management system of animals should be considered in addition to the effects of tranquilliser administration during capture operations.