Capture, restraint and transport stress in Southern chamois (*Rupicapra pyrenaica*)

Modulation with acepromazine and evaluation using physiological parameters

Jorge Ramón López Olvera

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Los Doctores SANTIAGO LAVÍN GONZÁLEZ e IGNASI MARCO SÁNCHEZ, Catedrático de Universidad y Profesor Titular del Área de Conocimiento de Medicina y Cirugía Animal de la Facultad de Veterinaria de la Universidad Autónoma de Barcelona, respectivamente,

CERTIFICAN:

Que la memoria titulada 'Capture, restraint and transport stress in Southern chamois (*Rupicapra pyrenaica*). Modulation with acepromazine and evaluation using physiological parameters', presentada por el licenciado Don JORGE R. LÓPEZ OLVERA para la obtención del grado de Doctor en Veterinaria, se ha realizado bajo nuestra dirección y, considerándola satisfactoriamente finalizada, autorizamos su presentación para que sea evaluada por la comisión correspondiente.

Y para que así conste a los efectos que sean oportunos, firmamos el presente certificado en Bellaterra, a 27 de Julio de 2004.

Firmado: Santiago LAVÍN GONZÁLEZ Firmado: Ignasi MARCO SÁNCHEZ

Sólo sé que no sé nada

Sócrates (Filósofo griego, 470-399 a. C.)

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1. ABSTRACT

The objective of this research was to assess capture, handling and transport stress in Southern chamois (*Rupicapra pyrenaica*) captured by means of drive-nets, as well as determine the viability of non-invasive techniques (heart rate, rectal temperature and saliva cortisol concentration) to measure stress response. Lack of previous reliable reference haematological and serum biochemical values for this species made necessary to establish reference values at capture to assess changes in these parameters. It was expected to determine the best stress indicators in Southern chamois from the data obtained. The effect of acepromazine on both capture and transport stress was also evaluated.

Southern chamois were captured by means of drive-nets and first blood sample taken at capture was used to establish reference values. Then the animals underwent a three-hour period of physical restraint or a transport procedure to study physical restraint and transport stress, respectively. All experiment groups were randomly divided in treatment (acepromazine) and control (saline) groups, and sex effect and its interaction with treatment was considered in the physically restrained group. Heart rate and rectal temperature were registered throughout the study in all groups, and blood samples were obtained every hour in physically restrained Southern chamois, whereas blood samples were obtained at capture, immediately before transport and immediately after transport in transported animals.

Capture results were overall similar or better than those reported previously for Northern chamois.

The first complete and reliable reference values for Southern chamois captured by means of drive-nets were reported, and sex, season, and age differences were found, suggesting the establishment of separate reference values for each situation.

Heart rate increased during the study only in control animals during transport, whereas temperature decreased over time in all groups. Erythrocytic parameters (RBC, haemoglobin concentration and haematocrit) decreased after capture in all groups. Leukocytes changed from initial lymphocytosis to neutrophilia and lymphopenia, with increasing total leukocyte count over time. Monocytes and band neutrophils increased over time in treated restrained and untreated transported Southern chamois. Cholesterol, triglycerides, lactate, creatinine, AP, chloride and potassium decreased from capture values during the study, whereas cortisol, total bilirubin, urea, and serum enzymatic activity (ALT, AST, CK and LDH) increased over time. Glucose increased during the first part of the study to decrease later, and sodium increased in untreated restrained animals and decreased in treated transported Southern chamois.

Differences in stress response between male and female Southern chamois for erythrocytic parameters, glucose, bilirubin, creatinine, chloride and potassium indicate a stronger catecholamine response in females, whereas lymphocytes, cortisol and ALT differences seem to point to a higher corticosteroid response in males.

Transport was a more stressful stimulus than physical restraint for Southern chamois, as indicated by higher values and lack of decrease over time in heart rate, erythrocytic parameters (RBC, haemoglobin concentration and haematocrit), creatinine, urea, ALT, CK, LDH, chloride and sodium.

Acepromazine improved animal welfare and decreased the risk for the life of captured and treated Southern chamois, which showed lack of increases in heart rate during transport, lower values and earlier stabilization of rectal temperature, higher decreases with significantly lower values for erythrocytic parameters, creatinine, urea, sodium and potassium and lower increases in serum enzymatic activity (ALT, AST, CK and LDH). Although some of the positive effects of acepromazine may be due to its central tranquilizing action, most advantages of its use were attributed to its α-adrenergic-blocking action on spleen, kidney and muscle blood vessels. Moreover, central effect of acepromazine seems to be influenced by the nature of the stressor, since monocytes, band neutrophils and cortisol increased in the treated group in restrained Southern chamois and in the control group in transported Southern chamois, suggesting a higher degree of excitement in these two groups. Acepromazine also had a stronger tranquilizing and protective effect on females, as demonstrated by temperature, ALT, AST, CK, LDH, chloride and potassium.

Saliva cortisol concentration was well correlated with serum total cortisol concentration and is a potentially useful noninvasive tool to assess stress response, provided collection problems are solved and the method is adequately validated.

2. INTRODUCTION

Wild ungulates are captured and transported for population restocking, game translocation or scientific purposes. Handling wild ungulates is increasingly becoming a cause of concern, since it supposes a threat to the life of the animal and it also has animal welfare implications. One of the main factors participating in the development of physiological disorders is stress.

Southern chamois (*Rupicapra pyrenaica*) is a game species belonging to the Subfamily *Caprinae*. Its population reached more than 55000 individuals in 2003, although one subspecies, the Apennine chamois (*R. p. ornata*), is considered vulnerable by the International Union for Conservation of Nature and Natural Resources (IUCN), with a population of only 400 individuals. Capture, restocking and translocation operations of both Southern and Northern chamois (*Rupicapra rupicapra*), a closely related species, have been carried out during the last century in different countries (France, Italy, Spain, New Zealand). Drive-nets have been widely used in different countries in Europe to capture both Southern and Northern chamois.

Normal physiological data of free-ranging animals are difficult to establish, due to the effects of capture stress on haematological and biochemical parameters, and information about values for haematological and biochemical data for the Genus *Rupicapra* is scarce, particularly for Southern chamois (*Rupicapra pyrenaica*). Haematological, serum biochemical and clinical parameters of wild animals provide information about their physiological status, and may be useful to assess disease conditions, stress due to capture or captivity, nutritional status, and also as a tool to monitor and evaluate the density and status of its population and therefore establish environmental management measures.

Reference values are needed in order to monitor captured animals, and will be very useful to better understand physiological changes induced by physical capture. Not only reference values at the moment of the capture have to be known, but also the normal evolution of haematological and biochemical parameters in stress situations, in order to detect deviations of the expected reaction which could indicate risk for the welfare, health or life of the animal. When capturing, handling or transporting wildlife, it is important to establish the most accurate way to measure stress to reduce mortality and improve their well-being. However, there is not a specific stress response to all stressors, and physiological changes may vary depending on the species and the stressor, so stress indicators must be established for every species and circumstance. Although tranquilizers can reduce stress response and its biological consequences in wild animals, physical capture can induce such a strong stress reaction that can overcome the effect of the drug.

In spite of being a common operation for more than 100 years, stress response to capture, transport and handling and its modulation by tranquilizers have not been studied in none of the *Rupicapra* species. Handlers should be familiar with normal responses and values of the species for which they are responsible and aware of the use of techniques and drugs which can reduce the risk for the life of the animal.

3. OBJECTIVES

The present research work formed part of the research project 'Assessment of capture and handling stress in the Southern chamois (*Rupicapra pyrenaica*)', founded by the Science and Technology Interministerial Comission (CICYT AGF99-0763-C02). The main general objective of the project was to assess physical capture, restraint and transport stress in the Southern chamois. Methodological and specific objectives related to this main purpose were:

1. Assessing the viability, in terms of performance and safety, of drive-nets to capture Southern chamois.

2. Establishing reference intervals for haematological and serum biochemical parameters for free-ranging Southern chamois captured by means of drive nets.

3. Describing the stress response to physical capture, restraint and transport in Southern chamois.

4. Determining the best indicators to measure stress related to capture with drive-nets and physical restraint in the Southern chamois

5. Assessing the effect of acepromazine on stress response to physical capture, restraint and transport by means of haematological, biochemical and clinical parameters.

6. Test the usefulness of noninvasive techniques (telemetric recording of heart rate and body temperature and determination of saliva cortisol concentration) to assess the stress response in Southern chamois.

4. LITERATURE REVIEW

4.1. GENERAL CHARACTERISTICS OF SOUTHERN CHAMOIS (*Rupicapra pyrenaica*)

4.1.1. Taxonomy

Southern chamois (*Rupicapra pyrenaica*) is a medium sized ruminant formerly considered a subspecies of Northern chamois (*Rupicapra rupicapra*). However, behavioural anatomical, morphological and, specially, genetic differences demonstrated by different enzymatic electrophoretic migration, allowed the differentiation of the two species (Nascetti *et al*., 1985; Catusse *et al*., 1996). Southern chamois includes three different subspecies: Pyrenean chamois (*R. p. pyrenaica*), Cantabrian chamois (*R. p. parva*) and Apennine or Abruzzo chamois (*R. p. ornata*) (Shackleton *et al*., 1997) (Figure 4.1).

Family *Bovidae*
- Subfamily *Caprinae*
  - Tribe *Rupicaprini:*
    - *Capricornis* (Serows)
    - *Naemorhedus* (Gorals)
    - *Oreamnos americanos* (Mountain goat)
    - *Rupicapra* (Chamois)
      - *Rupicapra rupicapra* (Northern chamois)
      - *Rupicapra pyrenaica* (Southern chamois)
        - *R. p.* pyrenaica (Pyrenean chamois)
        - *R. p. parva* (Cantabrian chamois)
        - *R. p. ornata* (Apennine or Abruzzo chamois)

Figure 4.1. Southern chamois taxonomy (adapted from Shackleton *et al*., 1997).

4.1.2. Status

Southern chamois population is estimated in more than 55000 individuals, with approximately 16000 Cantabrian chamois and 40000 Pyrenean chamois. Nevertheless, the Apennine chamois is considered vulnerable, with a population of only 350-400 individuals (Lovari, 1985; Blanco, 1998; Shackleton *et al*., 1997). During the second half of the XIX century and last century Northern chamois has been introduced in different countries (Norway, 1862; New Zealand, 1907 and 1913; France, Germany, Argentine, Russia and Poland) (Catusse *et al*., 1996). Distribution of Southern and Northern chamois is showed in Figure 4.2.

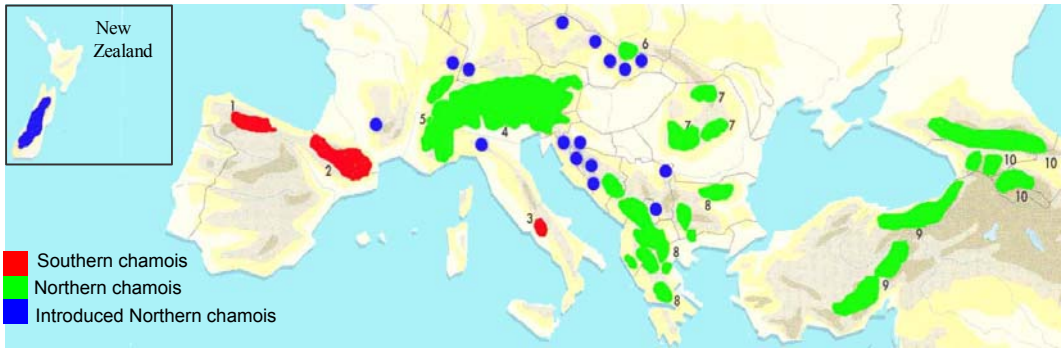


Figure 4.2: Chamois distribution (adapted from Catusse *et al*., 1996).

4.1.3. Morphological features

Southern chamois is smaller than Northern chamois, and different biometric measurements have been reported for both species (Delaunay, 1982; Pflieger, 1982; Poulain, 1990; ANCGG, 1992) (Table 4.1).

Table 4.1. Ranges of morphological measures for Southern and Northern chamois

		Southern chamois	Northern chamois
Total length (cm)		100 – 110	110 – 140
Height at the withers (cm)		69 – 75	70 – 85
Weight (kg)	Female	20 – 32	24 – 38
	Male	25 – 40	30 – 50 (max. 62)

Body weight also varies depending on subspecies, season, habitat, sex and age (Pflieger, 1982). Both sexes wear horns, which are black and straight finishing in a hook directed caudal and ventral. After the birth Southern chamois develops deciduous teeth which are progressively replaced by definitive teeth. Deciduous dental formula is (I 0-0/4-4, P 3-3/3-3) and the definitive one, appearing complete approximately at the age of 45 months is (I 0-0/4-4, P 3-3/3-3; M 3-3/3-3) (Pflieger, 1982; Ladini, 1990; Sáenz de Buruaga *et al*., 1991). Southern chamois can live up to 25 years in captivity, although it is difficult to find free-ranging individuals older than 15-18 years (Ladini, 1990).

4.1.4. Sex assessment

Sexual dimorphism is not very evident in Southern chamois, and different characteristics of the animal must be taken into account together, since a single character will not ascertain the sex with absolute certainty (Ponti, 1992; Catusse *et al*., 1996).

General aspect: Male is more heavily-built than female, with a deeper thorax. Width of the neck in the male is longer than length of the mandible, whereas in females the opposite relationship can be observed (Catusse *et al*., 1996) (Figure 4.3).

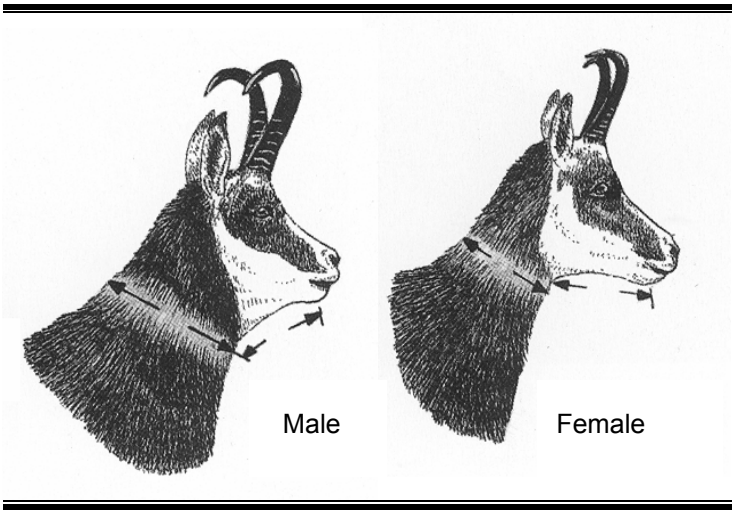


Figure 4.3. Neck and head in Southern chamois male and female (Catusse *et al*., 1996).

Horns: Male wears strong oval section horns with a more acute angle than female, whose horns are thinner and round sectioned (Sáenz de Buruaga, *et al*., 1991; Ponti, 1992) (Figure 4.4).

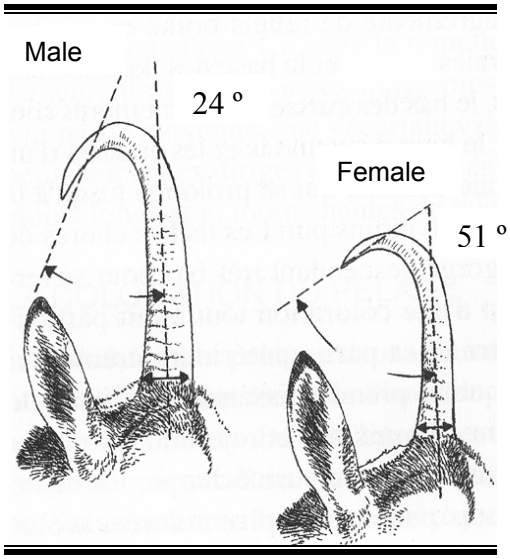


Figure 4.4. Differences in the angle between male and female horns (Catusse *et al*., 1996).

Coat: Winter coat is more contrasted in male than in females. Adult males (four years or more) show preputial hair and a mane over the dorsal midline, which is particularly evident in winter (Sáenz de Buruaga *et al*., 1991; Catusse *et al*., 1996)

Behaviour: The most evident behavioural difference between sexes is urinating position. Male urinates among its four legs, whereas female flexes its hindlimbs and urinates backwards, as shown at Figure 4.5 (Ladini, 1990; ANCGG, 1992).

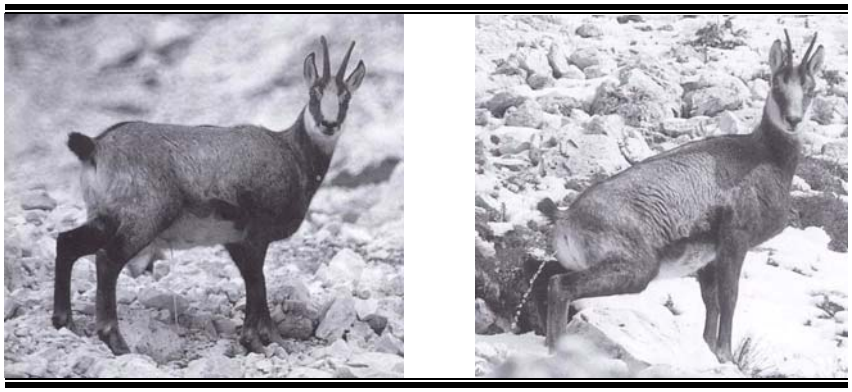


Figure 4.5. Urinating position in Southern chamois male and female (Ladini, 1990).

4.1.5. Age assessment

Horns: With the animal captured, horns are the main and easiest way to determine the age of Southern chamois, since each winter produces a ring which can be counted (Pérez-Barbería, 1994). Each growth segment represents a year in the life of the animal. First year is located in the curvature of the horn and usually it is difficult to observe. The segment of the second year is the longer one, and after it segments decrease in length up to the fifth year. The other segments are shorter, with one to three millimetres of length (Schröder and von Elsner-Schack, 1985) (Figure 4.6).

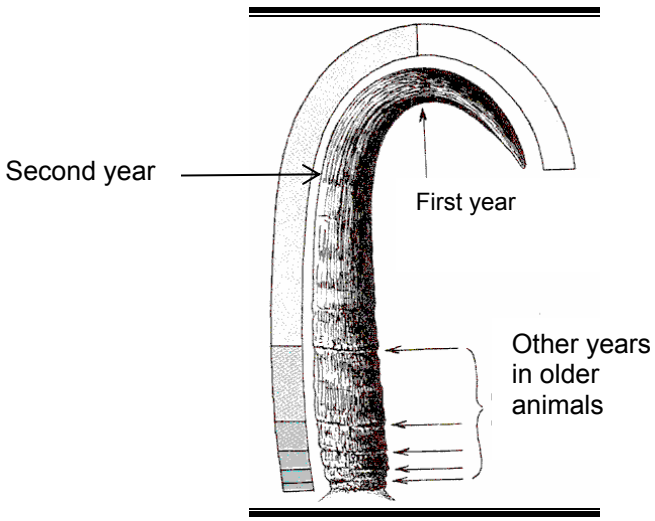


Figure 4.6. Assessment of age in Southern chamois through the horn (Catusse *et al*., 1996).

Dentition: Age of Southern chamois can also be assessed through the dentition, although it can be used only until 44-45 months of age and there is a considerable overlap in the apparition of new teeth (Ladini, 1990; Sáenz de Buruaga *et al*., 1991; Catusse *et al*., 1996). If it is not possible to capture the animal, broad age classes can also be established through general morphological criteria (Pflieger, 1982; Sáenz de Buruaga *et al*., 1991).

- Kid (< 1 year): smaller than adult, short horns almost without hook which attain half the length of the ears.
- Young (1 to 4 years): thin body, with forelimbs longer than chest, hooked horns which do not exceed the length of the ears.
- Adult (4 to 9 years): heavily-built body, forelimbs short when compared with thorax, mane and preputial hair in males, horns longer than ears.
- Old (more than 9 years): heavy and depressed impression, greyish coat, preputial hairs long and evident in males (up to 12 cm.).

4.1.6. Habitat and habitat use

Southern chamois inhabits alpine environments, mountain forests and subalpine pastures between 800 and 2600 metres. Altitude is not as important as orography for Southern chamois, since steep slopes are considered as secure refuges which proportion a fast flight. Lower altitude distribution limit is not related to biological reasons but rather to anthropogenic pressure (Ladini, 1990; Catusse *et al*., 1996).

Southern chamois moves from alpine pastures in summer, to obtain higher quality feeding, to lower altitudes in winter, when snow supposes an increase in energetic requirements, not only to keep body temperature, but also to move through their home range (Catusse *et al*., 1996; Blanco, 1998). In winter, Southern chamois look for places free of snow, establishing ‘refuge points’ from where they make short movements to feed (ANCGG, 1992; Catusse *et al*., 1996) (Figure 4.7). Females use higher altitudes and areas closer to escapes than males, probably as a protection for the kids (Pérez-Barbería, 1994). Camping area of Southern chamois oscillates from 20 to 100 hectares in males to 50 to 500 hectares in females, and is bigger in summer than in spring (ANCGG, 1992). Nutritional needs of female are bigger than those of males throughout most of the year except in the rut (autumn), in winter and spring due to gestation and in summer due to lactation and intraspecific competition, since they are more gregarious than males (Catusse *et al*., 1996).

Social organization of Southern chamois is characterized by sexual segregation except during the rut (Catusse et al., 1996). This is a gregarious species, and females, kids and young up to two years form herds. Young males also can form groups, although

normally males older than two years are solitaries (Ladini, 1990; Catusse et al., 1996). During rut, males join female groups, expel young males from the group and try to keep females grouped in a small area which they defend from other males (ANCGG, 1992).

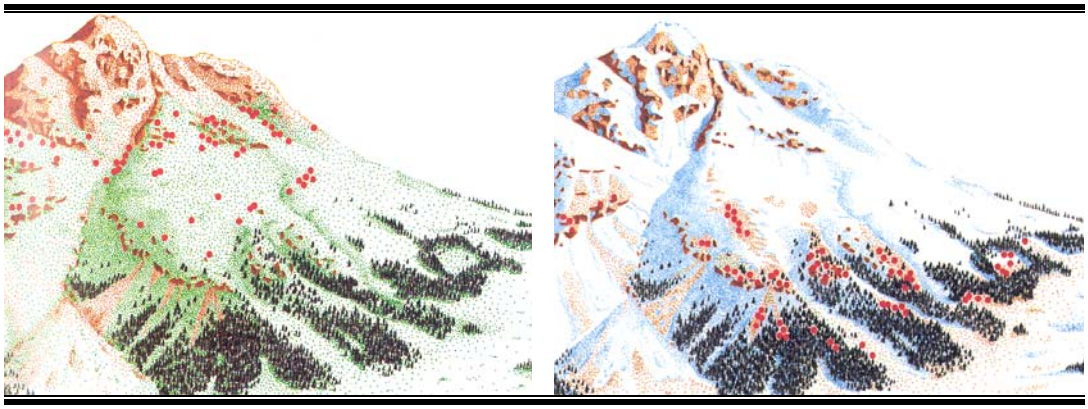


Figure 4.7. Summer and winter distribution of Southern chamois (Catusse *et al*., 1996).

4.1.7 Feeding

Southern chamois is herbivorous and feeds predominantly on herbaceous plants (grass and pulses), although up to 300 different plant species have been described in its diet (Catusse *et al*., 1996). Diet of Southern chamois is composed of herbaceous vegetables (50%), woody vegetables (35%), half woody vegetables (10%) and forest fruits (5%) (ANCGG, 1992) Transition from herbaceous diet from spring to fall to fibrous diet in winter provokes an important but progressive change in the size of the rumen, extremely small in winter (Marco *et al*., 1992). Southern chamois also feeds salt from rocks or that placed by stockbreeder for domestic animals or hunters to attract them (Catusse *et al*., 1996).

4.1.8 Reproduction

Southern chamois reaches sexual maturity at 18-20 months of age, although age of female at first delivery varies from three to five years and depends on population density. Population birth rate is also inversely proportional to population density, oscillating between 60 and 90% (Pflieger, 1982; Catusse *et al*., 1996; Blanco, 1998).

Rut takes place from the end of October to the beginning of December. Gestation takes 160 to 185 days and births are singles, concentrated between May 15th and June 15th. The newborn weights between 1.7 and 2.5 kg and attains 50 cm of length and 35 cm of height at the withers (Blanco, 1998; Catusse et al., 1996). Lactation lasts until September (Pflieger, 1982).

Mortality rate of the kids oscillates between 30 and 50% in normal conditions, although it can reach up to 70% in cold years (Catusse et al., 1996). Birth rate decreases and survival of kids increases with age of females, reaching 100% in 14 year-old females (Crampe, personal communication).

4.1.9. Population management

Optimal density for Southern chamois populations have been proposed to be 4-6 individuals/100 Ha in low altitude mountain environments, 8-10 individuals/100 Ha in medium mountain habitats (1700-2500m) and 5-8 individuals/100 Ha in alpine habitats (>2500 m). Sex-ratio oscillates between 1.5 and 2.5 females per male, due to higher game pressure on males. Lack of natural predators of Southern chamois makes population management rely almost exclusively on game management, although winter availability of food is also a natural regulating factor (ANCGG, 1992; Blanco, 1998). Game management can be calculated depending on the desired evolution of the population (Catusse et al., 1996) (Table 4.2):

Table 4.2. Population percentage to be hunted according to game planning (ANCGG, 1992)

Plan	Objective		Procedure
Quantitative	Stabilizing population		Hunting 12-15% of the population
Qualitative	Keeping sex and age balance	< 1 year: 20-25%	10-20% of animals hunted 1.2-3% of population
		2-3 years: 20-25%	40-50% of animals hunted 4.8-7.5% of population
		4-9 years: 40-45%	10% of animals hunted 1.2-1.5% of population
		> 9 years: 15-20%	30% of animals hunted 3.6-4.5% of population

4.2. CAPTURE METHODS

Capture can be another way to manage wild animal populations. Wild animal capture is not a purpose itself, but a way to carry out scientific studies (related to health, behaviour or population), to mark or translocate animals, to treat diseases or to obtain biological samples (Berducou, 1993; Pérez *et al*., 1997). In spite of being rather a frequent practice, capture is not a routine standard operation. Capture method must be chosen for every particular case, depending on animal density, environment, climatology, capture purpose, personnel available and experience of the capture group, and taken into account some general factors of each capture method, like safety and welfare of workers and animals, performance,

specificity, viability, total cost and cost per animal, among others (Jessup *et al*., 1988; Berducou, 1993; Gibert, 1993). For all those reasons, wild animal capture and handling has become an entire field of research endeavour (Tomkiewicz, 1982).

Capture methods can be classified as physical or chemical methods.

4.2.1 Physical methods

Physical methods are more effective than chemical methods to capture wild ungulates (Marco, 1995). Most used physical methods include:

- Box-trap. These are metal or wooden boxes of different sizes, with two doors better than one. A mechanism closes the door when the animal enters the trap. Traps can be baited, normally with salt but also with food for wild ungulates, to attract the animals to the box (Marco, 1995). The success of this technique depends on animal nutritional needs, and therefore will be more effective in cold winters (Jones, 1984).

- Corral traps. These are net, wire or wooden fences forming an enclosure, which are baited to attract the animals inside them. A hidden operator closes manually or by remote control the door of the enclosure when the animals to be captured are inside the trap. Animals are then immobilized by means of hand nets or teleanaesthesia. Entrance of the trap can be communicated with a cone-shaped passage. Different models of this trap have been used in Africa, where it is called ‘boma’ (Jones, 1984; Planton, 1993; Marco, 1995).

Both box and corral traps require heavy and difficult to transport material, and motor vehicles are needed. For this reason they are used mainly in extensive zoological collections, although they also can be used with free-ranging animals (Jones, 1984; Marco, 1995).

- Snares. These are nylon wires which close around the limb of the animal when it steps on the snare. They have a limit which prevents the strangulation of the limb. Snares are placed on the ways normally used by the animals (Marco, 1995). The use of elastic materials prevents the development of lesions (Struch and Baumann, 2000).

- Cannon nets. They are used in open areas baited with salt or food. Nets are shot from the floor over the animals by means of a system of cannons, trapping the animals beneath it (Marco, 1995).

- Drop-nets. These are square or round nets holding from 3-4 meters high poles over a plain and free of obstacles area. The area beneath the net is baited to attract the animals and when the animals to capture are beneath the net it is activated to fall on them. Animals trapped under the net must be unwrapped fast and effectively (Jones, 1984).

- Descending nets. These are nets wrapped at two meters of height by a wire holding from four or more poles, forming an enclosure. It is also baited to attract animals to the enclosure, and when animals are inside the area limited by the nets they are unwrapped and fall, closing the enclosure. Animals entangle into the net when they try to escape, and they must be rapidly unwrapped to avoid any complication (Montané, 1999).

- Drive-nets. These are 2-2.5 meters high per 50 meters long nets which can be connected to attain the length required. It is loosely fixed from poles, bushes or trees each 6-10 meters, and held down to the floor. It is important that enough net is laid on the floor to form a pocket in the direction the animals will run, so they will get entangled and will not be repelled by the net. A line of beaters tries to direct the animals towards the net, whereas another group of operators remains next to the net to restraint and unwrapped the animals once they are entangled, preventing them to escape or to be hurt (Jones, 1984; Marco, 1995).

- Hand nets. These are small nets held by one or two operators which pursuit the animal until it entangles into the net. It is used with newborn animals or with animals kept in captivity or caught in corral traps (Jones, 1984; Marco, 1995; Fernández-Arias *et al*., 1993).

All these capture methods have been used to capture Southern chamois and/or Northern chamois (Berducou, 1993; Boillot, 1993; Catusse *et al*., 1993; Delmas, 1993; Fernández-Arias *et al*., 1993; Hansen *et al*., 1993; Peracino and Bassano, 1993; Catusse *et al*., 1994; Meneguz *et al*., 1994; Marco, 1995; Struch and Baumann, 2000).

Individual capture methods (box-traps and snares) do not need many operators, but they are not selective (Marco, 1995). Another disadvantage of these methods is that they need constant surveying to prevent an animal to be trapped unnoticed and get hurt, although this problem can be solved with transmitters activated by the trap (Montané, 1999; Struch and Baumann, 2000).

Collective capture techniques (corral traps and nets except hand nets) allow the capture of more than one animal simultaneously. These animals can belong to the same social group, which can be interesting for some scientific studies. Collective capture methods are

also more selective than individual ones, since the moment they are activated can be chosen. Their main disadvantage is that they need more operators and heavier and more complicated material, making its transport difficult and restricting their use (Marco, 1995).

Physical capture methods described up to now are those used more commonly in Europe, but other capture methods have been used in those geographical areas with large populations of wild ungulates, like North America and Africa. Most spectacular are those employing helicopters to pursuit animals, either to direct them towards a net or an enclosure, to anaesthetize them with rifle, to shoot over them a net with a rifle specially adapted (net-gun) or even to jump over the animals directly from the helicopter (Tomkiewicz, 1982; Sullivan *et al*., 1991; Chardonnet, 1993; Planton, 1993). In a study comparing five different methods to capture bighorn sheep (*Ovis canadensis*), nets shot from a helicopter ('net-gun') was found to be the best method. It is a versatile method which can be used with many ungulate species (Kock *et al*., 1987a; Jessup *et al*., 1988), although none of the methods studied are free of risk and ulterior studies indicate that survival of animals captured with this method can be worse than it seemed at first (Kock *et al*., 1987b and 1987c; Jessup, 1999). These techniques are not viable in our continent, since their cost is elevated, they do not suit our orography and animal density is considerably lower, but also due to cultural and social reasons.

Table 4.3. Characteristics of different capture methods (Berducou, 1993).

Parameter	Nets and enclosures	Box trap	Snares
Staff safety	Good	Good	Good
Animal safety	Critical	Good	Variable
Specificity	Medium	Medium	Medium
Performance	Good	Low	Medium
Capture of groups	Easy	No	Possible
Sex and age selectivity	No	Scarce	No
Time of preparation	Medium	Bad	Excellent
Adaptability	Medium	Bad	Excellent
Investment	High	Medium	Low
Few operators	No	Possible	Possible
Time of work	Variable	Good	High
Showiness	High	Low	Medium
Discretion	Bad	Medium	Good
Time of the year	All	Summer	Winter

Capture methods may be assessed through different parameters listed in Table 4.3 (Berducou, 1993):

- Safety for the operators: Safety is similar for all the methods mentioned. A general advice is the use of gloves to avoid hurts in the hands caused by the horns.

- Safety for the animals:

  - Box trap is the safest technique, with a 3.3 % mortality rate reported. Cause of death is normally related to traumatism (bleeding due to horn loosing, vertebral lesions or loosing of teeth).

  - Snares mortality has been reported to be 5.8%, and it is related to asphyxia. Fractures and muscular damage can also arise, as well as freezing of the limb caught in the snare, which suggest the avoidance of this technique in intense cold periods (Catusse *et al*., 1994).

  - Nets and enclosures have the highest mortality reported, up to 12%. However, mortality rate varies depending on the type of net. Nets not surveyed constantly provoke up to 21% of deaths, whereas mortality in drive-nets and enclosures controlled by an operator can be as low as 3%. Death is usually related to neck fracture when the animal hits the net or asphyxia if the animal remains wrapped and unattended in the net with the neck twisted. Capture myopathy and shock due to stress have also been reported in animals captured with nets (Berducou, 1993; Meneguz *et al*., 1994).

- Specificity. Only operator-activated enclosures are completely specific for the species to be captured. Unwished captures have been reported for all the other methods.

- Performance. In general, performance depends on animal density, but there is controversy about the existence of statistical relationship between animal density and number of animal captured (Berducou, 1993; Meneguz *et al*., 1994). Drive-net is the most effective method in terms of days of capture to obtain an animal (0.67 days/chamois), followed by snares (2 days/chamois) and box -traps (2.94 days/chamois). If net is not combined with a battue, efficacy of nets is much lower, quite similar to that of box-traps (2.6 days/chamois) (Berducou, 1993).

- Capability to capture groups. Drive-nets and enclosures are the most adequate methods to capture groups, either, socially related or not.

- Sex and age selectivity. Enclosures are the most selective method. Nets, box and snares can not be selective, and sex ratio of animals capture will depend on the area the traps are placed.

- Seasonality. Nets and enclosures can be used almost all year round. Box traps are normally used form May to September and snares are more effective in winter (November to March), although they must be disconnected during intense cold.

- Cost. Nets and enclosures have the highest initial investment, whereas box-trap has a moderate cost and snares are the cheapest method. To keep costs reasonably low, collaboration of volunteers is an option in those methods requiring many personnel (Meneguz *et al*., 1994). Box traps and snares need not as much personnel as drive-nets and enclosures.

4.2.2. Chemical methods

Anaesthesia is a highly specific and selective method of individual capture. Chemical immobilization is particularly useful to capture aggressive or very stressful species, although anaesthesia does not prevent completely stress and capture myopathy (Harthoorn, 1982; Gauthier, 1993). Stress produced by chemical capture can be as intense as or even stronger than that related to physical capture, due to previous pursuit to inject the anaesthesia and subsequent pursuit to find the sedated animal (Jessup, 1999). When dealing with wild animals, anaesthesia has an added risk, since it is difficult to calculate the weight of the animal, we do not know its health status and adverse effects of the drug may arise. Anaesthetizing physically captured animals to diminish their stress can suppose an increased risk, since they are already stressed and anaesthetic may compromise furthermore their vascular and respiratory function, so this practice is not recommended and caution must be taken (Jones, 1984; Kock *et al*., 1987c; Klein *et al*., 1993; Ebedes and Raath, 1999).

Use of teleanaesthesia is limited due to a number of reasons. Although shot distance range for the equipments used can attain up to 80-120 meters, impact strength and accuracy limit the effective range of most equipments to 30-60 meters (Fowler, 1986; Gauthier, 1993; Nielsen, 1999). Teleanaesthesia is rarely used with free-ranging animals in Spain, but in other European countries its use is more extended, either due to a lower flight distance of animals in areas where hunting is forbidden or because physical capture methods itself are also forbidden, like in Austria (Montané, 2002).

Anaesthetic, dose and administration method depend on a series of factors that must be studied and taken into account for each anaesthetic procedure. These factors include species, sex, age, weight, body condition, physiological status, degree of excitement, shot distance, flight distance, season, weather, time of the day, orography, risk, and capture equipment available (Fowler, 1995; Kreeger, 1997; Nielsen, 1999).

The use of many different drugs has been reported to capture and anaesthetize wild animals, but the perfect drug is still to be discovered. Ideal anaesthetic should (Fowler, 1995):

- Have a high therapeutic index (lethal dose/effective dose).
- Be compatible with other drugs.
- Not irritate muscle tissue.
- Have a short induction period (currently it oscillates between 10 and 20 minutes).
- Have an available antagonist or reversal agent.
- Remain stable in solution without refrigeration.
- Have an effective dose low enough to be used in the small volume syringe necessary for dart injection.

4.3. HAEMATOLOGICAL AND SERUM BIOCHEMICAL REFERENCE VALUES

Haematological and serum biochemical values are well known for most domestic species, but there is a lack of knowledge about them for many wild species (Ursache *et al*., 1980; Kocan *et al*., 1981; Peinado, 1994; Lastras *et al*., 2000). Moreover, normal physiological data of free-ranging animals are difficult to establish, due to the effects of capture stress on haematological and biochemical parameters (Gibert, 1993). However, the development of new telemetric sampling techniques allows the collection of blood samples reducing the stress caused to the animal, thus obtaining values more similar to true physiological values (Wilklund *et al*., 1994, Waas *et al*., 1999, Säkkinen *et al*., 2004). To establish a reference interval it is necessary to fulfil some requirements (Solberg, 1999; Walton, 2001):

- Randomly selected reference individuals are needed to establish a reference interval. Reference individuals are healthy individuals which meet all the features of the population the reference interval is intended for.

- Sample size is one of the most important parameters, determining the percentage of the population that will be included in the interval obtained.

Distribution of biological data is asymmetric and biased, and data dispersion increases due to high variability associated to stress (Knox *et al*., 1992; Solberg, 1999). Over 60 reference individuals for parametric analysis or 120 for nonparametric analysis, influence of increasing number of observations starts to decrease (Lumsden, 1998). However, since biological parameters show high variability, a minimum of 120 individuals is necessary to establish an interpercentile reference interval, although required number of individuals depends on the desired percentage of the population to be included in the interval and the probability to include such percentage, as expressed in Table 4.4 (Walton, 2001). If less than 40 individuals are used to establish the reference interval and there are no outliers, best approach to 95% central percentile limits (percentiles 2.5 and 97.5) are the lowest and highest values observed, respectively (Lumsden, 1998).

Table 4.4. Sample sizes for tolerance intervals (Walton, 2001).

Probability of including specified proportion	Proportion of population to be included between extreme values of reference data			
	0.50	**0.90**	**0.95**	**0.99**
0.90	7	38	79	410
0.95	8	47	97	490
0.99	11	66	135	690

Haematological and biochemical parameters may vary depending on the capture method used, so it must be considered when comparing values with the reference interval for the species (Kock *et al*., 1987b; Peinado *et al*., 1993; Marco and Lavín, 1999). It has been suggested that separated reference values should be defined for sedated and non sedated animals (Cross *et al*., 1988). Drive-nets have been widely used in different countries in Europe to capture both Southern and Northern chamois, so reference values for this capture method are needed in order to monitor captured animals and will be very useful to better understand physiological changes induced by physical capture (Berducou, 1993; Catusse *et al*., 1993 and 1994; Fernández-Arias *et al*., 1993; Meneguz *et al*., 1994).

Establishment of reference intervals for haematological and serum biochemical parameters in a species does not determine its diagnostic value, since changes in one parameter may have different meanings in different species. A number of factors can affect these parameters (captivity, capture method, sex, age, season, physiological and nutrition status), and a conservative interpretation of the results obtained is recommended until more information on dynamics of these parameters in the new species is obtained (Williams *et al*., 1992; Walton, 2001). It has been suggested that separated reference values should be defined for sedated and unsedated animals (Cross *et al*., 1988).

Information about values for haematological and biochemical data for the Genus *Rupicapra* is scarce, particularly for Southern chamois. Only protein values of shot dead animals have been given for this species (Lastras *et al*., 2000). Some haematological and biochemical values have been described for Northern chamois, but are heterogeneous and proceed from animals in different situations of captivity or which have undergone different capture operations, which induces a variation of both haematological and biochemical parameters (Artois, 1987; Gibert, 1993; ISIS, 2002).

4.4. STRESS

Many definitions have been proposed for stress (or stress response). It has been defined as an unspecific reaction of the organism which has been exposed to a stressor (Selye, 1973), as an internal stimulus that initiates an adaptive change or a stress response in an animal (Breazile, 1987) and as the cumulative response of an animal resulting from interaction with its environment via receptors (Fowler, 1995). However, since stress was first used in biology in 1935, an acceptable definition has not been established yet (Cannon, 1935; Moberg, 1987). Failure to establish a clinical objective definition of stress comes from the inability to solve four major problems (Moberg, 1987):

- Determining the best biological value to measure stress.
- Lack of unspecific response to all stressors.
- Interanimal variability in the biological responses to stress.
- Failure to establish a correlation between stress measures and a meaningful impact on the animal's wellbeing.

Reaction of the organism to the contact with a stressor was first thought to be unspecific and identical regardless of the nature of the stressor. The reaction was described through a model called 'General Adaptation Syndrome' (G.A.S.), which included tree consecutive stages (Selye, 1946):

- *Alarm reaction*, during which defence mechanisms (physiological and biochemical) become active.
- *Resistance*, which is the stage of maximum adaptation of the organism through unspecific systemic changes.
- Exhaustion, when adaptive mechanisms collapse due to a prolonged stimulus.

However, although it has an unspecific component, variation of stress response depending on the stimulus was later demonstrated (Mason, 1971). Behavioural and central nervous system (CNS) factors, as well as predictability of the stimulus, also influence stress response (Mason, 1968 a and b; Weiss, 1972). Stress response was later divided in three phases different to those defined previously (Kagan and Levi, 1974):

- Perception of a stimulus as a threat for the homeostasis.

  Perception of a stimulus as a threat depends both on the nature of the stimulus and on the individual (Dantzer and Mormède, 1983; Wiepkema and Koolhas, 1993).

- Stress response itself.

  Recent research emphasizes the role of the Corticotrophin-Releasing Hormone (CRH) in the stress response (Dunn and Berridge, 1990; Taché and Rivier, 1993). CRH is secreted in the hypothalamus and the amygdale, and its presence in the CNS activates the behavioural response and the sympathetic-adrenal medulla (SA) and the hypothalamic-pituitary-adrenal cortex (HPA) axes, responsible of the behavioural and physiological components of the stress response, respectively (Chappell *et al*., 1986; Stratakis and Chrousos, 1997) (Figure 4.8).

  The behavioural response is the simplest way to avoid the stimulus, but if it is insufficient to alleviate the stress, then the animal activates two different physiological systems: the autonomic nervous system (SA) and the neuroendocrine system (HPA) (Moberg, 1987; Verde and Gascón, 1987).

  CRH activates the sympathetic autonomous nervous system which induces the release of catecholamines (epinephrine and norepinephrine) from the adrenal medulla and norepinephrine from postganglionic neurones of the sympathetic system. Adrenal medulla catecholamines secretion initiates one or two second after the stimulus is perceived, and catecholamines have a very fast catabolism and, consequently, a very short plasma half-life (McCarty, 1983; Ganong, 1998). Effects of catecholamines on the organism are listed in Table 4.5.

  CRH also provokes the secretion of adrenocorticotropic hormone (ACTH) from the hypophysis (Oliverio, 1987; Guyton and Hall, 2000). ACTH stimulates corticosteroid secretion from the adrenal cortex. Effects of corticosteroids on the organism are listed in Table 4.6. Circulating corticosteroids control ACTH and CRH

secretion through negative feed-back (Guyton and Hall, 2000). β-endorphin is released with ACTH (both derivate from the pro-opiomelanocortin - POMC -) (Guillemin *et al*., 1977; Rossier *et al*., 1977). β-endorphin is an endogenous opioid responsible of stress-induced analgesia (Hughes *et al*., 1975).

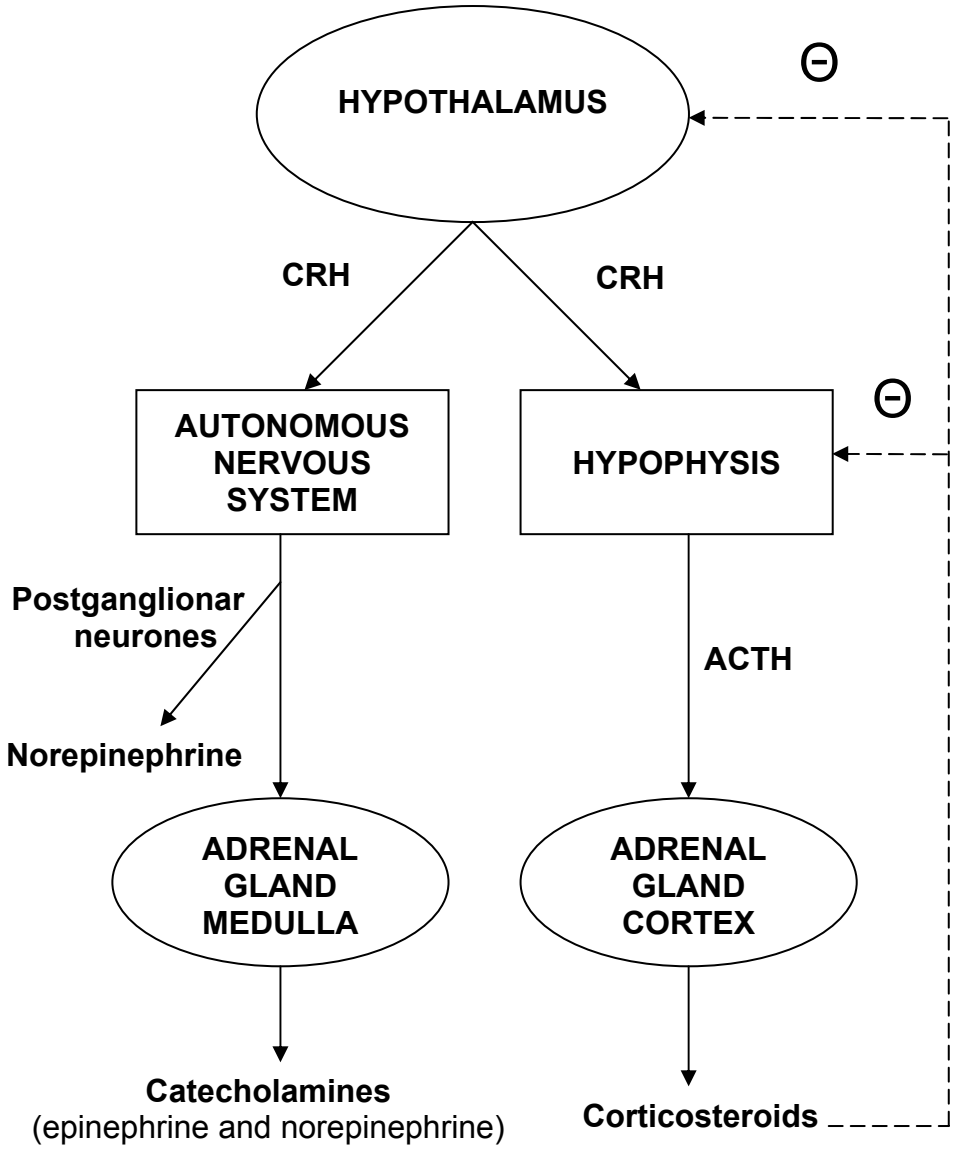


Figure 4.8. Activation of SA and HPA axes by CRH.

- Biological consequences of stress.

Biological consequences of stress arise due to changes in the biological functions caused by the hormones released in these two axes (Ganong, 1998;

Guyton and Hall, 2000). Biological consequences of stress have an adaptive purpose, intending to keep homeostasis balanced (Verde and Gascón, 1987; Fowler, 1995). However, they may lead to the development of a prepathologic state and, eventually, to a pathologic state. This prepathological state, when biological functions change but negative consequences of stress are still to arise, has been proposed to clinically define stress (Moberg, 1987). As a result of the impairment of biological functions due to stress, consequences for the animal well-being, health and life can arise (Kagan and Levi, 1974; Moberg, 1987; Verde and Gascón, 1987). Negative consequences of stress most commonly described include shock, trauma and capture myopathy in acute stress and immune system depression in chronic stress (Knottenbelt, 1990; Verde and Gascón, 1987; Verde and Gómez, 1987; Gibert, 1993; Fowler, 1995).

Table 4.5. Effects of catecholamine on the organism (Verde and Gascón, 1987).

Biochemical effects:
• Increase of glycogenolysis (increasing glycaemia, muscular lactic acid and oxygen consumption).
• Increase hepatic glycogenesis.
• Increase lipolysis (increase circulating non-sterified fatty acids (NEFA).
Vascular and muscular effects:
• Increase blood pressure and heart contractibility rate and strength.
• Dilate coronary arteries.
• Increase blood flow in skeletal muscle and viscera (brain, liver).
• Decrease blood flow in the skin.
• Smooth muscle: vasodilatation (β_2 receptors), vasoconstriction (α receptors), dilatation of the bronchial tubes, erection of the hair, relaxation of smooth muscle except in sphincters (digestive system and uterus).
Respiratory effects:
• Increase respiratory frequency and depth.
CNS effects:
• Euphoria, anxiety, fatigue and restlessness.
Effects on hypophysis:
• Release of ACTH and TSH

However, physiological changes registered during the stress response may not be harmful to the animal, but help him to cope with a potentially dangerous situation (Broom and Johnson, 1993; Guyton and Hall, 2000; Montané, 2002).Three different types of stress have been defined depending on its effects on animal welfare (Breazile, 1987):

- Eustress involves physiological responses which help the animal to cope with a potentially threatening situation
- Neutral stress is neither harmful nor helpful
- Distress evokes harmful responses capable of inducing pathologic changes.

Generally, distress only appears when the stimulus persists and stress response reaches its exhaustion phase, or when the stimulus induces directly discomfort or pain (Breazile, 1987).

Table 4.6. Effects of corticosteroids on the organism (Verde and Gascón, 1987).

Effects on carbohydrate metabolism:
• Stimulate hepatic glycogenesis.
• Prevent the uptake of glucose by peripheral tissue, increasing glycaemia.
Effects on protein metabolism:
• Stimulate hepatic synthesis of proteins.
• Increase proteolysis in peripheral tissues.
• Increase non protein nitrogen.
Effects on lipids metabolism:
• Increase lipolysis.
• Increase fat mobilization form reserves.
• Increase triglyceride synthesis in the liver.
• Increase lipemia.
Effects on electrolytic balance:
• Increase extra cellular sodium, chloride and bicarbonate concentrations.
• Decrease calcium, phosphorus and potassium concentrations.
Effects on erythropoietic system:
• Increase erythropoiesis.
• Decrease circulating lymphocytes and eosinophils.
• Increase circulating neutrophils.
Effects on bone system:
• Induce osteoporosis.
• Inhibit paratiroid hormone (PTH).
Effects on CNS:
• Inhibit gamma-amino-butyric acid (GABA) synthesis, increasing irritability of the individual.
Effects on inflammatory and immune activity:
• Stabilization of lysosomes.
• Prevent leukocyte diapedesis and phagocytosis.
• Decrease circulating lymphocytes.
• Limit prostaglandins formation.
• Decrease activity of fibroblasts and granuloma formation.
• Increase capillary tone and selective permeability.
Other effects:
• Increase pepsinogen and hydrochloric acid production in the gastric mucosa
• Increase pancreatic trypsinogen production.
• Inhibit ACTH and CRH secretion.

Although these physiological responses are common in the stress response, individual variability in the stress response associated to differences in the character of the animal has been postulated (Manteca and Deag, 1993; Erhard *et al*., 1999; Thodberg *et al*., 1999).

Stress response has an additive effect, which means that different stressors together can have the same effect that a single stressor of a higher intensity (Curtis, 1993). This feature is particularly important during wild animals capture, handling and transport, since animals are exposed to a number of stressors in a relatively short time.

4.4.1. Transport

Transport has been reported to be a more stressful stimulus than physical restraint (Montané, 2002). However, only limited information exists on the effects of transport in wild animals, which may react to handling in different ways than domestic species (Brelurut *et al*., 1991; Waas *et al*., 1997 and 1999; Montané *et al*., 2002)

Heart rate has been described both to decrease due to habituation and to increase due to movement during transport (Horalek and Jones, 1993; Waas *et al*., 1997 and 1999; Grigor *et al*., 1998). Increases in haematocrit have been related to dehydration in long transports with water deprivation, and increases in serum CK activity were attributed to physical demand during transport (Grigor *et al*., 1998). Differences in lactate and sodium evolution over time during transport have been reported, with some authors finding increases or decreases in their concentrations and other authors observing no changes during transport (Marco *et al*., 1997; Waas *et al*., 1997; Grigor *et al*., 1998). Serum cortisol concentration has been described to increase during transport, and it has also been reported to stabilize during transport after a 30 minute initial increase (Hartmann, 1988; Sartorelli *et al*., 1992; Waas *et al*., 1997; Grigor *et al*., 1998; Anderson *et al*., 1999a).

Overall, little is known about the physiological and psychological reactions of animals to transport (Waas *et al*., 1999). Contradictory results have been attributed to different type of road (Montané *et al*., 2002). However, differences in transport conditions, pretransport handling and duration of transport, it is, experimental design, could better explain contradictory results found in so many parameters.

4.5. STRESS RESPONSE ASSESSMENT

As aforementioned, assessing stress in an objective clinical way is a problem still unsolved. Many studies describe changes in biological parameters when capturing and handling wild ungulates, trying to detect the best indicators of stress, assess animal welfare and survival, and determine the less stressful way to capture and manipulate them (Franzmann and Thorne, 1970; Hyvärinen *et al*., 1976; Mautz *et al*., 1980; Rehbinder and

Edqvist, 1981; Kock *et al*., 1987a; Kock *et al*., 1987b; Kock *et al*., 1987c; Cross *et al*., 1988; Hattingh *et al*., 1988; Hattingh *et al*., 1990; Kock *et al*., 1990; Brelurut *et al*., 1991; Gibert, 1993; Horalek and Jones, 1993; Diverio *et al*., 1996a and b; Meneguz *et al*., 1996; DeNicola and Swihart, 1997; Goddard and Grigor, 1997; Marco *et al*., 1997; Waas *et al*., 1997; Grigor *et al*., 1998; Marco *et al*., 1998; Kock *et al*., 1999; Marco and Lavín, 1999; Waas *et al*., 1999; Read *et al*., 2000). However, it is difficult to establish a stress response pattern for every species and stressor, and even to identify the health and welfare meaning of the changes found in the biological parameters analyzed (Moberg, 1987; Rushen, 1991; Wiklund *et al*., 1994). Thus, stress indicators have to be established for each species and stressor, due to stress response variability.

Although behaviour is a good indicator of chronic stress (Broom and Johnson, 1993), it is difficult to assess in physically restrained animals, and presents the same problems of physiological parameters (Rushen, 1991). Most of the research directed to determine the stress of capture and handling in wild ungulates relies on the analyses of clinical (heart rate and body temperature), haematological and biochemical parameters. However, physiological measures also present problems to assess reactivity (Manteca and Deag, 1993):

- It is difficult to obtain meaningful measures of some physiological parameters.
- It is difficult to choose a relevant parameter.
- It is difficult to isolate the effect of the cause intended to study on the parameter analysed from other causes, which may have confounding effects.

Most recent studies used telemetric devices to obtain blood samples and to register heart rate or body temperature, avoiding the effect of stress due to handling and restraint (Wiklund *et al*., 1994; Moe and Bakken, 1997; Bakken *et al*., 1999; Waas *et al*., 1999). Alternative non-invasive techniques to assess stress and welfare in domestic and wild animals are also being developed, based mainly in cortisol and cortisol metabolites detection in faeces or saliva (Fell *et al*., 1985; Greenwood and Shutt, 1992; Monfort *et al*., 1998; Möstl *et al*., 1999; Denhard *et al*., 2001).

However, variations in the parameters studied are difficult to correlate a meaningful change in animal well-being. Variability for every species, individual and stressor makes it difficult to determine a single indicator for every circumstance (Moberg, 1987; Rushen, 1991; Wiklund *et al*., 1994). A different response in one or more physiological variables to that described for the same species and stressor is a poor prognostic indicator, meaning animal well-being and life are threatened (Knox *et al*., 1992). Thus, description and establishment of 'normal' physiological changes for each stressor and species is necessary, in order to be

able to detect deviations of any variable which could mean the development of a prepathologic state, as previously defined.

4.5.1 Heart rate

Heart rate has been widely used as an acute stress indicator, and it is considered an objective way to assess the autonomic nervous system response to stressors (Broom and Johnson, 1993; Hopster and Blockhuis, 1994). Catecholamines stimulation of β_1 adrenergic receptors causes an increase in heart contractibility and heart rate (Genuth, 1996), although in some species and in young ungulates stress may cause bradycardia instead of tachycardia (Hofer, 1970; Gabrielsen *et al*, 1977; Espmark and Langvatn, 1985). Nevertheless, physical activity and handling to detect heart rate can also increase it, which must be taken into account (Spodick, 1980; Broom and Johnson, 1993). Handling effect can be overcome with noninvasive telemetric methods (Hopster, 1998; Waas *et al*., 1999).

Heart rate has been used to assess stress in wild and domestic animals, but results have been contradictory (Franzmann and Thorne, 1970; Kock *et al*., 1987a; Horalek and Jones, 1993; Hopster and Blockhuis, 1994; Diverio *et al*., 1996a and b; DeNicola and Swihart, 1997; Waas *et al*., 1997; Grigor *et al*., 1998; Marco *et al*., 1998; Waas *et al*., 1999; Read *et al*., 2000). It has been proposed that heart rate variability could be a better indicator of individual capacity to respond to environmental demands and autonomous nervous system status than heart rate itself, since variability would indicate competition of sympathetic and parasympathetic effects on the sinusal node (Porges, 1985; Goldberger, 1991; Hopster and Blockhuis, 1994).

4.5.2. Body temperature

Body temperature is relatively stable in mammals, and is originated by muscular contraction, food assimilation and metabolism (Lusk, 1989). Increases in body temperature are considered a predictor of capture myopathy (Spraker, 1982). Temperature increases during capture, restraint and handling of animals, not only due to muscle contractions or physical activity but also due to stress-induced hyperthermia (SIH). Catecholamines increase basal metabolic rate by stimulating facultative thermogenesis, and corticosteroids also elevate body temperature, so SIH is related to the activation of the SA and the HPA axes (Trunkfield *et al*., 1991; Broom and Johnson, 1993; Groenink *et al*., 1994; Haskins, 1995; Genuth, 1996; Moe and Bakken, 1997; Ganong, 1998; Bakken *et al*., 1999). SIH is a fever mediated by prostaglandin E and interleukins 1 and 6 (Kluger *et al*., 1987; Briese and Cabanac, 1990; Kent *et al*., 1993; Le May *et al*., 1990). SIH is time dependent, and temperature increases to reach a stable high level (1 to 1.5 ºC higher than basal values) in

10 minutes to decrease and return to baseline after 60 to 90 minutes (Zethof *et al*., 1994; Moe and Bakken, 1997). Temperature increase is one of the most common problems found when capturing wild animals. It may lead to death and also plays an important role in the development of capture myopathy, so it must be carefully controlled throughout every wild animal capture and handling operations (Spraker, 1982).

Rectal temperature has been used to study stress response to capture, handling and restraint, trying to assess SIH (Franzmann and Thorne, 1970; Kock *et al*., 1987a and 1987b; Kock *et al*., 1990; Meneguz *et al*., 1996; Moe and Bakken, 1997; Marco *et al*., 1998; Bakken *et al*., 1999; Korhonen *et al*., 2000). Nevertheless, external temperature can decrease during a stress episode due to peripheral constriction, while core temperature is actually increasing (Duncan and Filshie, 1979). Moreover, rectal temperature is poorly correlated with core body temperature, and handling to measure rectal temperature can also elicit a SIH. Tympanic temperature has been proposed as an alternative measurement to study SIH (Drew, 1996; Moe and Bakken, 1997; Drew, 1998; Korhonen *et al*., 2000). Body temperature follows a diurnal fluctuation, which also must be considered when assessing animal welfare by means of body temperature (Broom and Johnson, 1993).

4.5.3. Blood parameters

Haematological and biochemical parameters most frequently analyzed in literature to assess stress response are hematocrit, red blood cell count and haemoglobin, within haematological parameters, and glucose, cortisol, total protein, urea, aspartate aminotransferase (AST), creatine kinase (CK) and lactate, within biochemical parameters. Cortisol has been used as a single indicator of stress (DeNicola and Swihart, 1997), although there is poor evidence that a change in cortisol must be noticeable whenever there is welfare problem, and it is still unknown whether it is the main daily levels or the secretion pattern of cortisol the responsible of its metabolic and immune consequences (Rushen, 1991). Moreover, it has been proposed that a combination of different haematological and biochemical parameters would be a better predictor of animal stress and welfare than cortisol alone (Kock *et al*., 1987b; Chapple *et al*., 1991; Knox *et al*., 1992). Catecholamines have a very short life once secreted in plasma, and their changes are only valuable in catheterized animals (Broom and Johnson, 1993).

Despite previous studies trying to relate changes in haematological and biochemical parameters and stress, lack of reference values for many species and differences in the capture method used make comparisons difficult (Kocan *et al*., 1981). Capture method (either physical or chemical), blood sampling method and effects of both methods on

haematological and biochemical parameters must be considered when using reference values (Wesson *et al*., 1979; Mautz *et al*., 1980; Kocan *et al*., 1981).

Haematology

Catecholamines released from the adrenal medulla due to sympathetic stimulation cause an increase in RBC, haemoglobin concentration and haematocrit. This effect is mediated by α-adrenergic receptors of catecholamines in the spleen capsule, inducing smooth muscle contraction and the release to circulation of erythrocytes stored in the spleen, and has been demonstrated in different species of wild ungulates. Spleen can store up to 25% of circulating erythrocytes (Turner and Hodgetts, 1959 and 1960; Hartwig and Hartwig, 1985; Cross *et al*., 1988; Chapple *et al*., 1991; Jain, 1993; Ganong, 1998). No increase in none of these three parameters is registered in splenectomized animals undergoing physical stress or injected with epinephrine (Walton, 1971; Cross *et al*., 1989; Jain, 1993). However, increases in plasma viscosity suggest that it could also be due, at least partially, to a reduction in plasma volume due to plasma passing from vessels to tissues (Wesson *et al*., 1979; Cross *et al*., 1988; Jain, 1993). Red blood cells stored in the spleen are bigger than circulating erythrocytes, since they mature in the spleen immediately after leaving the bone marrow (Wade, 1973; Cross *et al*., 1988; Peinado, 1994). So, MCV increases and, consequently, MCHC decreases, in physically captured and stressed wild ungulates (Wade, 1973; Cross *et al*., 1988; Kock *et al*., 1999). Lack of circulating reticulocytes in sedated or physically captured animals further demonstrates spleenorigin, and not bone marrow, of released erythrocytes (Hawkey, 1975; Cross *et al*., 1988).

Lower values for RBC, haemoglobin concentration and/or haematocrit have been previously described in sedated or anaesthetized wild ungulates (Wesson *et al*., 1979; Cross *et al*., 1988; Morton *et al*., 1993; Peinado *et al*., 1993; Marco and Lavín, 1999; Montané *et al*., 2002 and 2003). These lower values have been attributed to haemodilution, with concurrent decreases in total protein and fibrinogen concentrations and plasma viscosity, and spleen smooth muscle relaxation in sedated animals with the consequent splenic sequestration of erythrocytes (Turner and Hodgetts, 1960; Seal *et al*., 1972; Wesson *et al*., 1979; Kocan *et al*., 1981; Wilson and Pauli, 1982; Cross *et al*., 1988; Jain, 1993; Peinado *et al*., 1993; Marco and Lavín, 1999; Montané *et al*., 2002 and 2003). Smooth muscle relaxing effect of acepromazine, chlorpromazine, xylazine and halotane on spleen blocks muscle contraction and allows erythrocytes sequestration in spleen (Turner and Hodgetts, 1960; Cross *et al*., 1988; Jain, 1993). Variation of these parameters is related with the degree of excitement in physically captured animals and with induction time in sedated animals (Kocan *et al*., 1981).

Effects of stress response on leukocyte total and differential count follow a biphasic pattern. Catecholamines increase blood and lymph circulation, mobilizing leukocytes from the marginal pools (capillary beds and lymph nodes) into the circulation and inducing leukocytosis with neutrophilia and/or lymphocytosis. Splenic contraction has also been reported to be responsible of catecholamines-induced leukocytosis. Monocytes and eosinophils may increase or decrease. Catecholamines effect lasts 20-30 minutes. Corticosteroids induce leukocytosis with neutrophilia, lymphopenia and eosinopenia. Monocytosis may appear or not, depending on the species. Corticosteroid changes follow appear after catecholamine effects, and reach their maximum level 4 to 6 hours after the stress episode. Corticosteroids-induced neutrophilia is caused by release of mature neutrophils from the bone marrow, decreased diapedesis of circulating neutrophils to tissues and mobilization of neutrophils from marginal pool to blood. Lymphopenia is caused by lympholysis of steroid-sensitive T lymphocytes and sequestration of lymphocytes in extravascular sites. Eosinopenia could be caused by intravascular lysis, decreased release from bone marrow, spleen and liver sequestration and migration to lymphoid tissue of eosinophils (Cross *et al*., 1988; Jain, 1993; Duncan *et al*., 1994; Smith, 2000; Schultze, 2000; Taylor, 2000; Young, 2000). However, leukocyte response to corticosteroids of domestic ungulate species, where lymphocytes are the main white blood cell type, is less dramatic than in other domestic species (Jain, 1993; Duncan *et al*., 1994).

Platelet count also increases due stress, since they are stored in spleen similarly to erythrocytes (Cross *et al*., 1988; Jain, 1993).

Serum biochemistry

Hormones

Catecholamines (epinephrine and norepinephrine) are rapidly released after a stimulus and have a very short plasma half-life (McCarty, 1983; Ganong, 1998), so they can only be assessed in catheterized animals in laboratory conditions. Blood samples must be obtained within one minute from the beginning of the experiment (Broom and Johnson, 1993). In spite of its fast catabolism, increases in circulating catecholamines in wild ungulates due to capture and handling stress have been reported (Hattingh et al., 1988; Hattingh et al., 1990; Martucci et al., 1992).

Corticosteroids are steroid hormones produced by the adrenal cortex. Cortisol is the main corticosteroid in ungulates. In normal conditions, only 10% of blood cortisol is in the free form, which is the active form. At body temperature, 90% of plasma cortisol is bound to proteins; 70% to transcortin or corticosteroid binding globulin (CBG) and 20% to albumin.

However, during stress response free cortisol can increase up to 20-30% of total cortisol (Rijnberk and Mol, 1997).

Corticosteroids have been used to assess acute stress response both in wild and domestic animals (Thurley and McNatty, 1973; Franzmann *et al*., 1975; Rehbinder and Edqvist, 1981; Bubenik, 1982; Jessup *et al*., 1982; Spraker, 1982; Seal and Bush, 1987; Mitchell *et al*., 1988; Viñas *et al*., 1989; DelGiudice *et al*., 1990b; Hargreaves and Hutson, 1990; Hastings *et al*., 1992; Sanhouri *et al*., 1992; Morton *et al*., 1995; Waas *et al*., 1999). Controversy exists about the usefulness of cortisol to assess stress in wild ungulates, since some authors have found statistical differences in plasma cortisol levels depending on stress level or capture method, whereas others have not and suggest the use of a number of haematological and biochemical parameters as a more sensitive stress indicator (Franzmann *et al*., 1975; Bubenik, 1982; Jessup *et al*., 1982; Spraker, 1982; Kock *et al*., 1987 a, b, and c; Hattingh *et al*., 1988; Hattingh *et al*., 1990; Chapple *et al*., 1991; Morton *et al*., 1995; Waas *et al*., 1999). Although rapid rises of cortisol as a response to discrete stressing events have been described, cortisol seems not to be so sensitive to specific events like other biological parameters, but being more valuable to assess longer lasting situations of acute stress (Hastings *et al*., 1992; Waas *et al*., 1999). However, in spite of all the problems and variables mentioned, measures of corticosteroid responses are still considered of considerable value to assess short term stress effects on animal welfare (Broom and Johnson, 1993).

Corticosteroids concentration may be affected by many factors which have to be considered when trying to use it to assess stress (Rushen, 1991):

- Cortisol concentration follows a circadian, ultracircadian and seasonal rhythm of pulsatile secretion (Rijnberk and Mol, 1997; Ingram *et al*., 1999; Guyton and Hall, 2000), and baseline circadian variation may also affect the magnitude of cortisol increase in stress response (Broom and Johnson, 1993).

- It is still unknown if metabolic and immune effects of altered corticosteroid secretion depends upon mean dairy levels or upon the patterns (duration and amplitude) of the secretory episodes of cortisol.

- There is not a ‘pathological level’ for cortisol concentration, and it is still unknown what level of corticosteroids leads to immune or reproductive problems, although serum cortisol concentration of 5 μg/dl has been proposed as a pathological level for physically captured bighorn sheep (Kock *et al*., 1987b).

- Chronic stress can cause a sensitization of HPA axis, and animals submitted to chronic stress will display higher rises in serum corticosteroid concentration than animals not submitted to chronic stress.

- Handling of the animal to obtain blood samples evokes an increase in corticosteroids concentration. Delay before corticosteroids are released in the bloodstream is at least 2 minutes in most species, so blood sample must be collected within 2 minutes from the beginning of the blood sampling procedure (Broom and Johnson, 1993; Säkkinen *et al*., 2004).

- Increase of corticosteroid concentration may be due to factors other than stress. Physiological information of the animal and additional information about the stressor applied and surrounding circumstances should be also taken into account.

- There is strong interindividual variability in corticosteroid response (Moberg, 1985).

- As aforementioned, analytical problems may arise when trying to determine cortisol, since only the free form of cortisol is biologically active and most laboratory tests detect total cortisol (Rijnberk and Mol, 1997). However, total cortisol and unbound cortisol are correlated (Greenwood and Shutt, 1992).

Total protein

Cortisol may cause a decrease in serum total protein due to its catabolic effects (Kaneko, 1997b). However, contradictory results have been published about total protein changes due to stress: some authors have found no changes, whereas others have found a slight increase attributed to dehydration (Franzmann and Thorne, 1970; English and Lepherd, 1981; Kock *et al*., 1987a; Bush, 1993; Diverio *et al*., 1996a; Wolkers *et al*., 1994; Marco *et al*., 1997; Montané *et al*., 2002). A consistent finding in literature is a higher total protein concentration in animals physically restrained than in animals anesthetized, which may be due to differences in blood pressure, colloid osmotic pressure, capillary pressure, lymphatic circulation, haemodilution provoked by the anaesthetic agent or splenic contraction rather than higher stress in immobilized animals (Seal *et al*., 1972; Wesson *et al*., 1979; Peinado *et al*., 1993; Montané *et al*., 2002). Adults have higher protein concentration than young animals (Kaneko, 1997b). Electrophoretic fractions of proteins depend on many factors, and may be very useful to assess the general status of an individual. In domestic small ruminants (sheep and goat) electrophoretic fractions are classified as α, β_1, β_2 and γ; (Kaneko, 1997b). α_1, α_2, β and γ fractions have been reported for Southern chamois, and even α_1 has been divided in α_{1a} and α_{1b} (Cuenca *et al*., 1996; Lastras *et al*., 2000).

Metabolites

Glucose

In ruminants, glucose and digestible carbohydrates like starch are fermented to volatile fatty acids (acetate, 65%; propionate, 20%; butyrate, 10%) by the rumen micro flora, and virtually there is no glucose absorption in the digestive. Lactic acid produced in the rumen can also be a source of blood glucose, but the direct precursor for blood glucose is propionate. Catecholamines and corticosteroids increase serum glucose concentration activating glycogenesis and glycogenolysis (Kaneko, 1997a; Ganong, 1998; Guyton and Hall, 2000). However, a vigorous exercise previous to capture may produce a decrease in plasma glucose concentration. Repeated blood sampling have been proposed as a way to closer monitor glucose evolution, but sampling itself could elicit new changes in adrenal activity (Broom and Johnson, 1993). Overall, glucose levels are very variable, are not a sensitive measure and have little value as welfare or stress indicators (Wesson *et al*., 1979; Sartorelli *et al*., 1992; Broom and Johnson, 1993; Waas *et al*., 1999).

Stress-related increases in serum glucose concentration have been reported in wild and domestic animals (Franzmann and Thorne, 1970; Seal *et al*., 1972; Bush *et al*., 1981; Kocan *et al*., 1981; Rehbinder and Edqvist, 1981; Kock *et al*., 1987a; Hartmann, 1988; Hattingh *et al*., 1988; Mitchell *et al*., 1988; Viñas *et al*., 1989; Carragher *et al*., 1997; Marco *et al*., 1997), although decreases in glucose concentration, sometimes after a short transient increase, have also been described (Quirce and Maickel, 1981; Kent and Ewbank, 1986).

Cholesterol

Cholesterol is synthesized mainly in the liver, and in blood is transported by low density lipoproteins (LDL) and high density lipoproteins (HDL) (Bruss, 1997). Cholesterol levels vary depending on the diet (Coblentz, 1975; Bruss, 1997). Both catecholamines and corticosteroids have a lipolytic effect on the adipose tissue, stimulating fat mobilization and increasing circulating free fatty acids (Genuth, 1996; Guyton and Hall, 2000). Thus, an increase in plasma cholesterol concentration would be expected after a stress episode and some authors have found such increase (Franzmann and Thorne, 1970; Barrett and Chalmers, 1977; Marco *et al*., 1998; Marco and Lavín, 1999). However, other authors have found a decrease in cholesterol concentration, which was attributed to a deviation of cholesterol to corticosteroid synthesis (Locatelli *et al*, 1988; Saccon *et al*., 1992; Peinado *et al*., 1993; Paltrinieri *et al*., 1994; Marco *et al*., 1997).

Triglycerides

Similarly to cholesterol, triglyceride levels are expected to rise due to stress, as some authors have described in different species (Saccon *et al*., 1992; Arnemo *et al*., 1994; Marco and Lavín, 1999). Nevertheless, since responses appear not to be consistent, value of cholesterol and triglycerides as welfare indicators is still very doubtful (Seal and Hoskinson, 1978; Kocan *et al*., 1981; Broom and Johnson, 1993).

Bilirubin

Bilirubin is used as an indicator of hepatic disorder (Tennant, 1997). In stressful situations, its levels could increase due to hepatocellular damage and/or haemoglobin liberated due to haemolysis, although it is not proved (Bush, 1993). Nevertheless, bilirubin rises are rare in ruminants, since they have a high hepatic capacity to excrete bilirubin and extrahepatic mechanisms to eliminate bilirubin. In domestic ruminants, hyperbilirubinemia is related to haemolytic conditions rather than to hepatic problems (Duncan *et al*., 1994; Tennant, 1997). No differences in bilirubinemia have been found in wild ungulates due to stress (Marco *et al*., 1997 and 1998; Marco and Lavín, 1999).

Lactate

Lactate is synthesized from glycogen in the muscle and transported via blood to the liver, where it is used to synthesize glucose. Anaerobic glycogenesis is another source of lactate. In ruminants, lactate originates also from many rumen fermentation reactions. Nevertheless, most of blood lactate comes from muscular gycogenolysis (Kaneko, 1997a). Physical capture increases lactate concentration, due catecholamines induce muscle glycogenolysis and anaerobic metabolism due to physical exercise (Mitchell *et al*., 1988; Kaneko, 1997a; Ganong, 1998). Since lactate is produced in the muscle in anaerobic conditions, it could be an indicator of muscular hypoxia and fatigue, two components of the pathogenesis of capture myopathy muscular lesions (Spraker, 1982). Increases in lactate concentration during a stress episode have been previously reported in wild ungulates (Hattingh *et al*., 1988; Waas *et al*., 1997 and 1999).

Creatinine

Creatinine is formed from phosphocreatine, which is synthesized in the muscle, at a relatively constant daily rate of 1.6-2%. The quantity of creatinine formed each day depends of dietary intake, creatinine rate of synthesis and muscle mass. In ruminants, creatinine dietary intake is very low, and muscular mass and serum creatinine concentration are directly

correlated. Increased creatinine production has been observed after prolonged severe exercise, so factors affecting muscle mass could influence on creatinine daily production. Serum creatinine concentration follows a circadian pattern, which must be taken into account (Refsum and Stromme, 1974; Wolkers *et al*., 1994; Finco, 1997). Creatinine is excreted by the kidney and undergoes no reabsorption process, so it is considered a renal failure indicator (Finco, 1997). Increases in serum creatinine concentration have been reported in wild ungulates due to renal vasoconstriction induced by catecholamines (Harthoon, 1976). Increases in serum urea and creatinine concentrations in stressed animals could indicate renal hypoxia due to vasoconstriction, one of the main factors in the pathogenesis of the ataxic myoglobinuric syndrome of capture myopathy (Spraker, 1993).

Urea

Urea is the resulting metabolite of protein catabolism. It is synthesized in the liver and excreted in the kidney. Serum urea concentration depends on metabolic rate, diet and renal excretion, but influence of rumen metabolism on serum urea concentration is still unclear. Dehydration also can cause an increase in urea serum concentration (Franzmann and Thorne, 1970; Duncan *et al*., 1994; Finco, 1997). Although urea has been used to assess both hepatic and renal function, an increase in blood or serum urea is due to an accelerated rate of protein metabolism rather than to a decreased urinary excretion of urea. However, urea has been traditionally used as a renal function indicator. Urea levels could increase in physically captured animals as a consequence of increased muscular activity or increased protein catabolism induced by cortisol (Kopple and Coburn, 1974; Rehbinder and Edqvist, 1981; Finco, 1997; Kaneko, 1997b; Marco *et al*., 1998; Guyton and Hall, 2000). Elevated serum urea concentrations in wild and domestic ungulates due to stress and/or increased physical activity have been previously reported (Sealander *et al*., 1975; Hyvärinen *et al*., 1976; Kock *et al*., 1987a; Viñas *et al*., 1989; Williams *et al*., 1992).

Enzymes

Muscular enzymes increase in stressed animals due to increased cellular permeability and cellular damage (Spraker, 1993; Duncan *et al*., 1994). In domestic animals epinephrine and ACTH have been reported to increase muscular enzymes (Holmes *et al*., 1973; Sconberg *et al*., 1993). Although it is more suitable for long-term stress studies, enzyme serum activity may also be useful to assess acute stress (Broom and Johnson, 1993). Muscular enzymes are considered of prognostic value to detect capture myopathy by different authors, either due to an increase in muscle cells permeability or to muscle damage (myonecrosis or muscle rupture) (Chalmers and Barrett, 1982; Vassart *et al*., 1992; Williams and Thorne, 1996).

Creatine kinase (CK)

There are four CK isoenzymes, depending on the subunits they are composed of (M-muscle and B-Brain): CK_1 (CK-BB), located mainly in the brain; CK_2 (CK-MB); CK_3 (CK-MM), located mainly in heart and skeletic muscle; and CK-Mt, located in mitochondrial membranes. CK is considered a very sensitive indicator of muscle damage. Intramuscular injections, small muscular bruising or moderate muscular ischemia may cause increases of serum CK activity (Kramer and Hoffmann, 1997). For that reason, only large increases in serum CK activity are of clinical significance (Lewis and Rhodes, 1978). Short serum half-life of CK causes CK activity to rapidly return to normal after muscle damage. Complementing muscle-specific CK with less specific but longer circulating AST activity the problem can be solved. Thus, a small increase in serum CK with a marked rise in AST indicates muscle ischemia some days before the sampling. CK and AST are considered a good tool to assess muscle status and the most useful to make a diagnosis and a prognosis of capture myopathy (Chapple *et al*., 1991; Williams and Thorne, 1996; Kramer and Hofmann, 1997). CK is al well useful for welfare assessment, in combination with other measures (Broom and Johnson, 1993). CK has been used in a number of studies as physical stress indicator in wild ungulates (Seal *et al*., 1972; Seal and Hoskinson, 1978; Kocan *et al*., 1981; Kock *et al*., 1987a; DelGiudice *et al*., 1990b; Chapple *et al*., 1991; Peinado *et al*., 1993).

Aspartate aminotransferase (AST)

AST is a nonspecific but sensitive marker of soft tissue damage, frequently used to complement CK changes (Kramer and Hoffmann, 1997). CK and AST are more sensitive indicators of muscular damage than LDH and ALT (Chapple *et al*., 1991; Duncan *et al*., 1994). Larger increases of CK and AST in males or females have been related to a stronger stress response to capture and handling in males (Kent *et al*., 1980; Kock *et al*., 1987a; Chapple *et al*., 1991) or females (Sartorelli *et al*., 1991) of different species of wild ungulates.

Alanine aminotransferase (ALT)

ALT is a sensitive indicator of hepatic damage in some species, but in domestic ruminants it is less specific and has little diagnostic value (Kramer and Hoffmann, 1997). Nevertheless it may be useful to detect capture myopathy, since it is also found in muscle (Barrett and Chalmers, 1977; Vassart *et al*., 1992). Increases in this enzyme seem to be related to poor tissue perfusion, decreased heat dissipation and hypoxia, all of them components of the pathophysiology of capture myopathy (Spraker, 1993; Williams and Thorne, 1996). Contradictory differences in ALT serum activity have been found in wild

ungulates captured with physical or chemical methods (Marco *et al*., 1998; Marco and Lavín, 1999). ALT increases have been described after a stress episode in wild ungulates, related to hepatic damage or to physical stress (Vassart *et al*., 1992; Broom and Johnson, 1993; Montané *et al*., 2002 and 2003).

Lactate dehydrogenase (LDH)

LDH is a nonspecific enzyme, with five isoenzymes whose tissue distribution varies among species. However, LDH-5 isoenzyme is highly specific of skeletal muscle, and rises in LDH activity are highly correlated with CK, thus being a useful marker of muscle damage. However, isoenzyme LDH-5 increases are not only due to muscular exercise or damage, but are also related to animal's response to a stressor, since LDH-5 activity in plasma increases in animals not submitted to any muscular activity (Jones and Price, 1990 and 1992; Goddard and Grigor, 1997; Kramer and Hoffmann, 1997). LDH has been used as a physical stress indicator in different species of wild ungulates (Seal and Hoskinson, 1978; Bush *et al*., 1981; Kock *et al*., 1987a; DelGiudice *et al*., 1990b; Chapple *et al*., 1991; Peinado *et al*., 1993; Marco *et al*., 1997; Montané *et al*., 2002 and 2003). Adult animals show higher LDH serum activity than young ones, probably due to a higher size of the organs containing LDH (Bush *et al*., 1981; Chapple *et al*., 1991; Goddard and Grigor, 1997).

Alkaline phosphatase (AP)

AP is found in intestine (intestinal isoenzyme), kidney, liver and bone (unspecific isoenzyme), and also there is a termostable corticosteroid-induced isoenzyme (Kramer and Hoffmann, 1997; Rijnberk and Mol, 1997). However, it seems to have poor diagnostic value of tissue damage (Broom and Johnson, 1993).

Electrolytes

Chloride

Chloride is the major extracellular anion. It determines the osmotic pressure of extracellular liquid and participates in the acid-base balance. In normal conditions, plasma chloride concentration varies along with plasma sodium concentration. However, increases in chloride concentration are associated with a tendency towards acidosis (hyperchloremic acidosis), with increased renal chloride reabsorption to compensate the decrease of bicarbonate, whereas decreases in chloride concentration are related to metabolic alkalosis (Carlson, 1997; Autran de Morais, 2000). Acidosis is present in the pathogenesis of capture myopathy (Spraker, 1982), so chloride variation could not be related to sodium variation

during stress and serve as a useful indicator of acidosis and risk of developing capture myopathy. Higher chloride concentration has been related to physical capture or stress (Kock *et al*., 1987a; DeLiberto *et al*., 1989; Jalanka, 1989; Brelurut *et al*., 1991; DelGiudice *et al*., 1992; Williams *et al*., 1992; Peinado *et al*., 1993).

Sodium

Sodium is the main extracellular electrolyte and determines extracellular volume and osmolality. Serum sodium concentration depends on relative water balance, and varies along a relatively narrow interval. Sodium and water balance is regulated by Na^+,K^+-ATPase in the kidney, and sodium concentration is kept in a relatively narrow interval (Carlson, 1997; DiBartola, 2000). Increases in serum sodium concentration are caused by increased sodium intake, excessive water loss or decreased water intake, and opposite circumstances cause a decrease in serum sodium concentration (Bush, 1993). Catecholamine vasoconstriction on renal arterioles and effects on renal proximal tubule favour and stimulate sodium reabsorption (Carlson, 1997; DiBartola, 2000). So, sodium could be a diagnostic tool to evaluate catecholamines effect as well as vascular renal compromise. However, results about sodium changes due to stress are contradictory, since some authors have found changes whereas others have not. Moreover, among those who have found increases in serum sodium concentration following a stress episode, interpretation has also been contradictory, attributing such increases either to stress or to water deprivation (Hyvärinen *et al*., 1976; Kocan *et al*., 1981; Kock *et al*., 1987a; Peinado *et al*., 1993; Marco *et al*., 1998; Marco and Lavín, 1999; Montané *et al*., 2002). The lack of consistent findings about its relationship with stress, the narrow range of its variations and the fact that many other factors are also important in sodium concentration dynamics make it a poor stress and welfare predictor.

Potassium

Potassium is largely an intracellular ion, with good renal excretion. Na^+,K^+-ATPase is the responsible of keeping potassium distribution balanced, which has a paramount importance in neuromuscular and heart excitability (Carlson, 1997). In general, hypokalemia increases membrane potential, producing a hyperpolarization block resulting in weakness or paralysis. Hyperkalemia decreases membrane potential, causing hyperexcitability (Patrick, 1977). Catecholamines may cause an increase (α-adrenergic effect) followed by a decrease (β_2 effect) in serum potassium concentration, whereas acute administration of corticosteroids causes transitory hyperkalemia (Bia and DeFronzo, 1981). Exercise, massive muscular necrosis, decreased tissue perfusion, hypoxia and acute metabolic acidosis cause a translocation of potassium from intercellular fluid to extracellular fluid which increases serum

potassium concentration. Acute acidosis decreases urinary excretion of potassium, and renal failure induces hyperkalemia. Hyperkalemic changes in heart excitability can cause ventricular fibrillation to a myocardium already sensitized with catecholamines and lead to shock and peracute death (one of the clinical expressions of capture myopathy), so potassium should be considered as a definitive emergency indicator (Van Beaumont *et al*., 1973; Spraker, 1982; Carlson, 1997; DiBartola and Autran de Morais, 2000). Potassium increases have been related to physical capture or stress (Kock *et al*., 1987a; DeLiberto *et al*., 1989; Jalanka, 1989; Brelurut *et al*., 1991; DelGiudice *et al*., 1992; Williams *et al*., 1992; Peinado *et al*., 1993).

4.5.4. Cortisol in saliva

Efforts are done currently to find non-invasive ways to assess stress. Cortisol concentration can also be measured in saliva samples, where only its unbound form, which is capable of crossing membranes, is detected (Fell and Shutt, 1985; Greenwood and Shutt, 1992; Broom and Johnson, 1993; Rijnberk and Mol, 1997). Since free or unbound cortisol is the only biologically active form of cortisol, saliva cortisol could be a better indicator of the possible effects of the HPA axis on the animal organism than serum cortisol (Rijnberk and Mol, 1997; Chacón-Pérez *et al*., 2004). A more sensitive test is required to determine salivary cortisol, since cortisol concentration is lower in saliva than in plasma (Broom and Johnson, 1993). It has been proposed as a less invasive technique than blood sampling to avoid adrenal response elicited by handling when collecting blood samples, and new techniques to assess saliva cortisol are being developed (Rijnberk and Mol, 1997; Chacón-Pérez *et al*., 2004). Although restraint to obtain saliva samples may also elicit cortisol secretion and interfere with the results, techniques have been described which minimize handling and reduce stress response due to saliva collection, and allow sample collection during the first 4 minutes of handling, when basal values of salivary cortisol are still not affected by manipulation (Fell *et al*., 1985; Kobelt *et al*., 2003). Saliva cortisol shows a high correlation with free serum cortisol and it is also correlated with total serum cortisol, although the ratio between saliva and serum cortisol may be affected by stressful stimuli (Fell *et al*., 1985; Cook *et al*., 1996). Increases in saliva cortisol during a stress episode have been reported, although it seems to depend on the characteristic of the stimulus (Greenwood and Shutt, 1992; Cook *et al*., 1996; Beerda *et al*., 1998; Geverink *et al*., 1998).

4.6. TRANQUILIZERS

Tranquilizers have been recommended to reduce stress and risk to animal's health during physical capture, restraint and transport of wild ungulates (Ebedes and Raath, 1999; Montané *et al*., 2002 and 2003). Neuroleptics belong to several types of drugs and are defined by exerting similar effects in treated animals, and can be classified as (Ebedes and Raath, 1999; Nielsen, 1999):

- Short-Acting Neuroleptics (SAN), with immediate effects lasting from a few to 18 hours. Some examples are acepromazine, chlorpromazine, promazine, haloperidol, droperidol, azoperone and diazepam.

- Long-Acting Neuroleptics (LAN), which are effective for 3 to 7 days and longer after administration. These are fatty acid esters of the basic tranquilizer compounds dissolved in oil solutions. Zuclopenthixol acetate, perphenazine enanthate and pipothiazine palmitate belong to this group. Administration of LAN must be intramuscular.

Neuroleptics produce a calming effect and suppress spontaneous movements with retention of spinal reflexes and nociceptive and avoidance behaviours, but they do not produce immobilization or analgesia (Kreeger, 1997; Nielsen, 1999). Effects of neuroleptics in wild animals usually include (Ebedes and Raath, 1999):

- A general calming effect.
- Loss of interest in new and unnatural surroundings.
- Loss of fear of humans.
- Reduction of aggressive and dominant behaviour.

4.6.1. Acepromazine

Acepromazine is the common name for the 2-acetyl-10-(3-dimethylaminopropyl) phenothiazine, a phenothiazine belonging to the short acting neuroleptic group, with central nervous system effects by blocking post-synaptic dopamine receptors, as well as anticholinergic, antihistaminic, antispasmodic, and α-adrenergic blocking effects. Exact mechanism of action is not fully understood, but phenothiazines block post-synaptic dopamine receptors in the CNS and may also inhibit the release and increase the turnover rate of acepromazine. They are thought to depress portions of the reticular activating system, which assists in the control of body temperature, basal metabolic rate, emesis, vasomotor tone, hormonal balance, and alertness (Plumb, 1999). Pharmacokinetics of this drug has

been studied in the horse. It has a fairly high volume of distribution (6.6 L/kg), and is more than 99% protein bound (Ballard *et al*., 1982). Acepromazine is metabolized in the liver with both conjugated and unconjugated metabolites eliminated in urine. The onset of action is fairly low, requiring up to 15 minutes following intravenous administration, with peak effects seen in 30-60 minutes (Plumb, 1999). Clinical signs of acepromazine last 3 to 4 hours, but can still be present after 7 hours (Booth, 1982).

Acepromazine primary desired effect is its central tranqulizing action, but additional pharmacologic effects include antiemetic, antispasmodic and hypothermic actions. Acepromazine may decrease respiratory rate, but has little or no effect with regard to blood gas picture, pH or oxyhaemoglobin saturation. A dose-dependent decrease in haematocrit is seen within 30 minutes after dosing in domestic animals, probably due to increased splenic sequestration of erythrocytes. Acepromazine vascular effects consist in lowering arterial blood pressure and increasing central venous pressure, vagally induced bradycardia and transient sinoatrial arrest. Bradycardia may be negated by reflex tachycardia secondary to decreases in blood pressure (Plumb, 1999). It also has antidysrhythmic effects and a protective effect from ventricular fibrillation caused by halotane and epinephrine (Muir *et al*., 1975; Plumb, 1999).

Acepromazine has been widely used as preanaesthetic or sedative agent in wild ungulates (Cowan *et al*., 1962; Arnemo *et al*., 1993; Kreeger, 1997; Nielsen, 1999; Montané *et al*., 2002 and 2003). The recommended intramuscular sedation dose is 0.05-0.1 mg/kg for red deer (*Cervus elaphus*), domestic sheep (*Ovis aries*) and goats (*Capra hircus*) and large wild animals in general (Arnemo *et al*., 1993; Nielsen, 1999; Plumb, 1999). However, variability and unpredictability depending on initial emotional status of the animal in the response to the administration of acepromazine has been described in wild ungulates and domestic animals. Not enough tranquilization was achieved with acepromazine in hyperexcited cattle (*Bos taurus*), and no differences between lactating cattle treated with acepromazine and those injected with saline have been found (Cowan *et al*., 1962; Booth, 1982; Brearley *et al*., 1990). Chlorpromazine, a closely related phenothiazine, has been described to increase ACTH secretion and, consequently, cortisol secretion (Bruss, 1980).

Acepromazine has also been used to prevent rhabdomyolysis in horses (Andrews, 1994; Beech, 1994), thanks to muscular vasodilatation and delay in the transference of potassium from extracellular to intracellular space induced by the drug (Freestone *et al*., 1991).

5. MATERIALS AND METHODS

5.1. CAPTURE

Southern chamois were captured with 10x10 cm mesh drive-nets (Ziboni Ornitecnica, Bergamo, Italy). The net was 2.5 meters high and it was formed by 50 meters sections which were joined to attain a final mean length of 530 meters (range 275 to 650), hanging from trees and poles placed for this purpose. A total of 24 capture operations were carried out in alpine and subalpine environments, with altitudes ranging from 1700 to 2300 meters, in the National Game Reserves of Cadí (42º18' N, 1º54' E) and Freser-Setcases (42º22' N, 2º09' E), during Northern hemisphere early spring (March and April), summer (June and July) and autumn (October and November) of years 2000 to 2003. The net was placed on location and presented at least one or two days before the capture operation, and it was left on the ground. The net was picked up the morning of the capture operation by a group of people, composed by researchers and volunteers, which remained hidden and distributed along the net. A line of beaters, composed by personnel and rangers of the National Game Reserves of Cadí, Cerdanya-Alt Urgell and Freser-Setcases, drove the animals towards the net, where operators hidden beside the net assisted the animals as soon as possible. Once the animals fell trapped in the net, they were immediately blindfolded, unwrapped, their legs restrained and finally introduced in a 4x4 cm mesh transport sack net (Ziboni Ornitecnica, Bergamo, Italy).

5.2. EXPERIMENT DESIGN

5.2.1. Reference values

Once the animals where in the sack net, blood samples were immediately collected from the jugular vein of 75 free-ranging Southern chamois (43 adult males ->1 year old-, 12 young males -1 year old-, 18 adult females and 2 young females), with disposable 10 ml syringes and 21Gx1" needles. Two ml of each blood sample was placed in commercial tubes with anticoagulant (EDTA K_3) for haematological analyses, and the remaining was placed in serum collection tubes and allowed to clot at room temperature. Once the clot was formed, the sample was centrifuged at 1200 x G for 15 minutes and serum obtained was frozen at -20ºC until biochemical analyses were performed.

5.2.2. Capture and physical restraint stress

Forty free-ranging Southern chamois, 25 males (twenty adults and five young -one year old-) and fifteen females (thirteen adults and two young) formed the study group for the capture and physical restraint stress experiment. At the moment of the capture, nineteen

randomly selected Southern chamois, thirteen males (nine adult and four young) and six adult females, were injected intramuscularly with 2.5 mg of acepromazine maleate in 0.5 mL volume (Calmo Neosan® 5mg/mL, Smithkline Beecham, Madrid, Spain), receiving a dose of 0.108 ± 0.018 mg/kg (mean ± standard deviation). Other 21 animals, twelve males (eleven adults and one young) and nine females (seven adults and two young) received 0.5 mL of saline and were used as control group. Blood samples were collected from the jugular vein at the moment of the capture (time 1) and each hour thereafter for three hours (times 2, 3 and 4), using disposable 10 ml syringes and 21Gx1" needles. Blood samples were handled as described for the reference values experiment. Seventeen Southern chamois of the control group, twelve males (eleven adults and one young) and five females (four adults and one young), and eighteen belonging to the treated group, thirteen males (nine adults and four young) and five adult females, were fitted with telemetric heart-rate recording equipment (Polar Vantage NV™ and Polar S710i™, Polar Electro Oy, Kempele, Finland), which measured and recorded heart rate at 60 second intervals for a period of at least two hours. The chamois were also fitted with telemetric body temperature recording devices (Mätman datalogger®, Eltex of Sweden AB, Älmhult, Sweden). Rectal temperature was also measured and recorded every minute. Animals were physically restrained in the net bag until they were released three hours after capture at the same place where they were captured.

5.2.3. Transport stress

21 free-ranging Southern chamois from the captured Southern chamois, 18 males (14 adults and four young -one year old-) and three adult females, formed the study group for the transport stress experiment. At the moment of the capture, eleven randomly selected Southern chamois, ten males (nine adult and one young) and one adult female, were injected intramuscularly with 2.5 mg of acepromazine maleate in 0.5 mL volume (Calmo Neosan® 5mg/mL, Smithkline Beecham, Madrid, Spain), receiving a dose of 0.100 ± 0.020 mg/kg (mean ± standard deviation). Other ten animals, eight males (five adults and three young) and two adult females received 0.5 mL of saline and were used as control group. Blood samples were collected from the jugular vein at capture (time 1), immediately before transport (time 2) and immediately after transport (time 3) using disposable 10 ml syringes and 21Gx1" needles. Blood samples were handled as described for the reference values experiment. All the animals in the study were fitted with telemetric heart-rate recording equipment (Polar Vantage NV™ and Polar S710i™, Polar Electro Oy, Kempele, Finland), which measured and recorded heart rate at 60 second intervals throughout all the study period. The chamois were also fitted with telemetric body temperature recording devices (Mätman datalogger®, Eltex of Sweden AB, Älmhult, Sweden). Rectal temperature was also measured and recorded every minute. Animals were placed in a 4WD vehicle 108.6 minutes

± 14.3 (mean ± standard deviation) after capture and underwent a 102.4 minutes ± 11.1 (mean ± standard deviation) transport until they were released.

5.3. HEART RATE AND RECTAL TEMPERATURE

Once the battue was finished, the right thoracic and left precordial areas were clipped to install the transmitter of a heart rate recording device. Ultrasonography gel (Geleco®, Novartis, Barcelona, Spain) was used to ensure good contact of the electrodes with the skin. The receiver was placed in a collar around the neck, since it has to be less than 1.5 meters away from the transmitter. At that moment a temperature recording device was placed in the rectum of the animals, and both heart rate and temperature recording started. Both heart rate and temperature registers were saved using their respective software and converted to numeric values. To allow statistical analysis, mean value of every five minute period was calculated for both heart rate and temperature. Both heart rate and temperature registers were saved using their respective software and converted to numeric values. To allow statistical analysis, mean value of every five minute period was calculated for both heart rate and temperature.

5.4. HAEMATOLOGY AND SERUM BIOCHEMISTRY

Red blood cell count (RBC), white blood cell count (WBC), platelet count and haemoglobin concentration were determined by an electric impedance semi-automated analyser (Sysmex F-800, Toa Medical Electronics, Japan). Blood samples had to be previously diluted with an automated haemodiluter (AD-260, Toa Medical Electronics Co. LTD, Japón), which absorbed 0.2 mL of blood to realize a 1:500 dilution, which was used to determine leukocyte count and haemoglobin concentration. A 1:100 dilution was made from the first dilution to obtain a final dilution of 1:50000, which was used to determine erythrocyte count. Haemolyser (Quicklyser II, Sysmex Corporation, Kobe, Japan) was added to first haemodilution (after realizing the second haemodilution) to break erythrocytes. Both dilutions were manually homogenised before analysis. Limits of the cell size range were manually adjusted in each analysis to adapt the domestic animals designed analyser to the small cells of Southern chamois. Haematocrit was measured by the standard microhaematocrit method with a microhaematocrit centrifuge (Hawksley, Lancing, UK) at 14000xG during 6 minutes. Erythrocyte indexes, i.e. mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH), were calculated from the data obtained (RBC, haemoglobin and haematocrit). Differential leukocyte count was

performed by identifying 200 leukocytes on blood smears stained with a commercial Diff-Quick®-like stain (Química Clínica Aplicada S. A., Amposta, Spain).

All biochemical parameters were determined by means of an automated analyser (Cobas Mira®, Roche, Nutley, USA), except sodium and potassium, which were measured by flame photometry (Corning 410C®, Corning Medical Medfield, USA) and cortisol, which was analysed with an ELISA commercial kit (EIA-1887, DRG Instruments GmbH, Marburg, Germany). Boerhinger Manheim, S.A. reactives (Manheim, Germany), were used for most parameters, except for chloride, which was analyzed with Gernon reactives (Lindau, Germany) (Table 5.1). Protein electrophoretic fractions for the reference value study were determined as previously described (Lastras *et al*., 2000), and fractions analyzed were those described previously for this species (Cuenca *et al*., 1996; Lastras *et al*., 2000).

Table 5.1. Commercial kits used to realize serum biochemical analyses with COBAS MIRA®.

Parameter	Commercial kit
Aspartate aminotransferase (AST)	1 087 541[1]
Alanine aminotransferase (ALT)	1 087 568[1]
Creatine kinase (CK)	1 087 533[1]
Lactate dehydrogenase (LDH)	1 087 592[1]
Phosphatase alkaline (AP)	1 087 517[1]
Urea	1 360 604[1]
Creatinine	124 192[1]
Total biliubin	123 919[1]
Lactate	124 842[1]
Triglycerides	701 882[1]
Cholesterol	1 442 341[1]
Glucose	1 442 449[1]
Chloride	GN 1300[2]

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

5.5. SALIVA CORTISOL

Saliva samples were collected from 57 animals. Samples were obtained by letting the animals chew a piece of cotton. When the cotton was soaked, it was placed in an eppendorf tube. At the arrival to the laboratory, the eppendorf tube was centrifuged at 1200 x G for 20 minutes to recover the saliva from the cotton, and the saliva was then frozen at -20ºC until its analysis. Enough sample of adequate quality was obtained from only 37 animals. Southern

chamois with extreme serum or saliva cortisol concentrations, captured in spring or animals that behaved nervously during the collection of saliva samples were eliminated from the statistical study in order to obtain a group as homogeneous as possible. Therefore, only 29 of the 37 animals were included in the study. Saliva cortisol concentration was determined according to instructions with a specific ELISA commercial kit (SLV-2930, DRG Instruments GmbH, Marburg, Germany), which calculates saliva cortisol concentration by comparing optical density of the samples with a range of standards of known cortisol concentration.

5.6. STATISTICAL ANALYSIS

5.6.1. Reference values

A multivariate analysis of the variance was performed to detect statistical differences between groups, using the PROC GLM procedure of SAS® System for Windows V8 (SAS Institute Inc., Cary, USA). Factors considered in the model for each parameter were season, sex and age, and the interaction between these three factors. Group least square means were used due to the unbalanced distribution of animals. Minimum accepted significance level was at least $p<0.05$ for all parameters.

5.6.2. Physical restraint and transport stress

A repeated measures analysis of the variance was performed to detect statistical differences between groups and along time within groups, using the PROC MIXED procedure of SAS® System for Windows V8 (SAS Institute Inc., Cary, NC, USA). The main factor was treatment (acepromazine or saline) and the repeated factor was time. Season, sex and age and interactions among factors were included in the model in the physical restraint study. Least square means were used due to the unbalanced distribution of animals in the groups. Minimum accepted significance level was at least $p<0.05$ for all parameters.

5.6.3. Saliva cortisol

A correlation analysis determining the Pearson correlation coefficient and a regression analysis between serum cortisol and saliva cortisol concentrations were performed using the PROC CORR and the PROC REG, respectively, of the SAS® System for Windows V8 (SAS Institute Inc., Cary, NC, USA). The same analyses were performed on the logarithms of both concentrations, in order to reduce variance of the data.

6. RESULTS

6.1. CAPTURE

6.1.2. Performance

A total of 91 free-ranging Southern chamois (45 adult males ->1 year-, 12 young males -1 year old-, 20 adult females, 2 young females and 12 animals of undetermined sex and age) were captured. Two adult males were captured twice and another adult male was captured three times, which accounts for a total of 95 captures (Table 6.1).

Table 6.1. Performance of Southern chamois captures.

Capture operation	Date	Place	Animals captured (recaptured)
1/00	20/06/00	Coll de Pal-RNC Cadí	3
2/00	22/06/00		0
3/00	28/06/00		0
4/00	13/07/00	Masella-RNC Cadí	5
5/00	26/10/00		2
1/01	06/06/01	Masella-RNC Cadí	5
2/01	14/06/01	Rus-RNC Cadí	1
3/01	19/06/01	Coll de Pal-RNC Cadí	16 (1)
4/01	22/06/01		6 (1)
5/01	25/06/01		6 (1)
6/01	11/07/01	Masella-RNC Cadí	1
7/01	13/07/01		4
8/01	18/07/01	Rus-RNC Cadí	1
9/01	20/07/01		3
1/02	21/06/02	Coll de Pal-RNC Cadí	5 (1)
2/02	25/06/02		2
3/02	16/07/02	Masella-RNC Cadí	1
4/02	23/07/02	Font Cerdana-RNC Cadí	0
5/02	25/07/02		0
6/02	07/11/02	Masella-RNC Cadí	1
1/03	27/03/03	Vallter-RNC Freser-Setcases	2
2/03	02/04/03		3
3/03	10/04/03	Fontalba-RNC Freser-Setcases	20
4/03	30/04/03		8

At least one Southern chamois was captured in 83.3% (20 out of 24) of the capture operations. Mean performance was 3.96 Southern chamois captured per capture operation (range 0-20). Since each capture operation accounts for two days of work (one to prepare the net and the second to realize the capture), capturing a Southern chamois represented 0.51 days of work.

6.1.3. Specificity

No animal of other species than chamois were captured, so specificity was 100%.

6.1.4. Safety for the animals

Two animals (an adult female and an adult male) of the 95 captures of Southern chamois died, accounting for a mortality of 2.11%. Shock was the cause of dead of the female (RP-C-8). The male (RP-C-20) died by asphyxia when fell by second time in the net unnoticed and remained unattended with its neck twisted. Three more animals suffered lesions when falling in the net. A two year-old Southern chamois fell trapped in the net but could escape before any operator could get it, loosing the corneous sheath of the horn, which remained hanging in the net. A second animal (RP-C-11) was cut by the net in the inner aspect of its right forelimb. The lesion was sutured and local antibiotic (amoxicillin) administered. The three times captured male (RP-C-18) lost its ear tag the third time it fell in the net, cutting its right ear. These three animals account for a 3.16% accidents percentage, which added to mortality figure gives a total incidences percentage of 5.27%.

Animals were visually marked with ear tags (70), coloured plastic collars (24) and/or radio transmitter collars (3). 18 of the 27 collared animals have been observed at least one year after the capture, and one animal was recaptured at the same zone one year after being captured by first and second time twice in a week. No dead animals have been found.

6.1.5. Safety for the operators

Three operators suffered lesions during the 24 capture operations carried out. One operator was wounded in the leg by the horn of a chamois when trying to unwrap it from the net, and two more people suffered lesions in their knee when working on the animals.

6.1.6. Sex and age ratio

A total of 57 males and 22 females of Southern chamois were captured. These figures represent a sex ratio of approximately 3:1 for males, as shown in Figure 6.1.

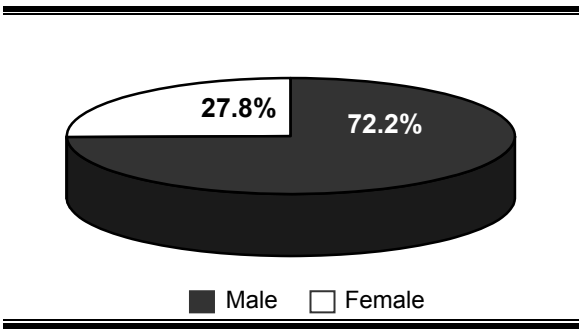


FIGURE 6.1. Sex-ratio of drive-net captured Southern chamois.

Animals from 1 to 14 years were captured, and no age class predominated over the others (Figure 6.2).

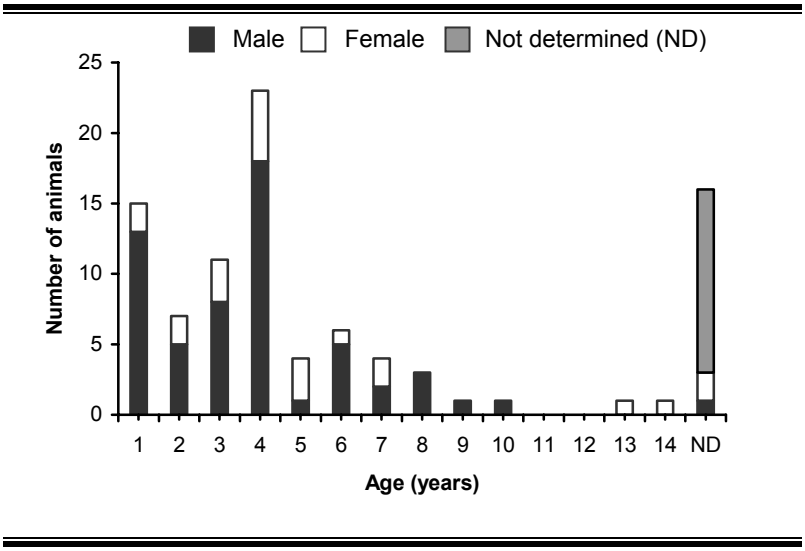


Figure 6.2. Sex and age of drive-net captured Southern chamois.

6.1.7. Group capture

More than one individual were captured in 15 of the 24 capture operations carried out (62.5%), whereas in 5 (20.8%) a single individual was captured and in 4 (16.7%) no Southern chamois was captured (Figure 6.3).

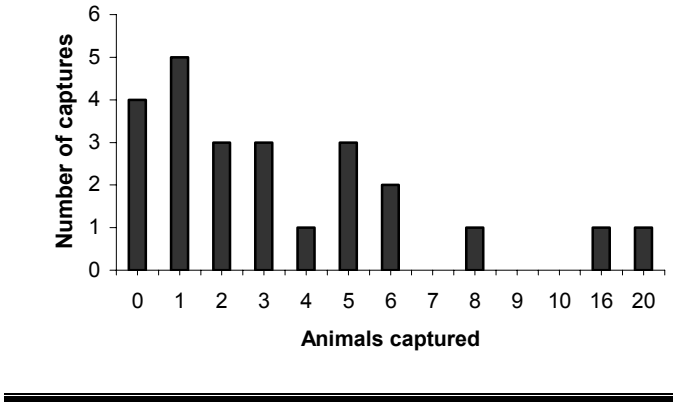


FIGURE 6.3: Performance of drive-net captures of Southern chamois.

All captured Southern chamois are listed in Table 6.2. First blood sample from all animals was used to establish reference values. Animals indicated as Reference in the list were not used for other studies.

Table 6.2: List of captured Southern chamois.

ANIMAL	SEX	AGE	DATE	STUDY	ANIMAL	SEX	AGE	DATE	STUDY
RP-C-1	Male	Adult	20/06/00	Stress	**RP-C-41**	Male	Adult	13/07/01	Stress
RP-C-2	Male	Adult	20/06/00	Stress	**RP-C-42**	Male	Adult	13/07/01	Stress
RP-C-3	Male	Young	20/06/00	Stress	**RP-C-43**	Male	Young	13/07/01	Stress
RP-C-4	Female	Adult	13/07/00	Transport	**RP-C-44**	Male	Adult	18/07/01	Stress
RP-C-5	Female	Adult	13/07/00	Transport	**RP-C-45**	Male	Young	20/07/01	Transport
RP-C-6	Female	Adult	13/07/00	Stress	**RP-C-46**	Male	Young	20/07/01	Transport
RP-C-7	Male	Young	13/07/00	Transport	**RP-C-47**	Male	Adult	20/07/01	Transport
RP-C-8	Female	Adult	13/07/00	Reference	**RP-C-48**	Male	Adult	21/06/02	Transport
RP-C-9	Female	Adult	26/10/00	Transport	**RP-C-49**	Male	Adult	21/06/02	Transport
RP-C-10	Male	Adult	26/10/00	Transport	**RP-C-50**	Male	Adult	21/06/02	Transport
RP-C-11	Male	Adult	06/06/01	Stress	**RP-C-51**	Male	Adult	21/06/02	Transport
RP-C-12	Male	Adult	06/06/01	Stress	**RP-C-52**	Male	Adult	21/06/02	Transport
RP-C-13	Male	Adult	06/06/01	Stress	**RP-C-53**	Male	Adult	25/06/02	Transport
RP-C-14	Male	Adult	06/06/01	Stress	**RP-C-54**	Male	Adult	25/06/02	Transport
RP-C-15	Male	Young	06/06/01	Stress	**RP-C-55**	Male	Young	16/07/02	Transport
RP-C-16	Male	Adult	14/06/01	Stress	**RP-C-56**	Male	Young	07/11/02	Stress
RP-C-17	Male	Adult	19/06/01	Stress	**RP-C-57**	Female	Adult	27/03/03	Stress
RP-C-18	Male	Adult	19/06/01	Stress	**RP-C-58**	Female	Adult	27/03/03	Stress
RP-C-19	Male	Adult	19/06/01	Stress	**RP-C-59**	Female	Young	02/04/03	Stress
RP-C-20	Male	Adult	19/06/01	Stress	**RP-C-60**	Female	Young	02/04/03	Stress
RP-C-21	Male	Adult	19/06/01	Stress	**RP-C-61**	Male	Adult	02/04/03	Stress
RP-C-22	Male	Adult	19/06/01	Reference	**RP-C-62**	Female	Adult	10/04/03	Stress
RP-C-23	Male	Young	19/06/01	Reference	**RP-C-63**	Female	Adult	10/04/03	Stress
RP-C-24	Male	Young	19/06/01	Reference	**RP-C-64**	Female	Adult	10/04/03	Stress
RP-C-25	Male	Adult	19/06/01	Reference	**RP-C-65**	Female	Adult	10/04/03	Stress
RP-C-26	Male	Adult	19/06/01	Reference	**RP-C-66**	Female	Adult	10/04/03	Stress
RP-C-27	Male	Adult	19/06/01	Reference	**RP-C-67**	Male	Adult	10/04/03	Stress
RP-C-28	Male	Adult	19/06/01	Reference	**RP-C-68**	Female	Adult	10/04/03	Stress
RP-C-29	Female	Adult	22/06/01	Stress	**RP-C-69**	Female	Adult	10/04/03	Stress
RP-C-30	Male	Adult	22/06/01	Stress	**RP-C-70**	Female	Adult	10/04/03	Stress
RP-C-31	Male	Adult	22/06/01	Stress	**RP-C-71**	Male	Adult	10/04/03	Stress
RP-C-32	Male	Adult	22/06/01	Stress	**RP-C-72**	Male	Adult	10/04/03	Reference
RP-C-33	Male	Adult	22/06/01	Stress	**RP-C-73**	Male	Adult	30/04/03	Stress
RP-C-34	Male	Adult	25/06/01	Transport	**RP-C-74**	Male	Adult	30/04/03	Stress
RP-C-35	Male	Adult	25/06/01	Transport	**RP-C-75**	Male	Young	30/04/03	Stress
RP-C-36	Male	Adult	25/06/01	Transport	**RP-C-76**	Male	Adult	30/04/03	Stress
RP-C-37	Male	Adult	25/06/01	Transport	**RP-C-77**	Male	Young	30/04/03	Stress
RP-C-38	Male	Adult	25/06/01	Transport	**RP-C-78**	Female	Adult	30/04/03	Reference
RP-C-39	Male	Adult	11/07/01	Stress	**RP-C-79**	Female	Adult	30/04/03	Reference
RP-C-40	Female	Adult	13/07/01	Stress	**RP-C-80**	Female	Adult	30/04/03	Reference

6.2. HAEMATOLOGICAL AND SERUM BIOCHEMICAL REFERENCE VALUES

Tables 6.3 and 6.4 show haematological and serum biochemical parameters for Southern chamois captured by means of drive nets, respectively. Central 95% interval is provided, as recommended (Lumsden, 1998; Walton, 2001), as well as sample size, mean, standard deviation, coefficient of variation and range. Tables 6.5 and 6.6 show haematological and serum biochemical values according to Southern chamois sex, season and age. Age group was only considered in males, since not enough young females were captured to form any group. Statistical differences were found in most of the parameters studied among the different groups. Single range and central interval are given, since range did not vary statistically among groups as mean did.

Table 6.3. Haematological values of Southern chamois captured by means of drive-nets.

PARAMETER	UNIT	N	Mean (SD)	CV (%)	Central 95% interval	Range
Red blood cells	$x10^{12}$/L	71	14.69 (1.42)	9.7	12.12 – 17.20	11.96 – 18.00
Haemoglobin	g/L	70	164.4 (10.4)	6.3	147.0 – 183.8	146 – 186
Haematocrit (PCV)	L/L	73	0.48 (0.03)	7.3	0.43 – 0.56	0.41 – 0.58
MCV	fL	71	33.23 (2.65)	8.0	28.9 – 38.78	27.6 – 40.1
MCHC	g/L	70	344.4 (11.3)	3.3	319 – 362	315 – 368
MCH	pg	70	11.34 (0.80)	7.1	9.9 – 12.9	9.7 – 13.4
Platelets	$x10^9$/L	51	269 (121)	45.0	135 – 538	133 – 589
White blood cells	$x10^9$/L	72	7.53 (3.57)	47.3	2.80 – 14.77	1.60 – 15.50
Lymphocytes	$x10^9$/L	72	5.26 (2.80)	53.3	1.63 – 11.61	1.25 – 13.13
Monocytes	$x10^9$/L	72	0.17 (0.11)	62.4	0.03 – 0.40	0 – 0.43
Neutrophils	$x10^9$/L	72	1.92 (1.09)	56.8	0.55 – 4.29	0.34 – 4.42
Band neutrophils	$x10^9$/L	73	0.01 (0.02)	255.2	0 – 0.07	0 – 0.12
Eosinophils	$x10^9$/L	71	0.14 (0.11)	76.0	0 – 0.35	0 – 0.38
Basophils	$x10^9$/L	73	0.01 (0.02)	310.8	0 – 0.08	0 – 0.13
Lymphocytes	%	73	69.2 (10.0)	14.5	51.3 – 85.1	47.0 – 87.5
Monocytes	%	73	2.4 (1.1)	48.2	0.5 – 4.6	0.0 – 5.0
Neutrophils	%	73	26.1 (9.6)	36.9	10.4 – 44.7	8.0 – 49.5
Band neutrophils	%	73	0.1 (0.3)	241.6	0.0 – 1.0	0.0 – 1.5
Eosinophils	%	71	2.0 (1.8)	89.7	0.0 – 5.5	0.0 – 11.0
Basophils	%	73	0.1 (0.4)	308.0	0.0 – 1.1	0.0 – 2.0

N = Number of samples analyzed; SD = Standard deviation; CV = Coefficient of variation
MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin;
MCHC = Mean Corpuscular Hemoglobin Concentration; PCV = Packed Cell Volume

Table 6.4. Serum biochemical values of Southern chamois captured by means of drive-nets.

PARAMETER	UNIT	N	Mean (SD)	CV (%)	Central 95% interval	Range
Cortisol	nmol/L	70	771.71 (497.00)	64.4	172.83 – 1981.57	93.14 – 2252.67
Cortisol log	nmol/L	73	2.83 (0.34)	11.9	2.24 – 3.48	1.97 – 3.76
Glucose	mmol/L	75	6.79 (2.11)	31.0	2.89 – 10.96	2.10 – 11.13
Cholesterol	mmol/L	70	2.15 (0.60)	27.7	1.14 – 3.34	1.01 – 3.44
Triglycerides	mmol/L	71	1.02 (0.40)	38.9	0.40 – 1.88	0.26 – 2.02
Total bilirubin	mmol/L	75	3.50 (1.48)	42.4	1.71 – 7.01	0.17 – 7.35
Lactate	mmol/L	73	20.75 (4.19)	20.2	12.24 – 27.38	9.45 – 29.62
Creatinine	μmol/L	74	113.01 (24.10)	21.3	70.72 – 159.12	70.72 – 167.96
Urea	mmol/L	75	9.58 (2.58)	27.0	4.55 – 14.16	4.05 – 14.40
ALT	UI/L	70	27.2 (10.6)	39.0	13 – 53	12 – 57
AST	UI/L	66	274.2 (93.2)	34.0	134 – 475	111 – 531
CK	UI/L	68	1093.1 (723.9)	66.2	254 – 2795	228 – 3432
LDH	UI/L	71	1313.1 (418.3)	31.9	769 – 2334	646 – 2338
AP	UI/L	75	465.5 (207.2)	44.5	101 – 893	73 – 964
Chloride	mmol/L	67	111.9 (6.6)	5.9	98.4 – 127.1	97.5 – 128.7
Sodium	mmol/L	64	146.5 (12.5)	8.5	122 – 169	116 – 177
Potassium	mmol/L	70	5.56 (0.90)	16.2	3.9 – 7.2	3.3 – 7.8
Total protein	g/L	75	69.5 (8.9)	12.8	53 – 86	50 – 93
Albumin	g/L	72	35.7 (6.1)	17.2	23.7 – 44.9	21.8 – 49.0
α1a-globulin	g/L	72	1.5 (0.6)	37.9	0.5 – 2.5	0.4 – 2.8
α1b-globulin	g/L	73	2.6 (0.6)	24.4	1.5 – 3.6	1.1 – 4.1
α2-globulin	g/L	73	5.6 (1.0)	17.5	4.0 – 7.6	3.4 – 8.0
β-globulin	g/L	67	2.9 (1.0)	34.0	1.6 – 4.9	1.5 – 5.8
γ-globulin	g/L	70	19.7 (4.9)	24.7	11.2 – 31.2	10.7 – 31.7
A/G ratio	-	73	1.12 (0.28)	25.2	0.65 – 1.66	0.48 – 1.68

N = Number of samples analyzed; SD = Standard deviation; CV = Coefficient of variation
CK = Creatine kinase; LDH = Lactate dehydrogenase; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALP = Alkanine phosphatase; A/G ratio = albumin/globulins

Table 6.5. Sex, age and season differences in haematological values of Southern chamois captured by means of drive-nets.

		SPRING						SUMMER					
		YOUNG MALE		ADULT MALE		ADULT FEMALE		YOUNG MALE		ADULT MALE		ADULT FEMALE	
PARAMETER	**UNIT**	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**N**	**Mean (SD)**
Red blood cells	$x10^{12}/L$	3	13.32 (0.72)^{a}	3	13.26 (0.89)^{a}	9	13.06 (0.81)^{a}	9	15.05 (1.46)^{b}	33	15.27 (1.12)^{b}	5	13.83 (0.54)^{a}
Haemoglobin	g/L	3	161.3 (5.5)^{ab}	3	161.0 (9.2)^{ab}	11	163.5 (8.9)^{ab}	9	161.7 (9.6)^{ab}	32	167.5 (11.5)^{a}	5	157.4 (2.9)^{b}
Haematocrit (PCV)	L/L	3	0.47 (0.015)^{ab}	3	0.46 (0.023)^{ab}	11	0.47 (0.027)^{a}	9	0.48 (0.033)^{ab}	33	0.49 (0.035)^{b}	5	0.47 (0.023)^{ab}
MCV	fL	3	35.57 (1.57)^{a}	3	34.47 (0.64)^{ab}	9	36.42 (2.13)^{a}	9	31.99 (3.57)^{b}	33	32.42 (1.97)^{b}	5	34.28 (1.56)^{ab}
MCHC	g/L	3	341.0 (2.0)^{ab}	3	352.7 (6.7)^{a}	11	350.1 (11.4)^{a}	8	342.8 (13.8)^{ab}	31	343.4 (8.9)^{a}	5	332.8 (16.8)^{b}
MCH	pg	3	12.13 (0.47)^{ac}	3	12.13 (0.29)^{ac}	8	12.64 (0.46)^{a}	9	10.79 (0.78)^{b}	33	11.04 (0.54)^{b}	5	11.38 (0.26)^{bc}
Platelets	$x10^{9}/L$	3	174 (64)^{a}	3	180 (37)^{a}	5	218 (45)^{a}	5	276 (146)^{ab}	23	272 (125)^{a}	5	398 (127)^{b}
White blood cells	$x10^{9}/L$	3	7.93 (1.64)^{ab}	3	3.30 (0.63)^{a}	11	6.40 (2.31)^{a}	9	10.40 (2.88)^{b}	33	6.61 (3.53)^{a}	4	12.25 (3.26)^{b}
Lymphocytes	$x10^{9}/L$	3	5.17 (0.33)^{ab}	3	2.46 (0.81)^{a}	11	4.06 (1.86)^{a}	9	6.95 (2.24)^{b}	33	4.56 (2.60)^{a}	4	9.96 (3.48)^{c}
Monocytes	$x10^{9}/L$	3	0.14 (0.13)	3	0.09 (0.03)	11	0.16 (0.11)	9	0.21 (0.08)	33	0.16 (0.10)	4	0.18 (0.17)
Neutrophils	$x10^{9}/L$	3	2.55 (1.53)^{ac}	3	0.68 (0.18)^{b}	11	2.06 (0.86)^{ac}	8	2.77 (1.04)^{a}	33	1.69 (1.01)^{bc}	5	2.29 (1.17)^{ac}
Band neutrophils	$x10^{9}/L$	3	0 (0)^{ab}	3	0.01 (0.01)^{ab}	11	0 (0)^{a}	9	0.01 (0.01)^{ab}	33	0.02 (0.03)^{b}	5	0 (0)^{ab}
Eosinophils	$x10^{9}/L$	3	71 (74)	3	57 (85)	11	112 (95)	9	137 (106)	32	155 (112)	4	211 (110)
Basophils	$x10^{9}/L$	3	0 (0)	3	9 (16)	11	6 (20)	9	0 (0)	33	12 (30)	5	0 (0)
Lymphocytes	%	3	67.0 (13.5)^{ab}	3	73.3 (12.5)^{ab}	11	62.5 (9.0)^{a}	9	67.1 (10.2)^{a}	33	68.5 (8.6)^{a}	5	79.4 (7.8)^{b}
Monocytes	%	3	1.7 (1.3)	3	2.8 (1.3)	11	2.5 (1.5)	9	2.2 (1.0)	33	2.5 (1.1)	5	1.8 (1.5)
Neutrophils	%	3	30.5 (11.7)^{ab}	3	21.3 (8.4)^{ac}	11	32.9 (8.3)^{b}	9	29.4 (10.4)^{ab}	33	25.7 (7.6)^{a}	5	16.7 (7.6)^{c}
Band neutrophils	%	3	0.0 (0.0)^{ab}	3	0.2 (0.3)^{ab}	11	0.0 (0.0)^{a}	9	0.1 (0.2)^{ab}	33	0.2 (0.4)^{b}	5	0.0 (0.0)^{ab}
Eosinophils	%	3	0.8 (0.8)	3	2.0 (3.0)	11	2.1 (1.7)	9	1.2 (0.9)	30	2.3 (1.6)	5	2.1 (1.3)
Basophils	%	3	0.0 (0.0)	3	0.3 (0.6)	11	0.1 (0.3)	9	0.0 (0.0)	33	0.2 (0.5)	5	0.0 (0.0)

N = Number of samples analyzed; SD = Standard deviation

MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; PCV = Packed Cell Volume

$^{a, b, c}$ Means with different superscript are significantly different from each other (at least $P<0.05$)

Table 6.6. Sex, age and season differences in serum biochemical values of Southern chamois captured by means of drive-nets.

		SPRING						SUMMER					
		YOUNG MALE		ADULT MALE		ADULT FEMALE		YOUNG MALE		ADULT MALE		ADULT FEMALE	
PARAMETER	UNIT	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
Cortisol	nmol/L	3	929.3 (210.5)^{ab}	3	694.4 (491.0)^{a}	11	802.8 (549.5)^{a}	8	795.7 (474.0)^{a}	3	604.8 (342.8)^{a}	5	1528.7 (404.8)^{b}
Cortisol log	nmol/L	3	2.96 (0.10)^{a,b}	3	2.75 (0.36)^{a,b}	11	2.82 (0.27)^{a,b}	8	2.84 (0.26)^{a,b}	3	2.75 (0.35)^{a}	5	3.17 (0.13)^{b}
Glucose	mmol/L	3	7.31 (1.22)^{ab}	3	6.76 (3.78)^{ab}	11	7.84 (2.23)^{a}	9	6.34 (1.67)^{ab}	3	6.33 (2.12)^{b}	5	8.03 (2.12)^{ab}
Cholesterol	mmol/L	3	2.53 (0.23)^{a}	3	2.36 (0.22)^{ab}	10	2.52 (0.40)^{a}	8	1.75 (0.60)^{b}	3	1.92 (0.47)^{b}	5	2.96 (0.82)^{a}
Triglycerides	mmol/L	3	0.77 (0.45)^{a}	3	0.91 (0.37)^{ab}	11	0.85 (0.12)^{a}	9	0.79 (0.51)^{a}	3	1.23 (0.41)^{b}	5	0.91 (0.26)^{ab}
Total bilirubin	µmol/L	3	4.62 (0.51)^{a}	3	3.9 (1.37)^{ab}	11	3.08 (1.71)^{ab}	9	2.74 (1.20)^{b}	3	3.59 (1.37)^{ab}	5	2.74 (1.71)^{ab}
Lactate	mmol/L	2	18.73 (3.92)^{ab}	3	16.38 (7.54)^{a}	10	20.37 (2.74)^{ab}	9	18.46 (2.88)^{a}	3	21.67 (4.40)^{b}	5	23.11 (1.93)^{b}
Creatinine	µmol/L	3	114.92 (8.84)^{ac}	3	147.63 (10.61)^{b}	11	137.02	9	99.01 (25.64)^{a}	3	106.96 (21.22)^{a}	5	102.54 (9.72)^{a}
Urea	mmol/L	3	8.49 (1.43)^{ab}	3	9.41 (4.95)^{ab}	11	8.20 (3.20)^{a}	9	7.81 (1.70)^{a}	3	10.88 (1.98)^{b}	5	8.36 (1.37)^{a}
ALT	UI/L	3	21.0 (3.0)^{ab}	3	19.3 (2.5)^{ab}	11	20.2 (6.1)^{a}	9	28.6 (11.4)^{b}	3	27.7 (9.7)^{b}	3	48.7 (6.8)^{c}
AST	UI/L	3	259.0 (72.7)^{ab}	3	335.0 (136.8)^{ab}	11	260.6 (57.2)^{a}	8	250.5 (74.0)^{a}	3	275.7 (108.8)^{a}	2	420.0 (69.3)^{b}
CK	UI/L	3	1185.0 (503.3)^{ab}	3	1373.0 (427.4)^{ab}	10	751.6 (453.8)^{a}	8	965.0 (807.6)^{ab}	3	1041.8 (750.5)^{ab}	5	1545.2 (735.4)^{b}
LDH	UI/L	3	1321.3 (155.0)^{abc}	3	1033.0 (406.3)^{bc}	11	928.6 (151.0)^{c}	8	1538.9 (452.0)^{a}	3	1360.0 (357.1)^{ab}	4	2089.8 (375.8)^{d}
AP	UI/L	3	543.0 (287.2)^{a}	3	396.0 (41.7)^{ab}	11	248.1 (119.6)^{b}	9	562.7 (149.6)^{a}	3	469.1 (188.3)^{a}	5	604.4 (190.3)^{a}
Chloride	mmol/L	3	108.2 (3.9)^{ab}	3	111.7 (3.3)^{ab}	11	112.7 (5.4)^{ab}	8	107.2 (5.2)^{a}	2	113.3 (7.5)^{b}	4	109.8 (9.2)^{ab}
Sodium	mmol/L	3	139.3 (2.1)^{a}	3	146.3 (13.7)^{ab}	11	145.8 (8.3)^{ab}	6	146.7 (10.6)^{ab}	2	144.21 (12.4)^{a}	4	159.5 (20.6)^{b}
Potassium	mmol/L	3	5.57 (0.21)^{ab}	3	5.83 (0.96)^{ab}	11	6.02 (0.72)^{a}	7	5.39 (0.87)^{ab}	3	5.23 (0.89)^{b}	5	5.76 (0.82)^{ab}
Total protein	g/L	3	67.0 (4.6)^{ab}	3	68.3 (5.0)^{ab}	11	73.2 (7.2)^{a}	9	64.2 (8.8)^{b}	3	69.3 (9.2)^{ab}	5	74.0 (13.0)^{ab}
Albumin	g/L	3	32.3 (4.8)	3	34.9 (5.5)	11	38.2 (6.6)	8	33.0 (6.6)	3	35.4 (6.4)	4	34.7 (7.9)
α1a-globulin	g/L	3	1.3 (0.6)	3	1.6 (0.1)	11	1.5 (0.4)	8	1.5 (0.6)	3	1.4 (0.6)	4	1.4 (0.9)
α1b-globulin	g/L	3	2.9 (0.4)	3	2.6 (0.6)	11	2.7 (0.5)	8	2.6 (0.7)	3	2.4 (0.7)	4	2.8 (0.4)
α2-globulin	g/L	3	5.6 (1.3)^{ab}	3	5.6 (0.7)^{ab}	11	5.3 (0.9)^{a}	8	4.7 (0.7)^{a}	3	6.0 (0.9)^{b}	4	5.1 (0.7)^{ab}
β-globulin	g/L	2	2.9 (0.0)	3	2.2 (0.3)	11	2.5 (0.7)	7	2.3 (0.4)	3	3.1 (1.2)	4	2.8 (0.7)
γ-globulin	g/L	3	18.3 (1.4)^{a}	3	20.0 (9.3)^{ab}	10	21.1 (2.7)^{a}	8	19.5 (3.4)^{a}	3	19.6 (4.8)^{a}	3	27.5 (7.1)^{b}
A/G ratio	-	3	0.99 (0.23)	3	1.17 (0.43)	11	1.13 (0.24)	8	1.06 (0.19)	3	1.13 (0.31)	4	0.86 (0.12)

N = Number of samples analyzed; SD = Standard deviation; A/G ratio = albumin/globulins

$^{a, b, c, d}$ Means with different superscript are significantly different from each other (at least P<0.05).

6.3. PHYSICAL CAPTURE AND RESTRAINT STRESS

6.3.1. Stress

Heart rate

Heart rate was superior in the treated group only at minute 5, and in control and treated groups from the beginning, but stabilized earlier in the treated group (35 minutes) than in the control group (80 minutes) (Figure 6.4). Individual variability of heart rate did not differ between control (16.41 ± 4.92% [mean ± sd]) and treated (16.09 ± 4.09% [mean ± sd]) group, but interindividual coefficient of variation was statistically higher in the control group (22.07 ± 4.21% [mean ± sd]) than in the treated group (18.30 ± 5.28% [mean ± sd]).

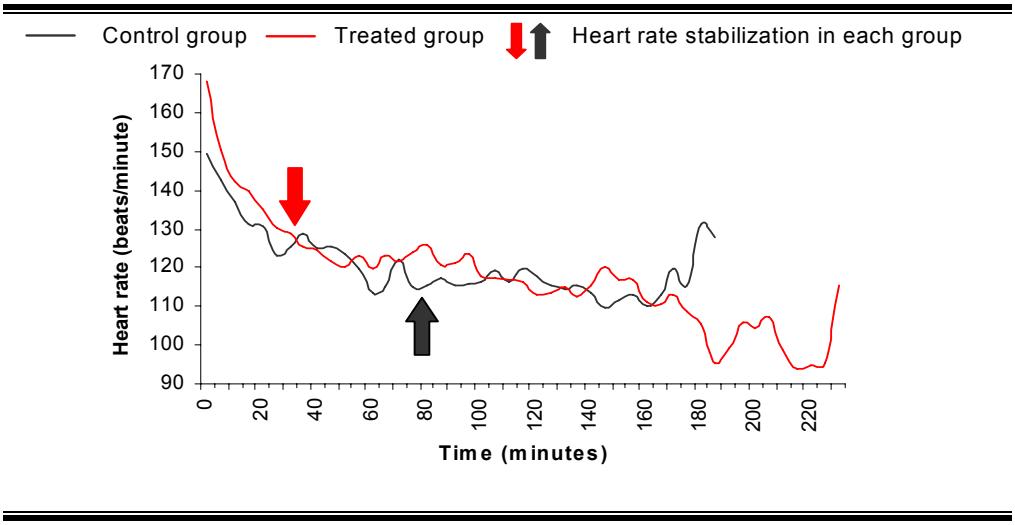


Figure 6.4. Heart rate of physically restrained Southern chamois.

Temperature

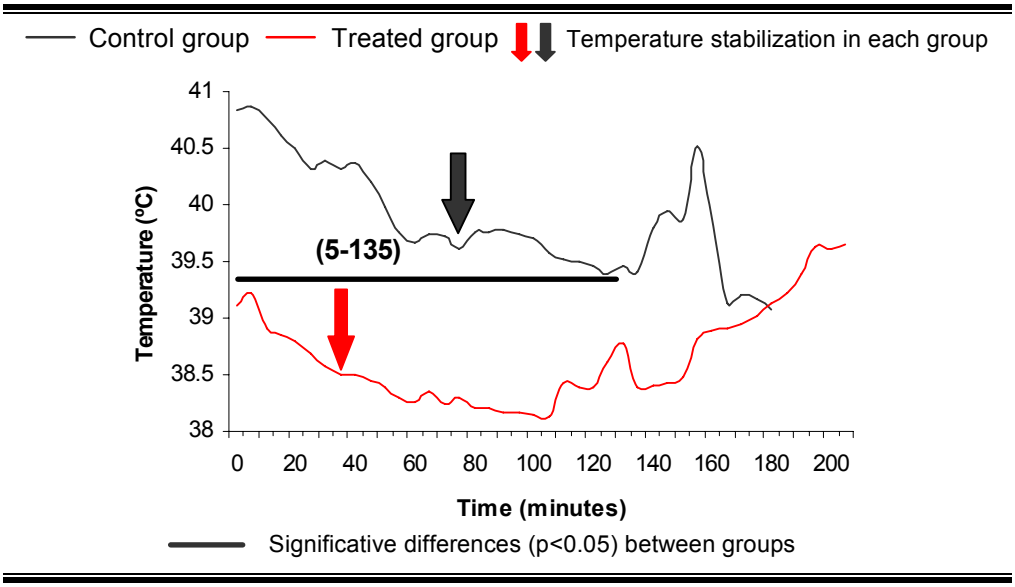


Figure 6.5. Rectal temperature of physically restrained Southern chamois.

Body temperature was higher in the control group from the start of the monitoring to minute 135. Body temperature decreased over time in both groups, but stabilized earlier in the treated group (35 minutes) than in the control group (75 minutes) (Figure 6.5).

Haematology

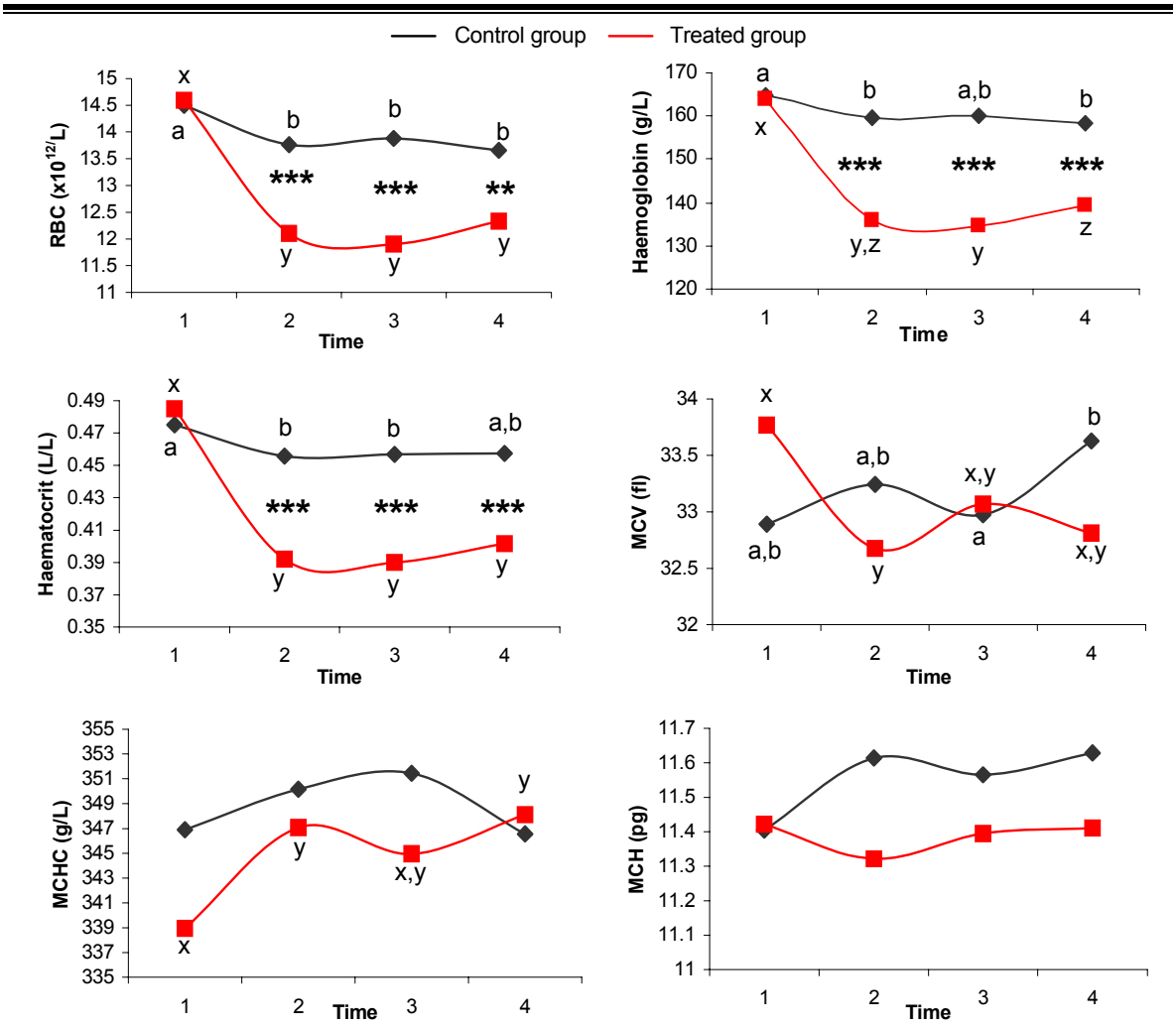
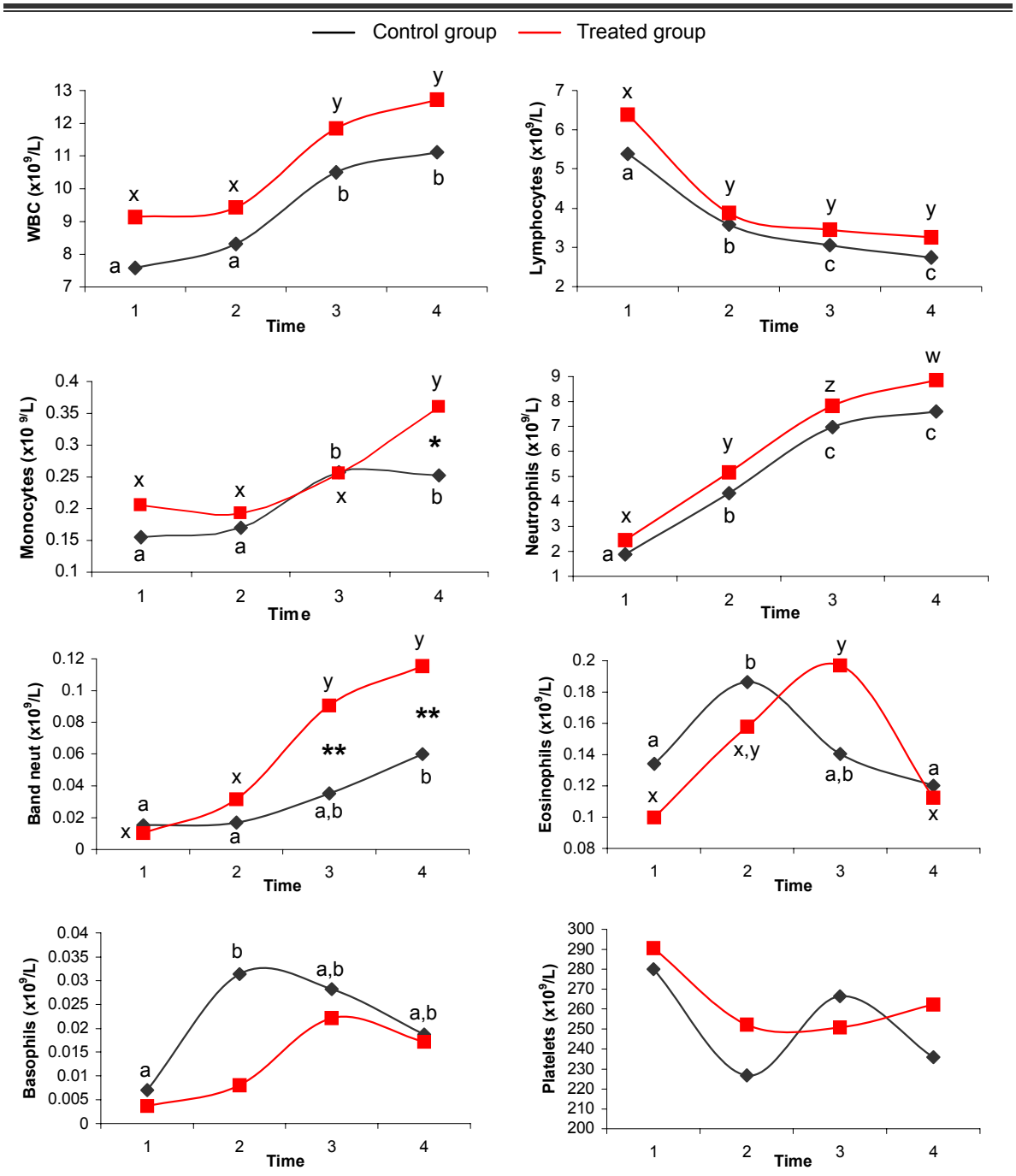


Figure 6.6: Erythrocyte parameters of physically restrained Southern chamois.

RBC, hemoglobin concentration and hematocrit decreased over time in both groups, and were significantly lower in the treated group from time 2 onwards. MCV decreased from time 1 to time 2 in the treated group and increased from time 3 to time 4 in the control group, whereas MCHC increased over time only in the treated group (Figure 6.6). WBC, monocyte, neutrophil, and band neutrophil counts increased over time in both groups, whereas lymphocyte count followed the opposite trend. Higher values at time 4 for monocytes and at

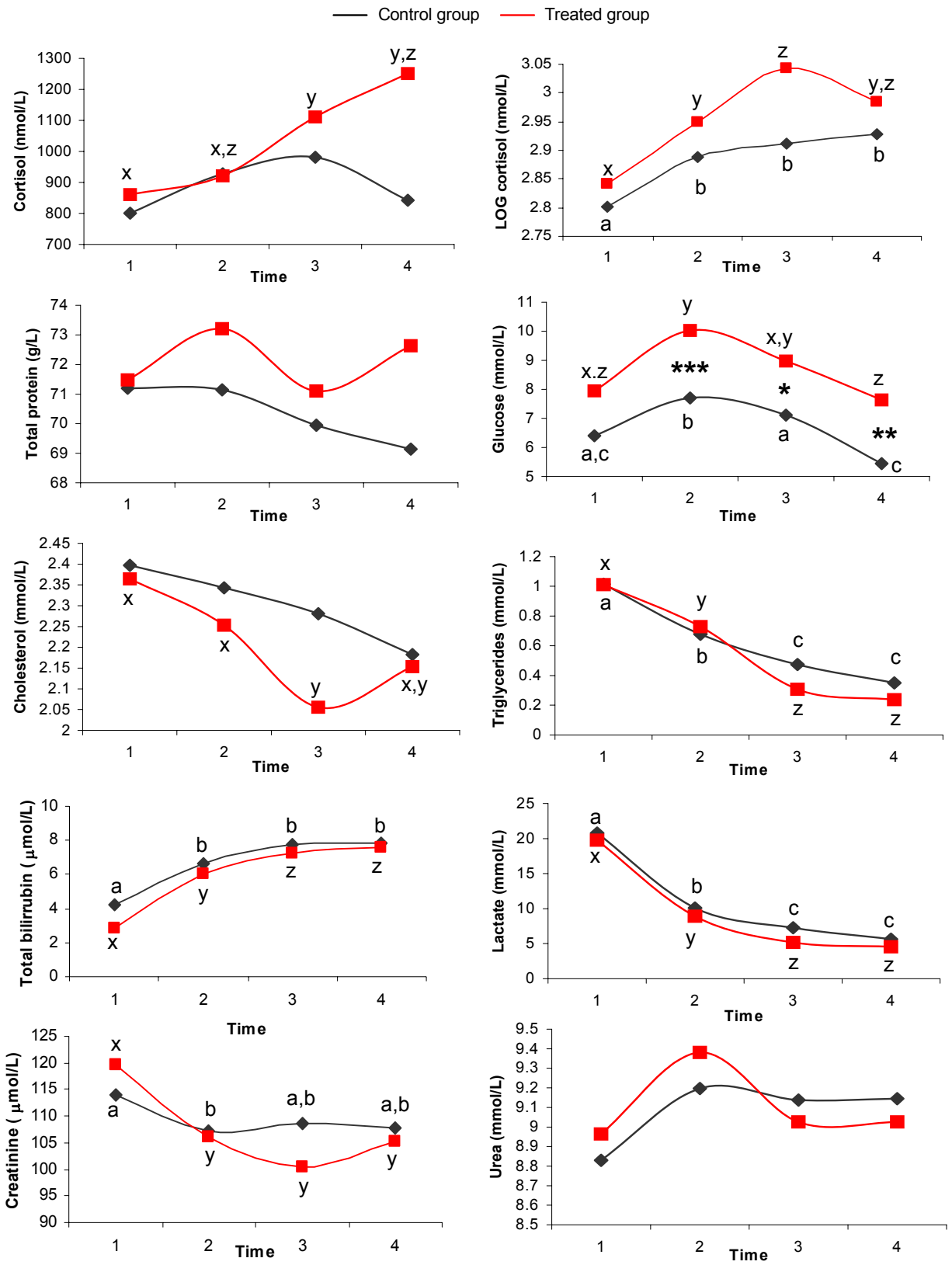
times 3 and 4 for band neutrophils were found in the treated group. Eosinophils increased over time and then decreased in both groups, but reached the maximum before in the control group (time 2) than in the treated group (time 3). Basophils increased from time 1 to time 2 only in the control group. Platelet count decreased from time 1 to time 2 only in the control group (Figure 6.7).



* Values are significantly different from each other between groups (* p<0.05; **p<0.01).
Means with different superscript are significantly different from each other in the same group

Figure 6.7: Leukocytes and platelets of physically restrained Southern chamois.

Serum biochemistry



* Values are significantly different from each other between groups (*p<0.05;**p<0.01;***p<0.005).
Means with different superscript are significantly different from each other in the same group

Figure 6.8. Cortisol and metabolites of physically restrained Southern chamois.

Serum cortisol concentration increased over time only in the treated group, but cortisol logarithm increased in both groups, although increase was more prolonged in the treated group. Serum glucose concentration increased to reach it maximum at time 2 and then decreased until time 4 in both groups, and treated group showed statistically higher values than control group from time 2 to time 4. Serum cholesterol concentration decreased from time 1 to time 3 only in the treated group. Serum triglycerides and lactate concentration decreased over time in both groups, whereas serum bilirubin concentration followed the opposite trend. Serum creatinine concentration decreased throughout the study in the treated group, but it only decreased from time 1 to time 2 in the control group (Figure 6.8).

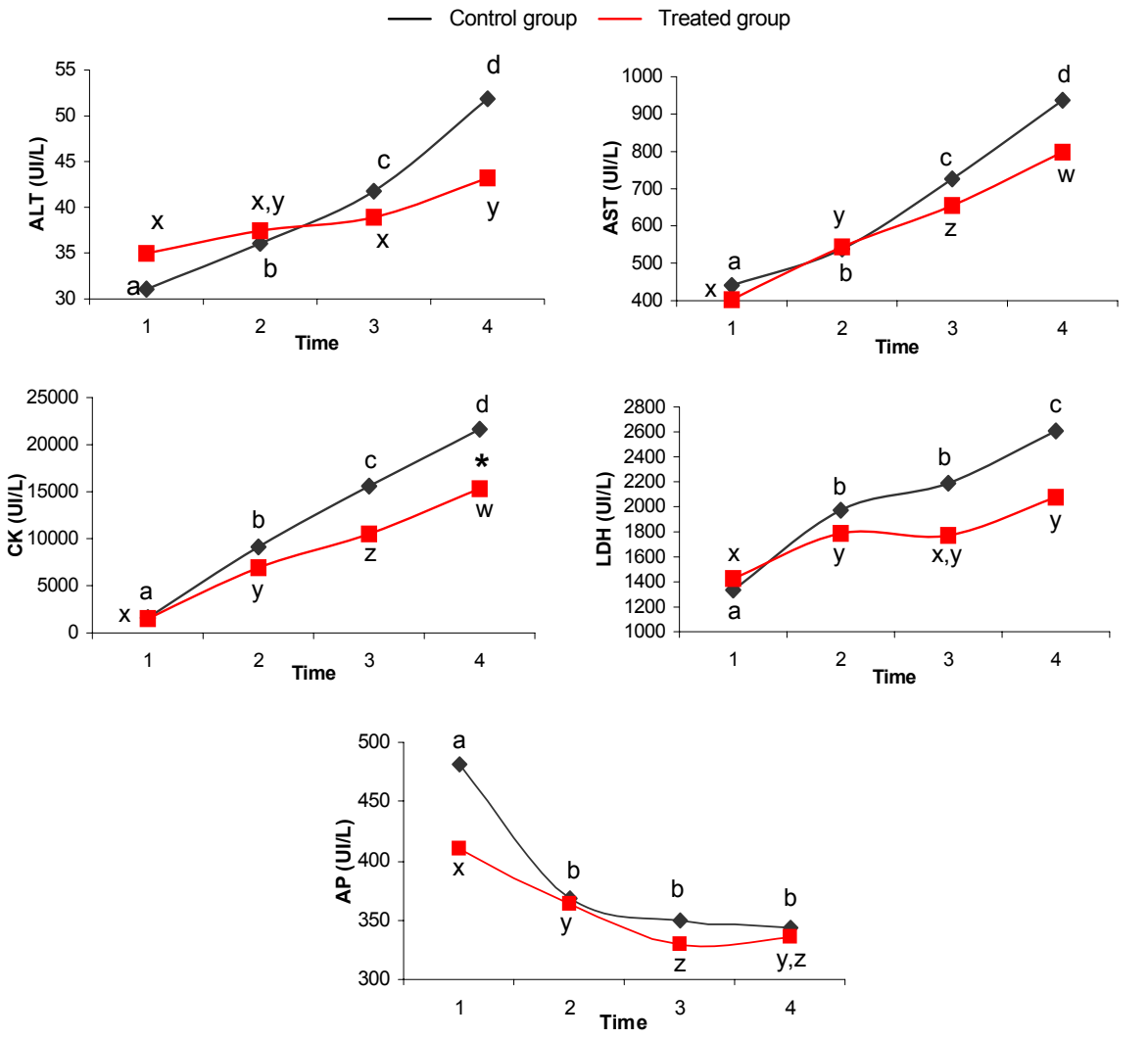
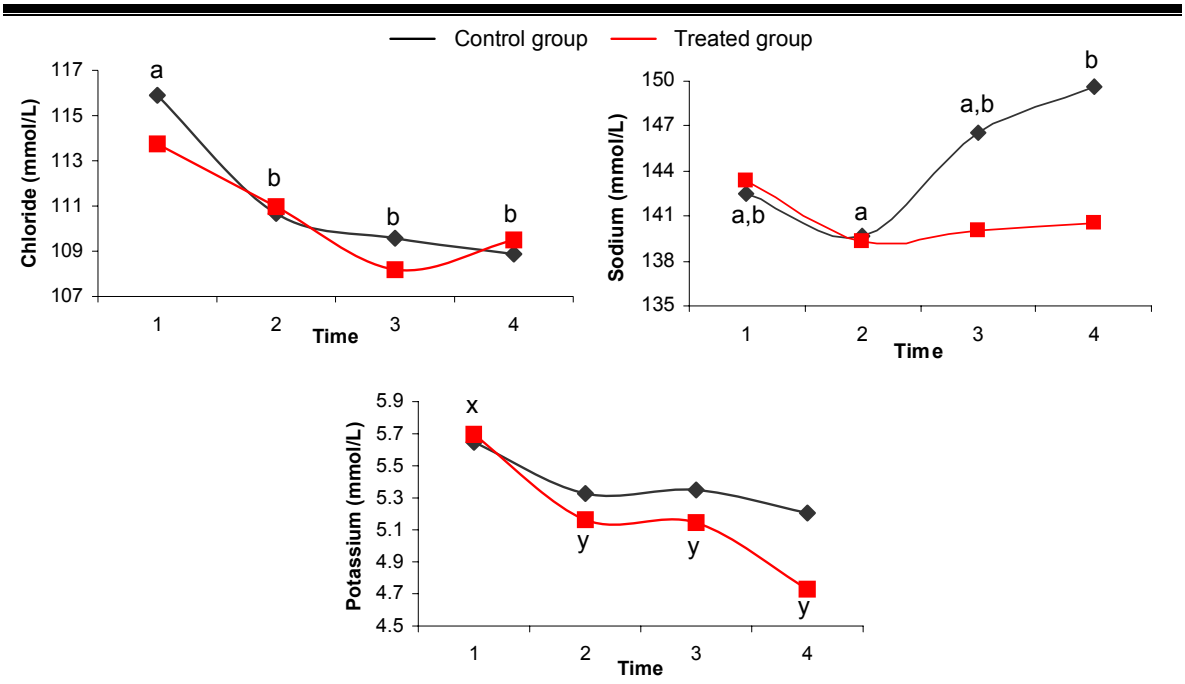


Figure 6.9. Enzyme activities of physically restrained Southern chamois.

Serum alanine aminotransferase (ALT) activity increased significantly in every period in the control group, whereas it only increased from time 3 to time 4 in the treated group. Serum aspartate aminotransferase (AST) and creatine kinase (CK) activities increased in every period in both groups, and significantly higher values for CK at time 4 were found in the control group. Serum L-lactate dehydrogenase (LDH) activity increased from time 1 to time 2 and from time 3 to time 4 in the control group, but it only increased from time 1 to times 2 and 4 in the treated group. Serum alkaline phosphatase (AP) activity decreased over time in both groups, but stabilized earlier in the control group (time 2) than in the treated group (time 3) (Figure 6.9).



Means with different superscript are significantly different from each other in the same group

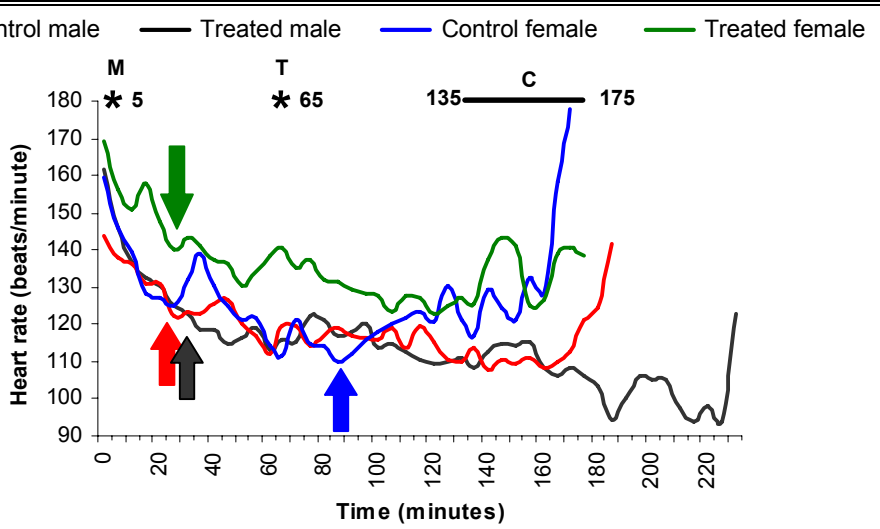
Figure 6.10. Electrolytes of physically restrained Southern chamois.

Serum chloride concentration decreased over time in the control group and remained stable in the treated group. Serum sodium concentration followed the opposite trend, remaining also stable in the treated group but increasing from time 2 to time 4 in the control group. Serum potassium concentration decreased only in the treated group, and remained stable in the control group (Figure 6.10).

6.3.2. Sex differences

Heart rate

Sex group data showed a decreasing heart rate that stabilized between minutes 25 and 30 in all groups but control females, whose heart rate did not stabilized until minute 85. Differences between control and treated animals within sex groups were found only in males at minute 5, but control females showed a superior heart rate than control males from minute 135 to minute 175. Differences between treated males and females were only present at minute 65 (Figure 6.11).



M Statistical differences between control and treated males; **T** Statistical differences between treated males and females; **C** Statistical differences between control males and females.

Heart rate stabilization in each group.

Figure 6.11. Heart rate of restrained Southern chamois according to sex and treatment.

Temperature

Body temperature stabilized later in both control groups (minute 55 for both sexes) than in treated groups (minute 10 for females and minute 35 for males), and increased at the end of the study period, during pre-release manipulation, in control males and treated females. Body temperature was lower in treated females than in control females during almost all the study period (from minute 5 to minute 125), whereas males only showed this difference from minute 5 to minute 55 and at minute 160. Treated females showed statistically significant lower temperatures than treated males from minute 15 to minute 30 and from minute 80 to minute 125 (Figure 6.12).

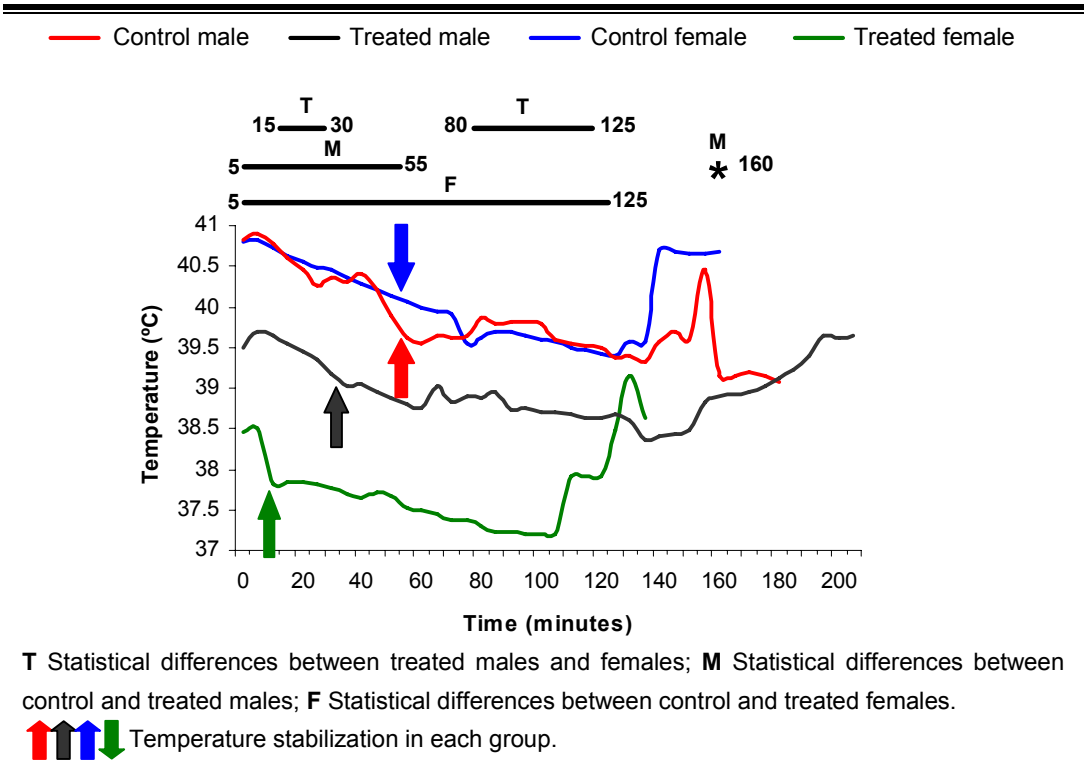


Figure 6.12: Rectal temperature of restrained chamois according to sex and treatment.

Haematology and serum biochemistry

Treatment effects on the parameters studied were influenced by sex, which is represented in Figures 6.13 to 6.20. Serum cortisol concentration and ALT activity increased and serum triglycerides and creatinine decreased over time in both groups of males, remaining stable in the groups of females except for creatinine, which increased from time 1 to time 2 in control females. RBC, hemoglobin concentration, hematocrit, lymphocyte count and potassium concentration decreased over time in all groups except in control females. MCV increased over time only in control females, and monocyte count increased from time 3 to time 4 in all groups but control females. When expressed as time 1 ratio due to differences between both female groups at time 1, MCHC also increased in all groups but control females. Serum bilirubin concentration reached its maximum before in control females (time 2) than in the other three groups (time 3). Serum AST and LDH activities and glucose and sodium concentrations increased and serum AP activity decreased over time in all groups but treated females. Serum CK activity increased in all groups, with control males showing statistically significant increases for each interval, control females and treated males lacking increases from time 2 to time 3 and treated females showing statistical differences only from time 1 to time 4. Eosinophils increased from time 1 to time 2 only in control males, and serum

chloride concentration decreased over time in treated males. MCHC absolute value increased in control males and treated females. Serum total protein concentration increased in treated males and decreased in treated females from time 1 to time 2.

Sex differences were stronger than treatment effects in some of the parameters studied. Both groups of females had higher MCV, MCH and creatinine values and lower urea concentrations than the respective male groups throughout the study. At the beginning of the study, females had higher total protein concentration (both treated and control groups), MCHC and potassium concentration (control groups), and cholesterol concentration (treated groups) than males. On the other hand, males had superior RBC (both groups), triglycerides concentration (control groups) and AP activity (treated groups) than females at time 1. Females showed higher values than males during the study period for hemoglobin and bilirubin (control groups) and cholesterol, sodium and potassium (treated groups) concentrations. Treated females had higher MCHC, band neutrophils count, and glucose and chloride concentrations and lower serum ALT, AST, CK and LDH activities than treated males at the end of the study period (time 4). Similar chloride differences were also present in the control groups.

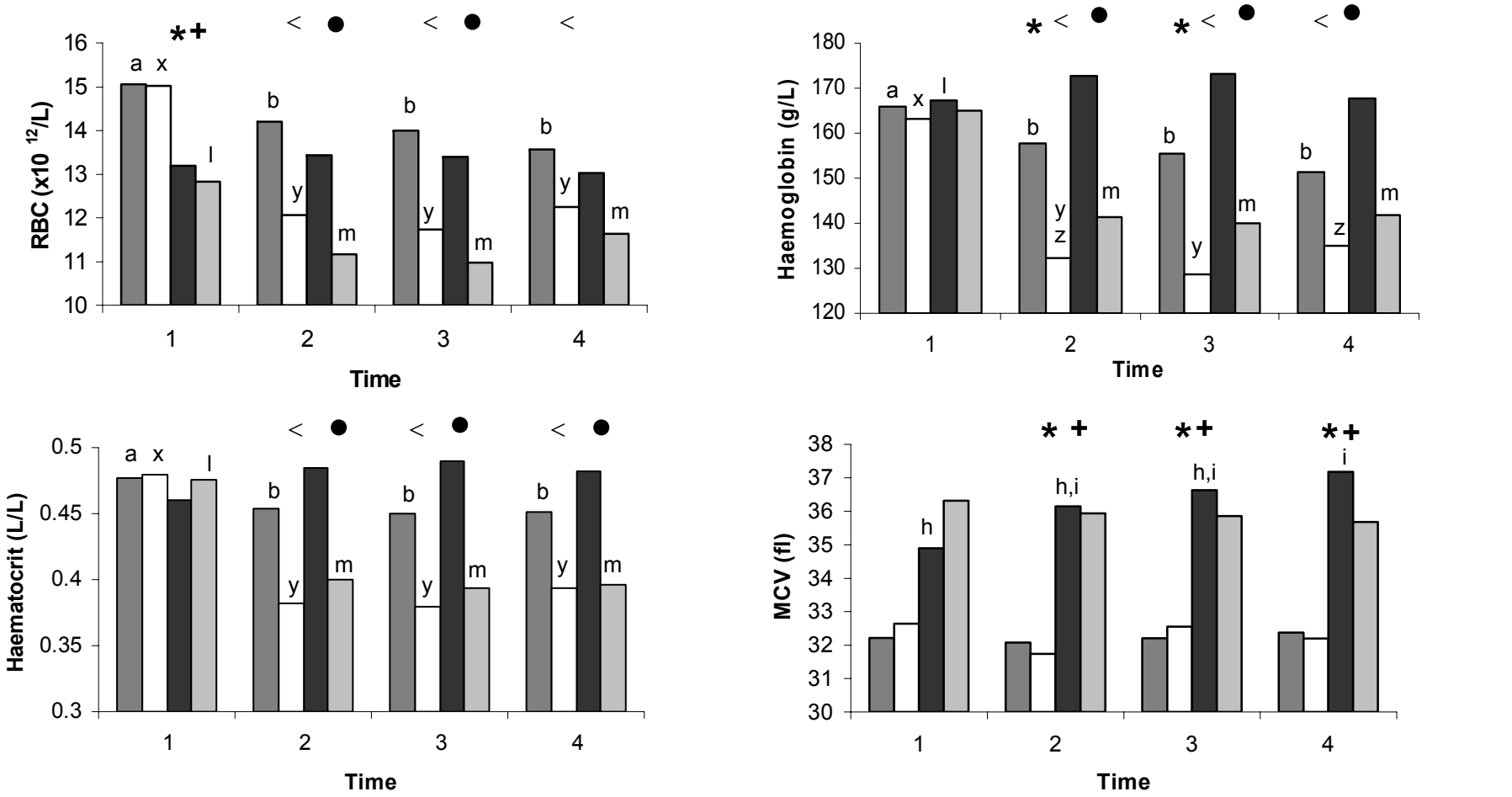


Figure 6.13: RBC, haematocrit, haemoglobin and MCV of Southern chamois. Control males; Treated males; Control females; Treated females. ✱ Statistical differences ($p<0.05$) between control males and control females; ✚ Statistical differences ($p<0.05$) between treated males and treated females; < Statistical differences ($p<0.05$) between control males and treated males; ● Statistical differences ($p<0.05$) between control females and treated females. Columns with different superscript are significantly different ($p<0.05$) from each other in the same sex and treatment group.

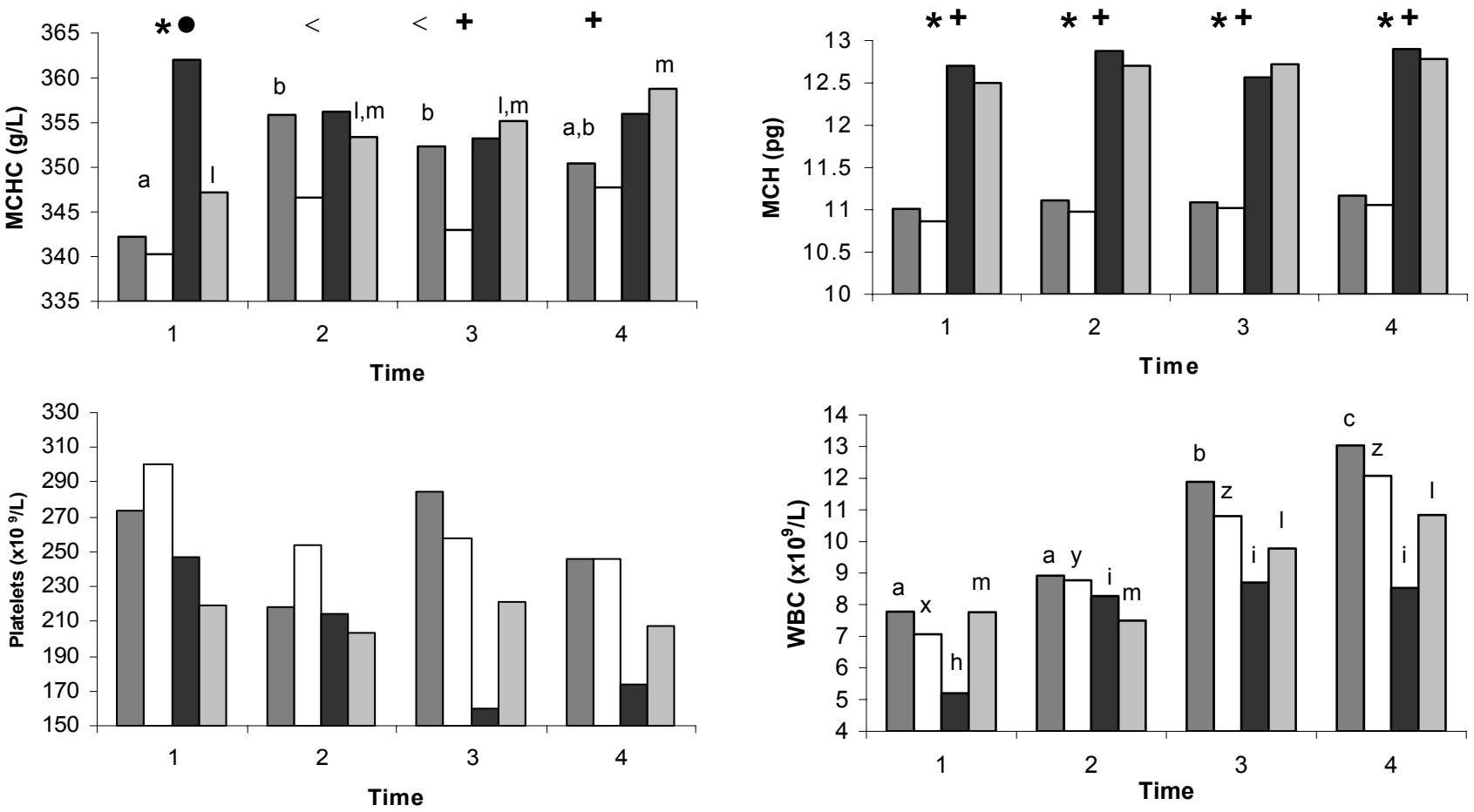


Figure 6.14: MCHC, MCH, platelet and leukocyte counts of Southern chamois. Control males; Treated males; Control females; Treated females. ✱ Statistical differences ($p<0.05$) between control males and control females; ✚ Statistical differences ($p<0.05$) between treated males and treated females; < Statistical differences ($p<0.05$) between control males and treated males; ● Statistical differences ($p<0.05$) between control females and treated females. Columns with different superscript are significantly different ($p<0.05$) from each other in the same sex and treatment group.

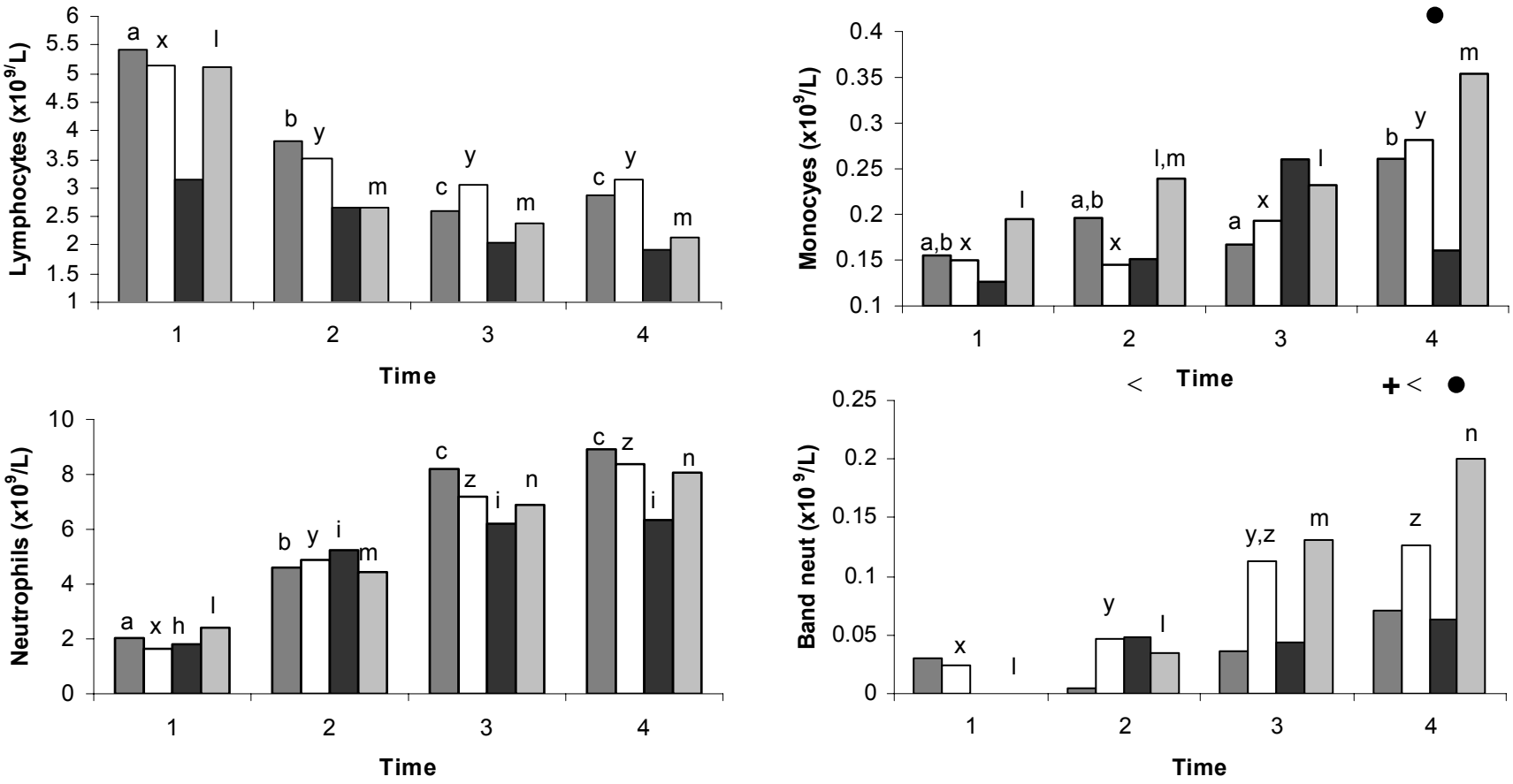


Figure 6.15: Lymphocytes, monocytes and neutrophils of Southern chamois. Control males; Treated males; Control females; Treated females.
✱ Statistical differences ($p<0.05$) between control males and control females; ✚ Statistical differences ($p<0.05$) between treated males and treated females;
< Statistical differences ($p<0.05$) between control males and treated males; ● Statistical differences ($p<0.05$) between control females and treated females.
Columns with different superscript are significantly different ($p<0.05$) from each other in the same sex and treatment group.

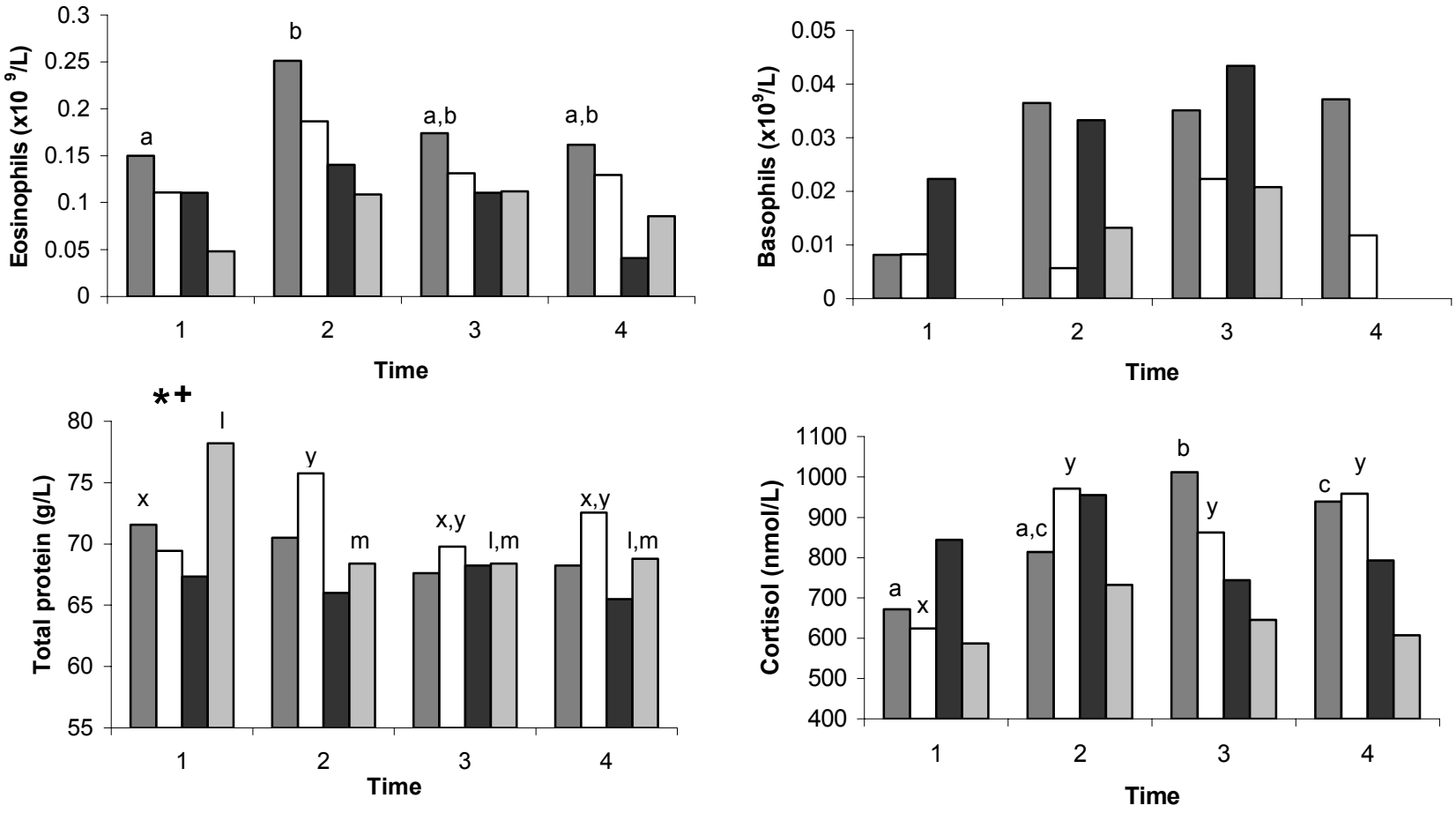


Figure 6.16: Eosinophils, basophils, protein and cortisol of Southern chamois. Control males; Treated males; Control females; Treated females.
* Statistical differences ($p<0.05$) between control males and control females; + Statistical differences ($p<0.05$) between treated males and treated females;
< Statistical differences ($p<0.05$) between control males and treated males; ● Statistical differences ($p<0.05$) between control females and treated females.
Columns with different superscript are significantly different ($p<0.05$) from each other in the same sex and treatment group.

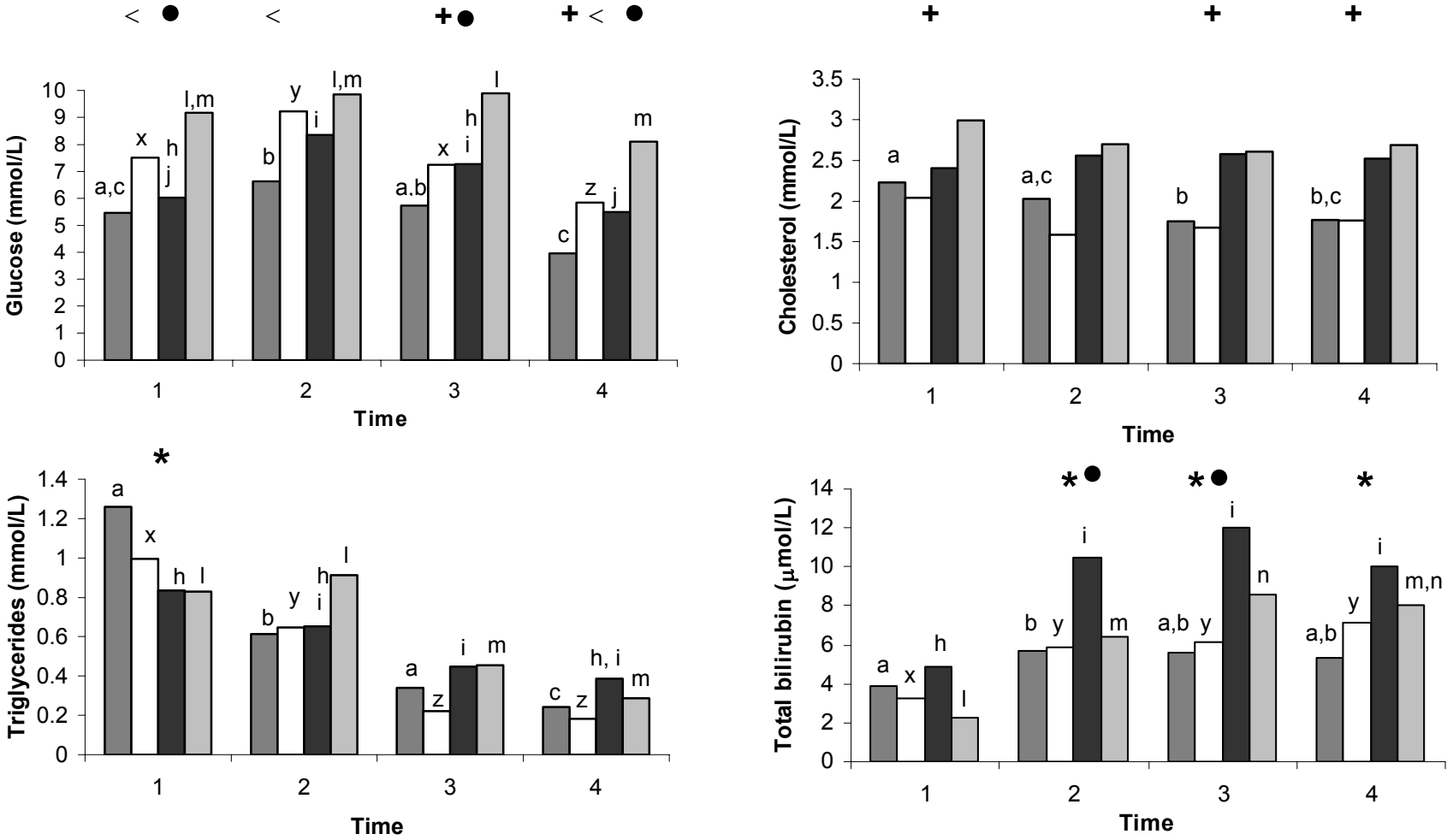


Figure 6.17: Glucose. lipid and bilirubin concentrations of Southern chamois. Control males; Treated males; Control females; Treated females.
✱ Statistical differences (p<0.05) between control males and control females; ✚ Statistical differences (p<0.05) between treated males and treated females;
< Statistical differences (p<0.05) between control males and treated males; ● Statistical differences (p<0.05) between control females and treated females.
Columns with different superscript are significantly different (p<0.05) from each other in the same sex and treatment group.

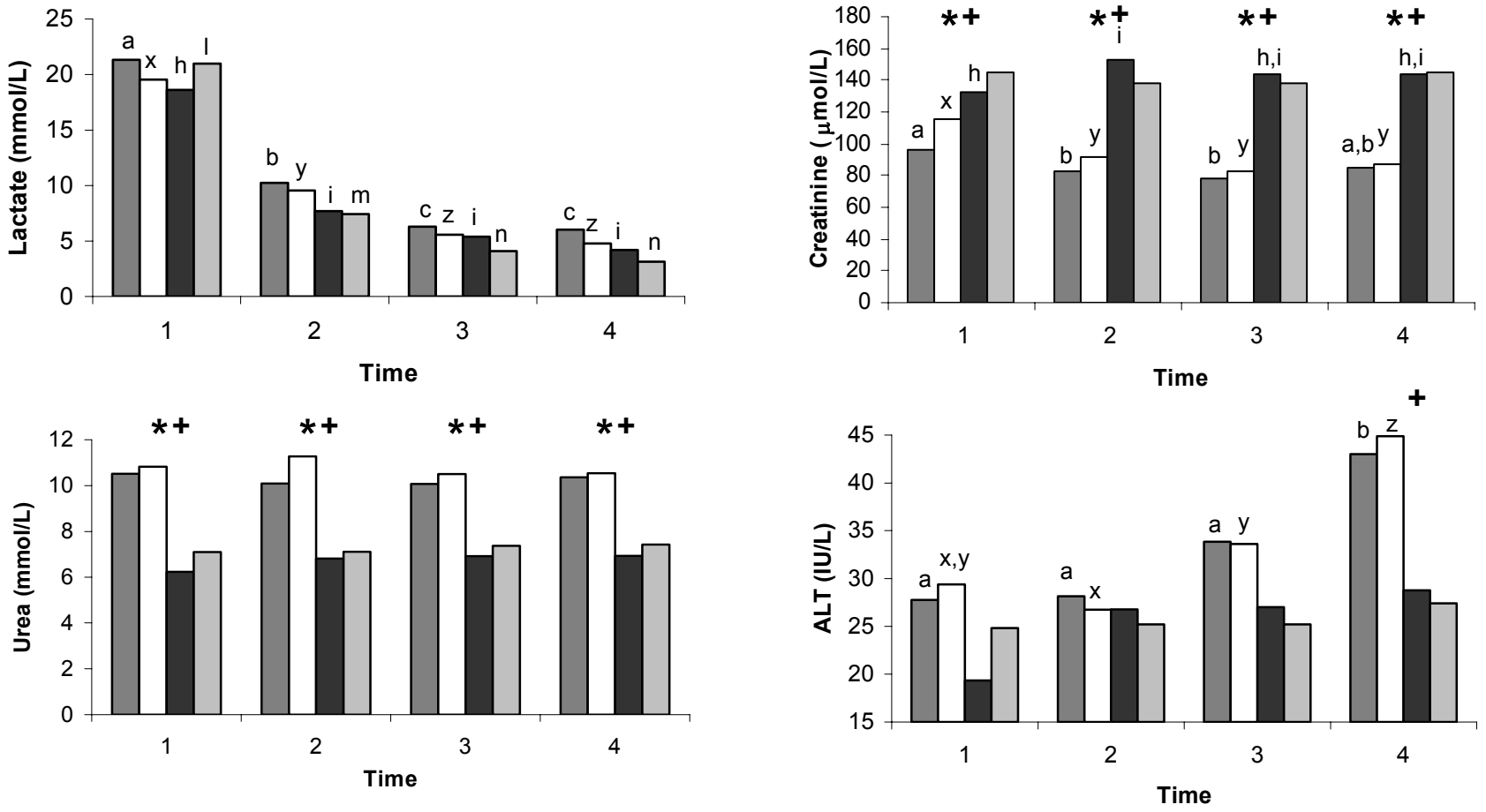


Figure 6.18: Lactate, creatinine, urea and ALT of Southern chamois. Control males; Treated males; Control females; Treated females.
✱ Statistical differences ($p<0.05$) between control males and control females; ✚ Statistical differences ($p<0.05$) between treated males and treated females;
< Statistical differences ($p<0.05$) between control males and treated males; ● Statistical differences ($p<0.05$) between control females and treated females.
Columns with different superscript are significantly different ($p<0.05$) from each other in the same sex and treatment group.

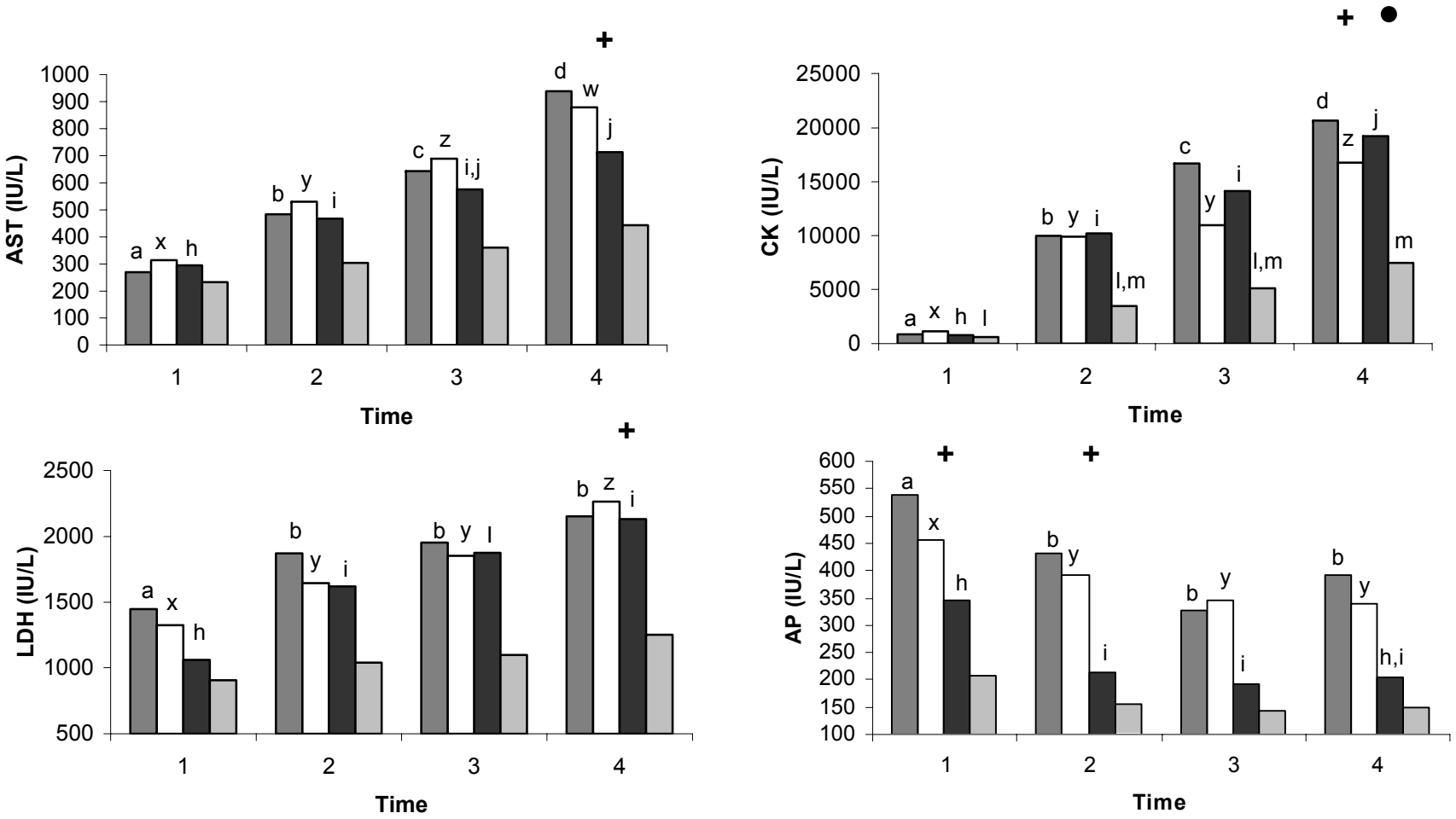


Figure 6.19: AST, CK, LDH and ALP activities of Southern chamois. ■ Control males; □ Treated males; ■ Control females; □ Treated females.
✱ Statistical differences ($p<0.05$) between control males and control females; ✚ Statistical differences ($p<0.05$) between treated males and treated females;
< Statistical differences ($p<0.05$) between control males and treated males; ● Statistical differences ($p<0.05$) between control females and treated females.
Columns with different superscript are significantly different ($p<0.05$) from each other in the same sex and treatment group.

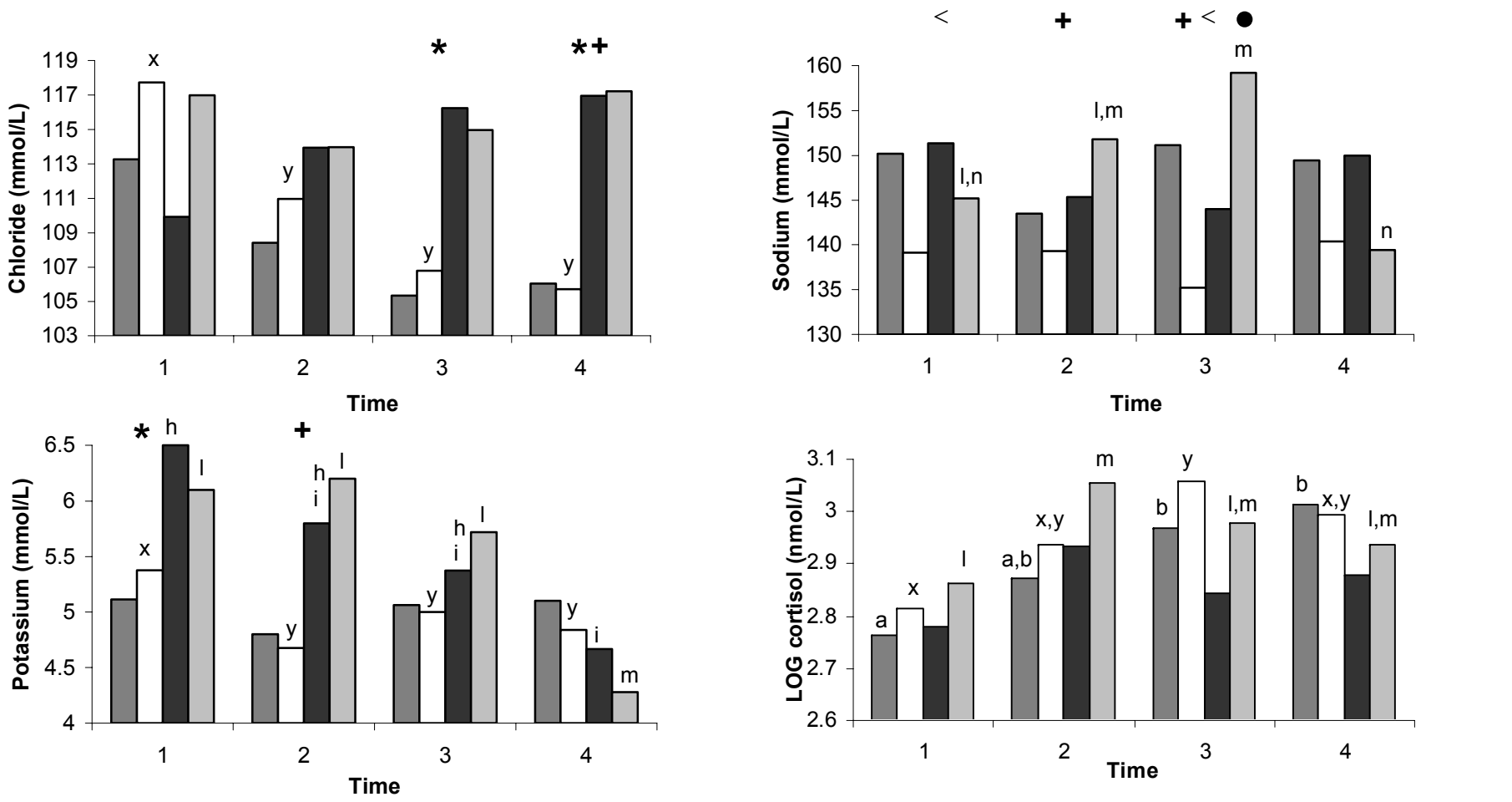


Figure 6.20: Serum electrolyte concentrations and cortisol logarithm of Southern chamois. Control males; Treated males; Control females; Treated femal

✱ Statistical differences ($p<0.05$) between control males and control females; ✚ Statistical differences ($p<0.05$) between treated males and treated females;

< Statistical differences ($p<0.05$) between control males and treated males; ● Statistical differences ($p<0.05$) between control females and treated females.

Columns with different superscript are significantly different ($p<0.05$) from each other in the same sex and treatment group.

6.4. TRANSPORT STRESS

6.4.1. Heart rate

After fitting the heart rate recording devices, heart rate decreased in the treated and control Southern chamois during the first 10 minutes. Heart rate decreased from the beginning of the monitoring (minutes 5 to 10) to the end of the transport (minutes 160 and 175) in the treated group, whereas it remained stable before transport and then increased during the transport period (minutes 85 to 155) in the control group. Statistically significant ($p<0.05$) differences between treated and control groups were found 85 and from 105 to 155 minutes after fitting the heart rate recorders, during the transport (Figure 6.21). Individual variability of heart rate did not differ between control (23.44 ± 9.61% [mean ± sd]) and treated (19.45 ± 6.27% [mean ± sd]) group, but interindividual coefficient of variation was statistically higher in the control group (30.70 ± 10.31% [mean ± sd]) than in the treated group (22.12 ± 7.78% [mean ± sd]).

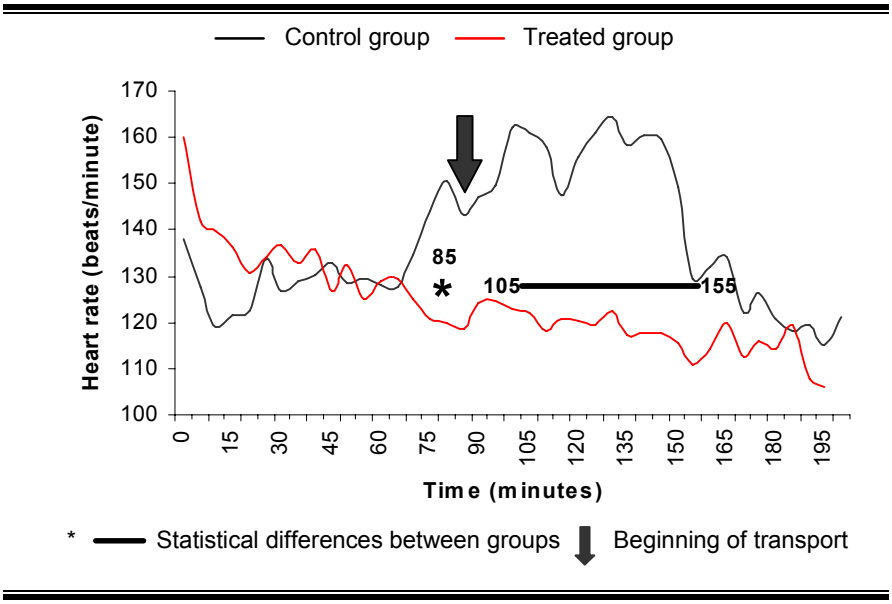


Figure 6.21. Heart rate of transported Southern chamois.

6.4.2. Temperature

Control group showed higher body temperature than treated group from minute 5 to minute 85. Body temperature decreased over time in both groups, but stabilized earlier in the treated group (15 minutes) than in the control group (75 minutes) (Figure 6.22).

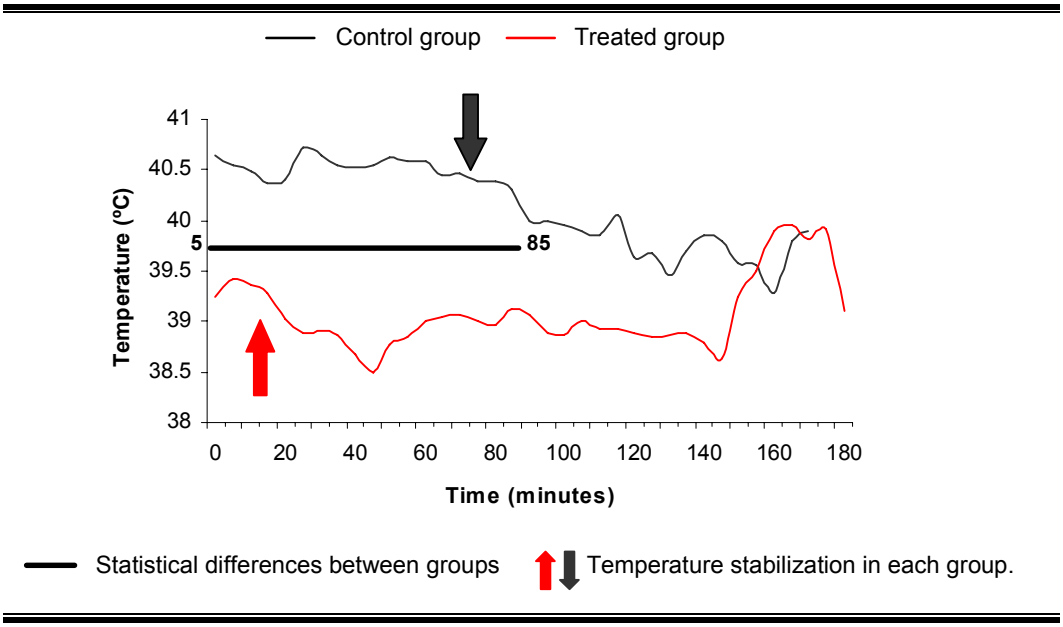
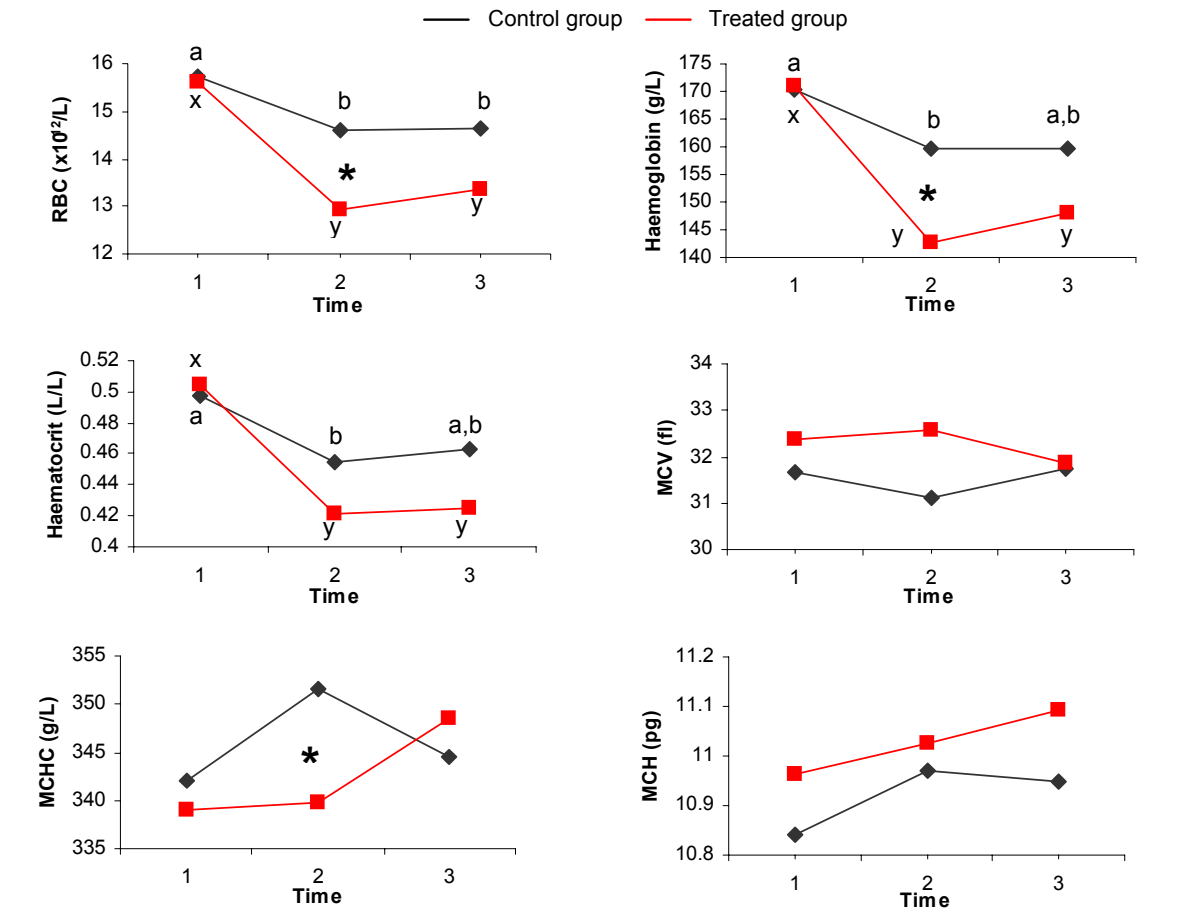


Figure 6.22. Rectal temperature of transported Southern chamois.

6.4.3. Haematology

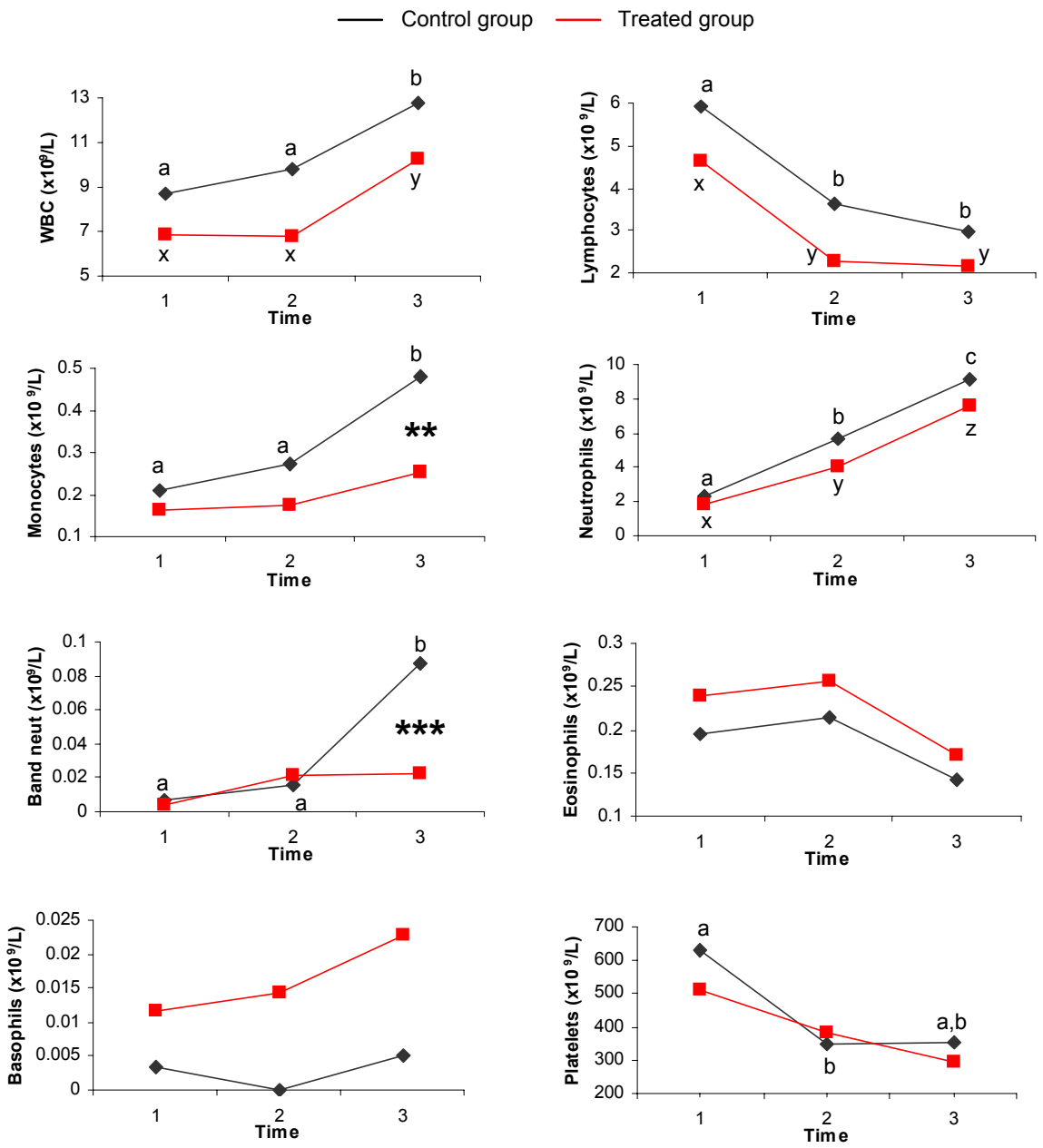
RBC, haemoglobin concentration and haematocrit decreased from capture (time 1) to pretransport sample (time 2), and then remained stable across transport in both groups. Both RBC and hemoglobin concentration were significantly lower in the treated group at time 2, but this difference was not observed at time 3. MCHC was higher in the control group at time 2 (Figure 6.23). Total leukocyte count remained stable during the pretransport period and increased throughout the transport in both groups, whereas lymphocyte count decreased during pretransport period to remain stable during transport. Neutrophils increased consistently in every period in both groups. Monocytes and band neutrophils stayed stable in both groups before transport, and increased during transport only in the control group. Significant higher values for both parameters were found in the control group at time 3. Platelet count decreased in the control group before transport, whereas it remained stable throughout all the study period in the treated animals (Figure 6.24).



* Values are significantly different from each other between groups ($p<0.05$).

Means with different superscript are significantly different from each other in the same group

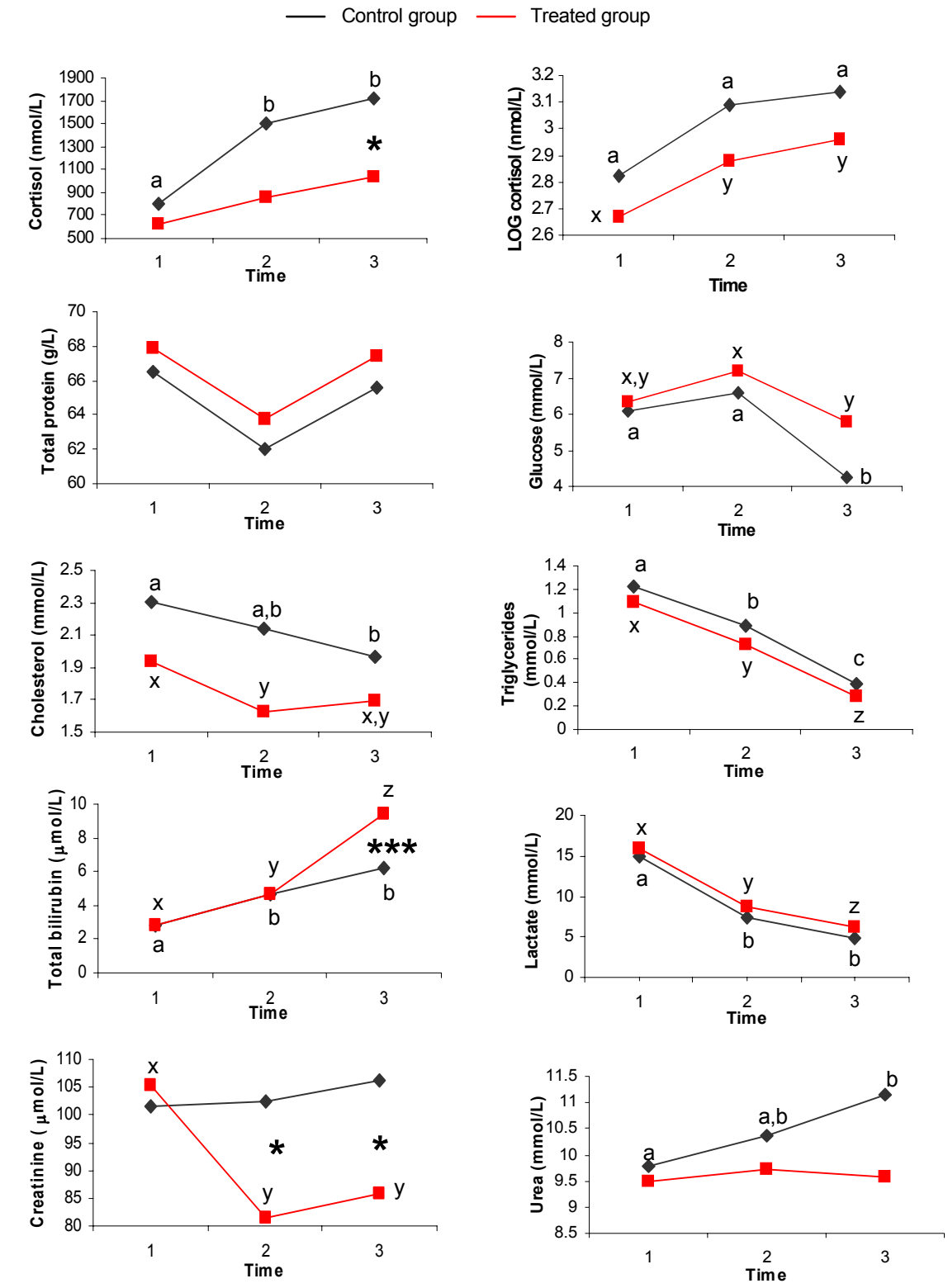
Figure 6.23. Erythrocyte parameters of transported Southern chamois.



* Values are significantly different from each other between groups (**p<0.01; ***p<0.005).
Means with different superscript are significantly different from each other in the same group

Figure 6.24. Leukocytes and platelets of transported Southern chamois.

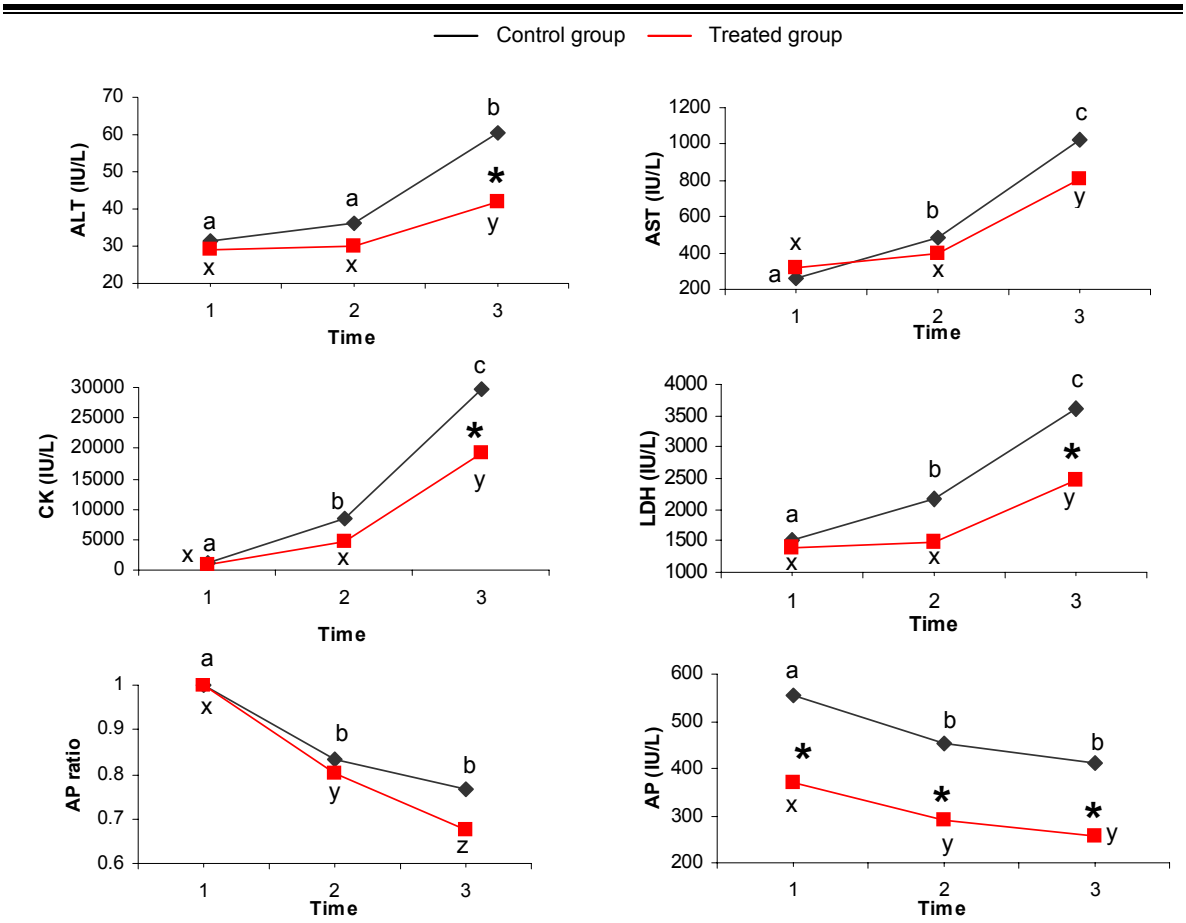
6.4.4. Serum biochemistry



* Values are significantly different from each other between groups (*p<0.05;*p<0.005).
Means with different superscript are significantly different from each other in the same group

Figure 6.25. Cortisol and metabolites of transported Southern chamois.

Serum cortisol concentration increased before transport and then remained stable in the control group, whereas it did not vary in the treated group. Control animals showed statistically significant higher serum cortisol concentrations than treated animals at time 3. Serum glucose concentration decreased during transport in both groups. Serum cholesterol concentration decreased from time 1 to time 3 in the control group, whereas in treated animals it decreased before transport but remained stable during transport. Serum triglyceride concentration decreased significantly along time in all periods and groups. Total bilirubin increased in both groups during the pretransport period, but during transport increased only in the control group, reaching statistically significant higher values in control animals at time 3. Serum lactate concentration decreased both before and during transport in the treated group, whereas it decreased only before transport in the control group. Serum creatinine concentration decreased before transport only in the treated group, showing statistically higher values in the control group at times 2 and 3. Serum urea concentrations increased from time 1 to time 3 in the control group, remaining stable in the treated group (Figure 6.25).

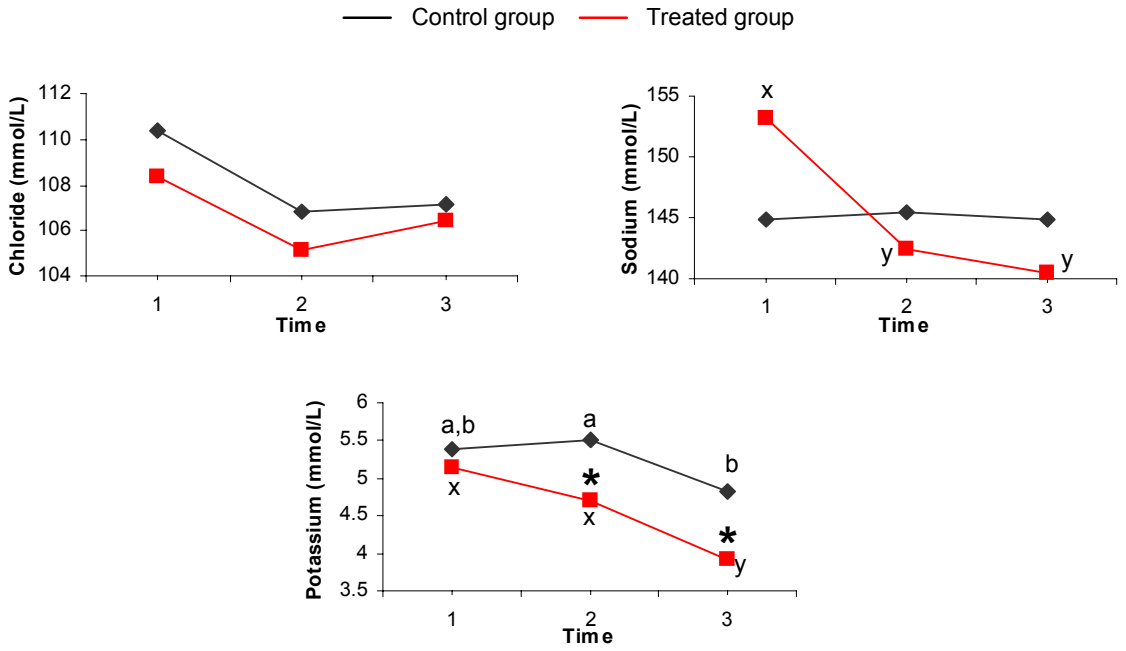


* Values are significantly different from each other between groups ($p<0.05$).
Means with different superscript are significantly different from each other in the same group

Figure 6.26: Enzyme activities of transported Southern chamois.

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK) and L-lactate dehydrogenase (LDH) activities increased before and during transport in the control group, whereas they only increased during transport in the treated group, except for ALT, which also increased before transport in the treated group. Statistically significant higher values in the control group were found for ALT, CK and LDH activities at time 3. Serum AP activity, expressed as time 1 ratio, decreased both before and during transport in the treated group, whereas it decreased only before transport in the control group (Fiure 6.26).

Serum sodium concentration remained stable throughout all the study period in the control group, whereas it decreased before transport in the treated group. Serum potassium concentration decreased during transport in both groups, and statistically significant higher values appeared at times 2 and 3 in the control group Figure 6.27).



* Values are significantly different from each other between groups ($p<0.05$).
Means with different superscript are significantly different from each other in the same group

Figure 6.27. Electrolytes of transported Southern chamois.

6.5. SALIVA CORTISOL

Both concentration and logarithmic values obtained with the reference cortisol standards fit the expected curve (Figures 6.28 and 6.29).

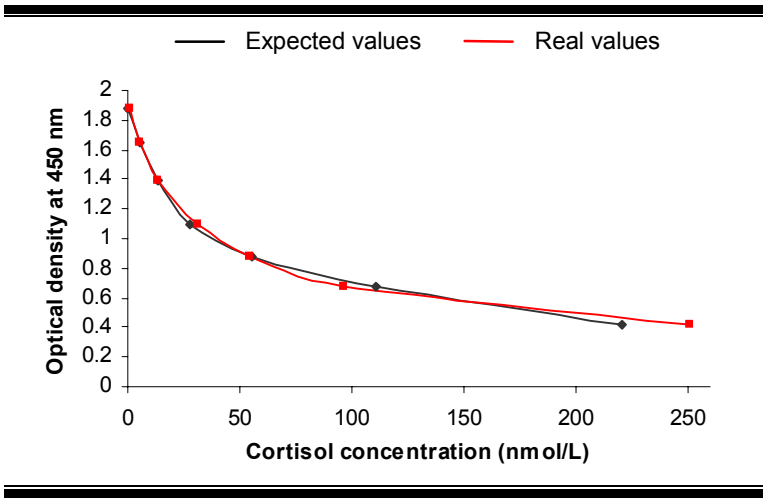


Figure 6.28. Real and expected curve for saliva cortisol standards concentration.

Concentration curve maximum slope is found below a concentration of 50 nmol/L, and between logarithmic values 1 and 2.5. These intervals include most of the range of saliva samples analysed.

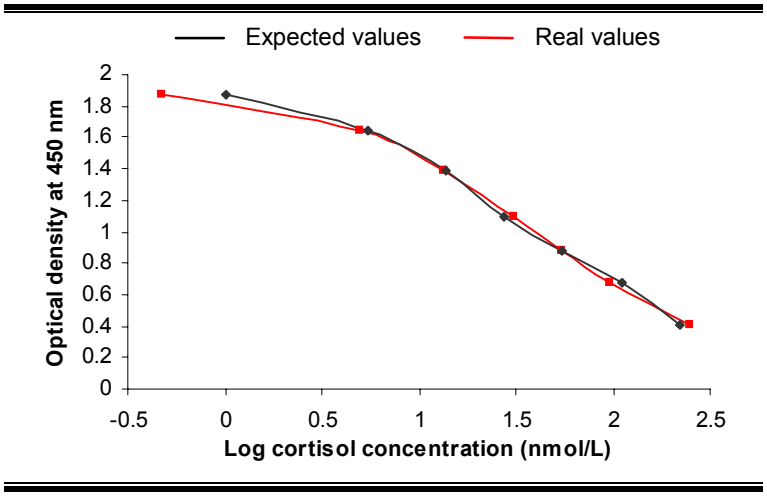


Figure 6.29. Real and expected curve for saliva cortisol standards logarithm.

Mean saliva cortisol concentration of the 29 samples studied was 24.6 nmol/L, which is approximately 50 times lower than serum cortisol concentration of the same samples. Pearson correlation coefficients and R^2 values of saliva and total serum cortisol concentrations and logarithms showed a good correlation between saliva and serum cortisol values (Table 6.7 and Figure 6.30).

Table 6.7. Saliva and serum concentration and logartithms analysed.

	Unit	N	Mean (SD)	Range	R (Pearson)	Adjusted R^2	P
Saliva cortisol	nmol/L	29	24.6 (11.3)	12.4 – 59.4	0.78987	0.6100	0.0126
Serum cortisol	nmol/L	29	1210.8 (889.3)	378.8 – 3929.6			
Log saliva cortisol	nmol/L	29	1.35 (0.17)	1.09 – 1.77	0.67221	0.4308	0.0432
Log serum cortisol	nmol/L	29	2.99 (0.28)	2.58 – 3.59			

N = Number of samples analyzed; SD = Standard deviation; CV = Coefficient of variation

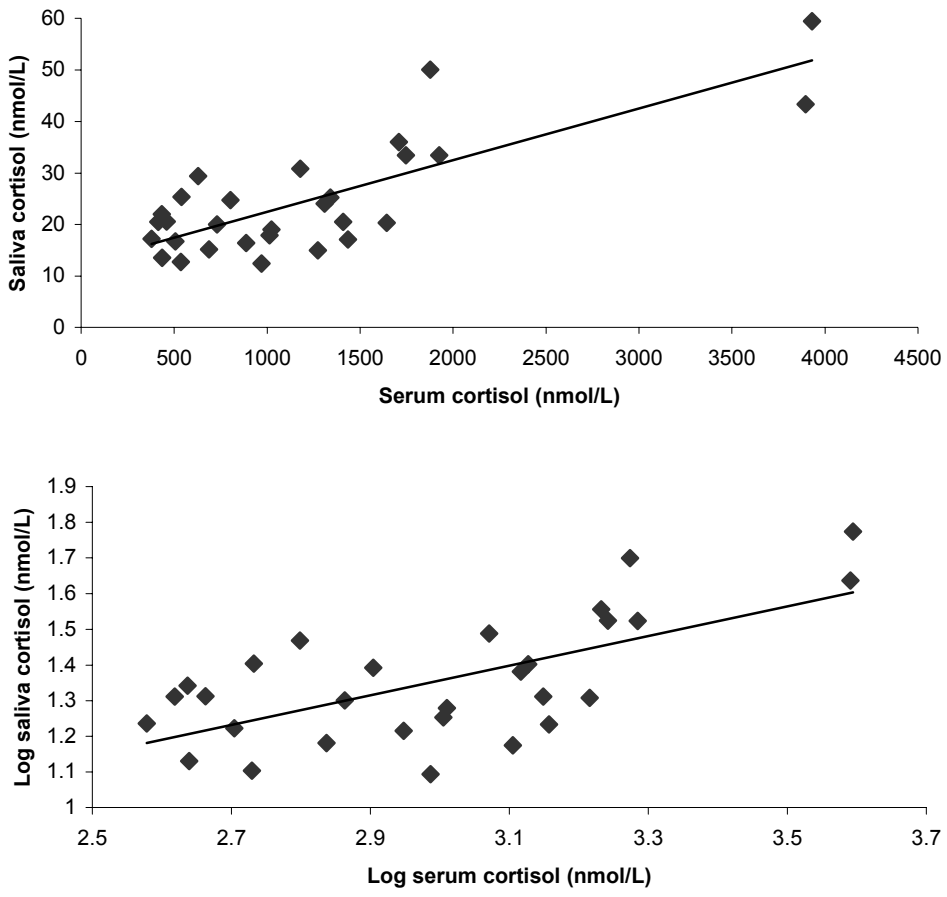


Figure 6.30. Correlation of serum and saliva cortisol concentrations and logarithms.

7. DISCUSSION

7.1. CAPTURE

7.1.2. Performance

Performance of Southern chamois capture with drive nets (3.96 Southern chamois per capture operation) has been superior to that described previously for Northern chamois with the same capture method (2.6 Northern chamois per capture operation), although a performance of 4.8 animals per capture operation was obtained by one of the teams capturing Northern chamois (Meneguz *et al*., 1994). In terms of days of work, the 0.51 days of work per Southern chamois obtained in our study are right in the middle of the interval described for drive-nets, which oscillates between 0.33 and 0.67 days per animal, and is fairly superior to the performance of snares (from 2 days to 170 hours per chamois), box-trap (2.94 days per chamois) or drive-nets without battue (2.6 days per chamois) (Berducou, 1993; Struch and Baumann, 2000).

7.1.3. Specificity

Specificity was 100%. Southern chamois was the only species captured. This result can be compared to operator-activated capture methods, and it is superior to the 73% reported previously for Northern chamois captured with drive-nets or the 67% obtained when capturing Northern chamois with snares (Berducou, 1993; Meneguz *et al*., 1994; Struch and Baumann, 2000). This specificity may be attributed to the selection of high altitude capture zones, where densities of ungulates different to Southern chamois is low, and to the experience of beaters (pushing towards the net only chamois) and the operators in the net (preventing other ungulates to run into the net by showing themselves before). During the capture operations, nonungulate wild mammals like Eurasian red squirrels (*Sciurus vulgaris*), European hares (*Lepus europaeus*) and red foxes (*Vulpes vulpes*), and birds like thrushes (*Turdus sp*.) were observed crossing the net through the mesh without any problem.

7.1.3. Safety for the animals

Total risk for the animals, measured as mortality rate plus lesion rate, was 5.27%, lower than that described for Northern chamois captured by means of drive-nets (Gibert, 1993). Mortality rate, 2.11%, was also lower than previously described for Northern chamois, which varied between 3.5 and 3.8% (Gibert, 1993; Meneguz *et al*., 1994). Causes of death registered in our study (shock and asphyxia) have already been described (Gibert, 1993).

7.1.4. Safety for the operators

Drive-net was overall safe for operators when capturing Southern chamois. Every capture operation involved between 15 and 35 people working on the field. Lesions registered during capture operations are related to the species (horn wound) and the steep environment where it inhabits (knee lesions), and are due to unavoidable risks when dealing with Southern chamois.

7.1.5. Capacity to capture groups

As demonstrated by the results obtained, drive-net was adequate to capture groups of Southern chamois. This fact may be interesting to mark animals of the same group in order to carry out behavioural or population studies which require identification of individuals.

7.1.6. Sex and age selectivity

Sex ratio of captured identified animals (57 males and 22 females) does not correspond with sex-ratio in the population. Spatial segregation is a characteristic of Southern chamois, and females use higher altitudes than males, probably as a protection measure for the kids (Pérez-Barbería, 1994). Preference in the use of alpine forest were most captures were carried out by males could explain the biased sex-ratio of captured Southern chamois.

Age of animals captured ranges from 1 to 14. Kids were not captured, since they were newborn or females were still pregnant in the time most captures were carried out. Moreover, lack of developed horns in the kid makes more difficult that they get wrapped in the net, and they can fly away when the net falls in the moment their mother is captured. At least one kid fell in the net but escaped in a few seconds, and another escaped on the opposite direction of the net when its mother was captured.

Overall, drive-net was an effective and safe method to capture Southern chamois. The main problem of the method is that it requires many operators to carry out the battue and to stay beside the net to assist the animals. In our study, this problem was overcome thanks to the participation of volunteers and the collaboration of the rangers of the National Hunting Reserves of Cadí, Cerdanya-Alt Urgell and Freser-Setcases. However, the most effective capture, with 20 Southern chamois trapped in the net, was carried out by a total of only 15 people, so people required can be reduced with experience and good planning of the capture.

7.2. HAEMATOLOGICAL AND SERUM BIOCHEMICAL REFERENCE VALUES

To our knowledge, these are the first haematological and serum biochemical values reported for Southern chamois, and the first ones for the Genus *Rupicapra* determined on a systematic basis. The establishment of reference values makes possible the assessment of newly captured animals, thus allowing the evaluation of stress response compared to previously existing data (Kocan *et al*., 1981). Ranges given can include more than 90% of the population with a 99% of probability for most of the parameters analyzed (Walton, 2001). To establish reference values, reference individuals are needed. These individuals must be not only healthy, but they also have to meet criteria which resemble those of the population values are referred to (Solberg, 1999). All animals were captured with the same capture method, drive nets, so haematological and biochemical values variation due to capture method, as described for other ungulate species, was avoided (Kock *et al*., 1987b; Cross *et al*., 1988; Marco and Lavín, 1999). Groups were formed according to those factors that may influence haematological and biochemical parameters, like season, age and sex (Delgiudice *et al*., 1992; Abaigar, 1993; Marco *et al*., 1997), thus enabling the establishment of more accurate and representative reference intervals for each group.

Results obtained were overall similar to those described previously for other species belonging to the *Caprinae* Subfamily (Peinado *et al*., 1993; Pérez *et al*., 2003) and showed lower coefficient of variation and a narrower interval than those data reported previously for the *Rupicapra* Genus (Artois, 1987; Gibert, 1993; ISIS, 2002). This fact proofs the standardization of our capture method and the usefulness of these reference values for animals in the same circumstances. The Southern chamois of our study had higher RBC, serum cholesterol, triglyceride and enzyme activity (CK, LDH, AST and AP) than those described for Northern chamois (ISIS, 2002). These differences could be due to diet and capture stress, since animals used for ISIS reference data may be in captivity and undergo different capture operations, including anaesthesia, which could further explain the differences found. Leukocyte differential count was also different to that reported for Northern chamois (Gibert, 1993; ISIS, 2002), with higher values for lymphocytes and lower values for neutrophils in the Southern chamois of our study. These differences may be related to a stronger adrenalin response in free-ranging Southern chamois, inducing a higher relative number of lymphocytes. Serum cortisol values were also elevated when compared to those described previously for other physically captured wild ungulates (Delgiudice *et al*., 1990a and b; Carragher *et al*., 1997; Waas *et al*., 1997 and 1999; Montané *et al*., 2002 and 2003) but similar to those described in stressed farmed red deer (Diverio *et al*., 1996a). Elevated values and high variability of serum cortisol indicate a strong response of the adrenal cortex with high individual differences in the stress response in the Southern chamois.

Statistical differences among sex, season and age groups may be due either to differences in the basal values of each group, in the stress response of the animal or a combination of both factors, so interpretation of these differences must be cautious.

Both groups of males showed superior values for RBC in summer than the rest of the groups. Haemoglobin and haematocrit concentration were higher in males in summer than in females in summer and females in spring, respectively. Superior RBC and haemoglobin concentration in males have been reported for rusa deer (*Cervus timorensis russa*), and males of Spanish ibex (*Capra pyrenaica*) showed higher haematocrit values than females, agreeing with our results (Audigé, 1992; Pérez *et al*., 2003). Physiologic requirements during late pregnancy and lactation produce a decrease in circulating erythrocytes, haemoglobin and haematocrit (Jain, 1993), which could explain the lower values of females. Similar seasonal changes in RBC have also been described in bison (*Bison bison*), and were attributed to changes in nutrition status (Hawley and Peden, 1982). This could be the explanation for the higher RBC values of males in summer when compared to males in spring.

In general, MCV and MCH are correlated, due to the slight variation of MCHC among species and individuals (Jain, 1993). Young males and adult females in spring showed higher values for MCV, and MCH was superior in spring in all groups. Similar seasonal variations in MCV and MCH have been reported in white-tailed deer (*Odocoileus virginianus*), and were attributed to a macrocytic (and hypocromic, according to our results) anaemia due to a deficient nutrition status after the winter period. Higher MCV and MCH values have also been related to an increase of the haematological effects of capture in summer due to higher temperatures (DelGiudice *et al*., 1990c and 1992; Rietkerk *et al*., 1994). Adult females in summer showed lower MCHC values than all the other adult groups. Different studies dealing with haematology of wild ungulates have found no sex differences in MCHC (Abaigar, 1993; Rietkerk *et al*., 1994; Borjesson *et al*., 2000; Pérez *et al*., 2003). Higher MCHC values have been found in summer in mountain gazelles (*Gazella gazella*), which agrees with our results (Rietkerk *et al*., 1994). Like for MCV and MCH, seasonal variation of MCHC has also been related with undernutrition and anaemia (Delgiudice *et al*., 1992).

Platelet count values of females in summer were higher than those of summer males and all the spring groups. Few references about platelet count in wild ungulates exist, and no differences related to sex, age and physiological status have been found for this parameter in dama (*Gazella dama mhorr*) and Cuvier's (*Gazella cuvieri*) gazelles (Abaigar, 1993).

Adult females and young males in summer presented the highest values for WBC and both absolute and relative lymphocyte count, showing sex and age differences with adult males in summer and, in the case of adult females, also season differences with spring females. Adult females in spring had more neutrophils than adult males in the same season. Lymphocyte count in females in summer was even superior to young males in the same season. These sex differences could be due either to higher physiological levels of WBC in females or to a more marked leukocytosis, lymphocytosis and neutrophilia due to a stronger adrenalin stress response of females, since differences in WBC parameters have been related to differences in capture method and stress response (Rietkerk *et al*., 1994; Marco and Lavín, 1999). Changes in WBC according to changes in female physiological status have been reported in dama gazelles, with lactating females showing superior leukocyte count than pregnant ones (Abaigar, 1993). This agrees with our results, since WBC was higher in Southern chamois females in summer (when lactating) than in spring (when pregnant). However, no season differences in lymphocyte count have been described when differences in leukocyte counts were present (Abaigar, 1993). Young males in summer had higher values for WBC, lymphocytes and neutrophils when compared with adult males. The adrenalin response is more common and marked in young animals (Jain, 1993), and this could explain the higher leukocyte values of young animals. Higher WBC and lymphocyte values in younger animals have been reported in Spanish ibex and bighorn sheep (Kock *et al*., 1987b; Abaigar, 1993; Pérez *et al*., 2003). No basophils were found in any female sample, whereas only young males in spring, of all the male groups, lacked basophils. Nevertheless, significant differences were only found between adult males in summer and adult females in spring, due to variations in sample size of each group.

Serum cortisol concentration has been traditionally considered as a good stress indicator in wild animals, even as a single parameter (Franzmann *et al*., 1975; Rehbinder and Edqvist, 1981; Morton *et al*., 1995; Waas *et al*., 1999). Serum cortisol levels were higher in adult females than in adult males during summer season, which could indicate a superior stress response in lactating females. Higher cortisol concentrations in adult females when compared to adult males have been described in bighorn sheep (Kock *et al*., 1987b).

Serum glucose concentration was lower in adult males in summer than in adult females in spring. Variation in glucose levels depending on the physiological status of females have been reported for Cuvier's gazelles (Abaigar, 1993).

Serum cholesterol and triglycerides concentrations depend largely on the diet (Bruss, 1997), although increases of these parameters in wild ungulates due to physical capture stress have also been described (Franzmann and Thorne, 1970; Barrett and Chalmers, 1977; Marco and Lavín, 1999). Increases in serum cholesterol and triglycerides

concentrations have been associated with fat mobilization and lipolysis during negative energy balance, which is known as fasting hyperlipidemia (DelGiudice *et al*., 1990c; Bruss, 1997). Higher cholesterol values of young males in spring agree with this physiological variation. Differences in cholesterol and triglycerides ratio among our age and sex groups and with other species could indicate differences in diet, fat metabolism or nutrition status along the year. Both groups of males in summer showed lower cholesterol values than all the other groups except adult males in spring. Similar seasonal variations of cholesterol levels have been reported for bison and opposite changes (higher cholesterol values in negative energy balance) have been found in female white-tailed deer, although not always significant differences have been found between summer and spring values (Hawley and Peden, 1982; DeLiberto *et al*., 1989). Higher values for serum cholesterol levels in females have been reported for Spanish ibex and pronghorn neonates (Barrett and Chalmers, 1979; Pérez *et al*., 2003), which agrees with our results in summer samples. Nevertheless, contradictory results have been reported, with adult male pronghorns having higher serum cholesterol levels than females (Barrett and Chalmers, 1977). Adult males in summer showed higher serum triglycerides concentration than both groups of young males and adult females in spring. Opposite differences have been found in Spanish ibex, with young animals and females presenting higher values than adult animals and males, respectively (Pérez *et al*., 2003).

Serum bilirubin values were higher in young males in spring than in summer. Bilirubin rises are rare in ruminants, since they have a high capacity of bilirubin elimination, either through hepatic or non-hepatic mechanisms. No season differences in serum total bilirrubin concentration have been described in previous studies dealing with wild ungulates (Abaigar, 1993).

Both adult summer groups had superior serum lactate levels than young males in summer and adult males in spring. Muscular glycogenolysis caused by capture and handling stress and an increase in anaerobic metabolism due to exercise increase serum lactate concentration (Hattingh *et al*., 1990; Kaneko, 1997a). According to our data, adult animals of both sexes seem to have a higher production of lactate in summer than young animals, which could be due to a higher muscular glycogenolisis and/or anaerobic muscular metabolism. Season differences between both adult male groups point to a higher physiological compromise related to stress in summer, which could be due to summer higher temperatures. Few references about serum lactate levels in free-ranging wild ungulates exist (Marco *et al*., 1997; Montané *et al*., 2002 and 2003).

Serum creatinine concentration in both adult groups in spring was superior to all the groups in summer. Adult males in spring also had higher levels than young males in the same season. Elevations in serum creatinine levels in wild ungulate species have been

related to nutritional deprivation, which agrees with our higher values in early spring (DelGiudice *et al*., 1990c and 1992). Since serum creatinine levels are directly related to the muscular mass of the animal (Finco, 1997), the higher values of our adult males when compared to young males in spring were expected. Higher serum creatinine concentration in adult bighorn sheep when compared to young ones has been reported (Kock *et al*., 1987b).

Adult males in summer had higher serum urea concentration than adult females in both seasons and young males in summer. No season differences in serum urea nitrogen have been previously found in female white-tailed deer, coinciding with our data (DeLiberto *et al*., 1989). Serum urea concentration depends largely on the diet, although the influence of rumen metabolism is still unclear (Finco, 1997). Higher values of blood urea nitrogen in adult animals have already been reported in dama gazelles and fallow deer (*Dama dama*), coinciding with our results, although the opposite trend has been found in bighorn sheep (English and Lepherd, 1981; Kock *et al*., 1987b; Abaigar, 1993). Male and female Southern chamois use different habitats and altitudes except in the rut (Pérez-Barbería, 1994), and this could explain our summer sex differences in urea values, since diet could also change with the habitat.

Adult females in summer showed the highest values for serum creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AP) activities, whereas adult females in spring had the lowest values of all the groups for the same parameters. Values of adult females in summer were also statistically higher than those of both groups of males in the same season for LDH, AST and ALT, whereas no sex differences were found in spring. Higher CK and AST activities in summer have been reported for reindeer (*Rangifer tarandus tarandus*), and they have been attributed to changes in nutritional condition (Hyvärinen *et al*., 1976). Changes in CK activity have also been related to higher mobility of the animals within their home range (De Meneghi *et al*., 1990), and this could also explain our differences between spring and summer, since Southern chamois movement area is bigger in summer than in spring, when snow covers an important part of their habitat (Catusse *et al*., 1996). In chital deer (*Axis axis*) females, pregnant hinds showed lower ALT activity than lactating ones, which agrees with our results, but they also had higher LDH and AP activity, opposite to our data (Chapple *et al*., 1991). Sex-related differences in serum LDH and ALT activity have not been previously reported in other species of wild ungulates, although higher LDH levels in males, opposite to our results, have been reported in pronghorns (Seal and Hoskinson, 1978; Peinado *et al*., 1990; Zaugg *et al*., 1993; Pérez *et al*., 2003). Higher activities of AST in males, opposite to our summer data, have been described in red deer, pronghorn and bighorn sheep (Seal and Hoskinson, 1978; Kent *et al*., 1980; Kock *et al*., 1987b). CK, LDH, AST and ALT have been widely used as physical stress indicators in different species of wild ungulates (Chapple *et*

al., 1991; Peinado *et al*., 1993; Ranucci *et al*., 1996; Marco *et al*., 1997; Montané *et al*., 2002 and 2003). Higher values of females in summer for all four enzymes could then indicate a higher muscular damage due to more compromised physiology during lactation or a higher stress level of females in summer than males in the same season and females in spring. Higher AP activities in young animals due to increased bone isoenzyme have been repeatedly reported in different species of wild ungulates (Barrett and Chalmers, 1977; Kock *et al*., 1987b; Peinado *et al*., 1990; Chapple *et al*., 1991; Kramer and Hoffmann, 1997; Marco *et al*., 1997; Borjesson *et al*., 2000; Pérez *et al*., 2003), but this difference was not clear in our data, since differences in spring values could be related to sex as well as age. No age differences in AP levels have been found in dama and Cuvier's gazelles, which is more similar to our results (Abaigar, 1993).

Serum chloride concentration may vary depending on hydration status and blood acid-base balance (Carlson, 1997). Serum chloride concentration only was different between young and adult males in summer, with adult males showing higher values. Lack of age differences in chloride concentration has been reported in Spanish ibex, bighorn sheep and bison (Zaugg *et al*., 1993; Borjesson *et al*., 2000; Pérez *et al*., 2003).

Adult females in summer had superior serum sodium concentration than adult males in summer and young males in spring. Coinciding with our results, higher serum sodium levels have been reported for bison females when compared to males (Zaugg *et al*., 1993). Increases in sodium concentration are caused by increased sodium ingestion, diminished water intake or increased water loss (Bush, 1993), and catecholamines increase renal sodium reabsorption (DiBartola, 2000).

Serum potassium concentration was superior in adult males in summer than in adult females in spring. Differences in potassium levels in females depending on their physiological status have been reported in dama gazelles (Abaigar, 1993). Potassium levels can increase due to vigorous exercise or muscular necrosis, but they also may be falsely increased due to haemolysis or high WBC or platelet counts. Dietary deficit can be the cause of lower serum potassium concentrations (Carlson, 1997).

Our results for serum total protein and electrophoretic fractions were overall slightly superior to those described previously for Southern chamois shot dead (Lastras *et al*., 2000). Physical capture has been reported to cause an increase in serum protein and electrophoretic fractions in wild ungulates (Peinado *et al*., 1993; Marco and Lavín, 1999), which could be the explanation for our higher levels compared to dead animals. Serum total protein concentration was rather stable across the groups, and only young males in summer showed lower values than adult females in spring. This difference is difficult to explain, since

it could be due to three different factors: age, sex and/or season, and for all three factors differences in total protein levels have been reported (Kock *et al*., 1987b; DeLiberto *et al*., 1989; Chapple *et al*., 1991; DelGiudice *et al*., 1992; Abaigar, 1993; Borjesson *et al*., 2000; Pérez *et al*., 2003).

Differences in protein electrophoretic fractions were only found for α2-globulin and γ-globulin. Adult males in summer showed higher α2-globulin concentration than young males in the same season and females in spring, whereas adult females had the higher γ-globulin concentration in summer, statistically superior to all the other groups except adult males in spring. Higher α2-globulin values in young red deer stags and in female Spanish ibex, opposite to our results, have been reported (Kent *et al*., 1980; Pérez *et al*., 2003), as well as variation of α2-globulin concentration in females depending on their physiological status (Chapple *et al*., 1991; Abaigar, 1993). Higher γ-globulin levels in females have been reported in red deer and chital deer, which agree with our results (Kent *et al*., 1980; Chapple *et al*., 1991). Age differences in γ-globulin levels have been reported both for males and females (Peinado *et al*., 1990; Chapple *et al*., 1991), but we did not found this effect in our groups of males and we had no group of young females to compare with adult ones. However, differences in γ-globulin concentration depending on the season (it is, the physiological status) have not been found in chital deer or dama and Cuvier's gazelles, unlike our data (Chapple *et al*., 1991; Abaigar, 1993).

To summarize, the haematological and serum biochemical data of Southern chamois are similar to those of other species of the *Caprinae* Subfamily. Values given are influenced by the capture method, and this must be taken into account when using these intervals as a reference for animals captured with another capture method or kept in captivity. Differences among groups suggest the existence of strong sex, season and age differences in most of the parameters analyzed. Some of the parameters traditionally considered as stress indicators (WBC, lymphocyte values, platelets count, CK, LDH, AST and ALT) seem to point to a higher stress level in females in summer than in the other groups. Further studies clarifying the influence of sex, age and season all the year round, as well as the effect of stress, capture method, diet and physiological and nutritional status will help to provide more accurate and specific reference intervals.

7.3. PHYSICAL CAPTURE AND RESTRAINT STRESS

7.3.1. Heart rate and rectal temperature

Polar® heart rate monitors have been already successfully used to measure heart rate in domestic and wild animals (Sloet van Oldruitenborgh-Oosterbann *et al*., 1988; Evans and Rose, 1986; Hopster and Blockhuis, 1994; Montané *et al*., 2002 and 2003). Heart rate has been used as an acute stress indicator, since it assess the response of the autonomic nervous system to stressors in an objective way (Broom and Johnson, 1993; Hopster and Blockhuis, 1994), although the use of heart rate variability has been proposed as a better stress indicator than basic heart rate (Porges, 1985). Physical activity related to capture also causes an increase in herat rate (Broom and Johnson, 1993). Heart rate decreased from the beginning of the monitoring and then remained stable during the stress study. Initial heart rate decrease was probably due to the ending of physical activity and the stop of manipulation of the animals. The lack of differences between control and treated groups in Southern chamois in both mean heart rate and individual variability agree with those data reported previously for bighorn sheep (*Ovis canadensis*) and wapiti (*Cervus elaphus*) (Kock *et al*., 1987a; Read *et al*., 2000), and may be due to reflex tachycardia secondary to hypotension caused by acepromazine (Plumb, 1999), as described previously in roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) (Diverio *et al*., 1996b; Montané *et al*., 2003). The earlier stabilization of heart rate in the treated group has already been described in deer treated with phenotiazines, and were attributed to the tranquilizing effect of the drug (Diverio *et al*., 1996b; Montané *et al*., 2003). Interindividual variability in the stress response has already been described (Moberg, 1987), and the tranquilizing effect of the drug could also explain the lower interindividual variation of heart rate in the treated group, with control animals displaying a less uniform response.

Captured animals may experience an increase in body temperature not only due to physical activity previous to capture, but also due to stress-induced hyperthermia (SIH) (Broom and Johnson, 1993; Moe and Bakken, 1997; Bakken *et al*., 1999). Hyperthermia participates in the pathophysiology of capture myopathy, an iatrogenic capture-related syndrome (Chalmers and Barrett, 1982; Kock *et al*., 1987a; Seal and Bush, 1987; Williams and Thorne, 1996). SIH is time dependent, and temperature increases to reach a stable high level (1 to 1.5 ºC higher than basal values) in 10 minutes to decrease and return to baseline after 60 to 90 minutes (Zethof *et al*., 1994; Moe, 1996; Moe and Bakken, 1997; Bakken *et al*., 1999). Continuous monitoring of rectal temperature with Mätman® probes avoid rises in temperature caused by repeated measures when using conventional thermometers (Moe and Bakken, 1997). These probes have also been used in wild animals (Montané *et al*., 2002 and 2003). Temperature monitoring of Southern chamois in our study started 56 ± 23

minutes after capture, when capture related SIH started to decline, as seen in the decrease of temperature over time in all groups. Rectal temperature decrease of stressed Southern chamois agrees with reported dynamics for stress-induced hyperthermia. The ending of capture related physical activity could also participate in the decrease of body temperature, but physical activity only accounts for a small percentage of total temperature increase (Bakken *et al*., 1999). However, Southern chamois were physical restrained, which was also a stressful stimulus, and temperature was probably still increased over basal values throughout the whole study period. Acepromazine did not avoid SIH, as body temperature followed the same trend in both groups, due probably to the fact that SIH is an anticipatory stress response already activated when acepromazine is administered (Montané, 2002). Nevertheless, acepromazine shortened capture related SIH, as demonstrated by earlier temperature decrease and stabilization in the treated groups (males and females), and also decreased SIH intensity, since body temperature was lower in treated animals during most of the study period. Hypothermia is an additional pharmacologic action of acepromazine by depressing the reticular activating system, and also vasodilatation induced both by central and α-adrenergic effects of acepromazine could help to dissipate heat and participate in decreasing body temperature in treated animals (Plumb, 1999). This hypothermic effect of acepromazine may be useful to modulate SIH in stressed animals and avoid the development of capture myopathy, since hyperthermia and metabolic acidosis are the two central factors of capture myopathy pathogenesis (Chalmers and Barrett, 1982; Williams and Thorne, 1996).

7.3.2. Haematology and serum biochemistry

Catecholamines are released from the adrenal medulla due to activation of the sympathetic nervous system during the stress response. Increases in RBC, haemoglobin concentration and haematocrit have been related to the effect of catecholamines on α-adrenergics receptors in the splenic capsule, inducing smooth muscle contraction and the release to circulation of erythrocytes stored in the spleen (Cross *et al*., 1988; Ganong, 1998; Jain, 1993), and also to a reduction in plasma volume (Wesson *et al*., 1979; Cross *et al*., 1988). Decreases of haematocrit value after the catecholamine effect has been previously described, although this effect has not been observed in erythrocyte count and haemoglobin concentration (Wesson *et al*., 1979; Montané *et al*., 2003). All three parameters decreased after capture in physically restrained Southern chamois. Lower values for RBC, haemoglobin concentration and/or haematocrit have been previously described in sedated or anaesthetized wild ungulates (Wesson *et al*., 1979; Cross *et al*., 1988; Peinado *et al*., 1993; Marco and Lavín, 1999; Montané *et al*., 2002 and 2003), which agrees with our results. These differences have been attributed to haemodilution, with concurrent changes in total protein concentration (Wesson *et al*., 1979), but spleen relaxation in sedated animals with

the consequent splenic sequestration of erythrocytes has been reported as the cause in most cases (Turner and Hodgetts, 1960; Cross *et al*., 1988; Peinado *et al*., 1993; Marco and Lavín, 1999; Plumb, 1999; Montané *et al*., 2002 and 2003). Although acepromazine causes hypotension and vasodilatation (Plumb, 1999), which could lead to haemodilution, differences in RBC, haemoglobin concentration and haematocrit can be explained by the α-adrenergic effect of acepromazine, since haemodilution in treated animals was not confirmed by decreases in total protein and sodium concentrations. Erythrocytes stored in the spleen have a higher MCV (and, consequently, a lower MCHC) than circulating ones, since they mature in spleen after being released from the bone marrow (Wade, 1973; Cross *et al*., 1988). MCV decrease and MCHC increase in treated group and MCV increase in control group over time further confirm spleen origin of RBC, haemoglobin concentration and haematocrit increases.

Stress leukogram induced by catecholamines in cattle is characterized by leukocytosis with neutrophilia and/or lymphocytosis, monocytosis and mild eosinopenia (Taylor, 2000). However, captured Southern chamois showed an intense total and relative leukocytosis in the first sample, during catecholamine stimulation, and no neutrophilia. Catecholamine effects last 20 to 30 minutes, and are followed by corticosteroids. Corticosteroids induce leukocytosis with neutrophilia, lymphopenia and eosinopenia, reaching its maximum 4-6 hours after the stimulus. Monocytosis may appear or not, depending on the species (Jain, 1993; Duncan *et al*., 1994). Southern chamois developed this leukogram during the study, with increasing total leukocyte numbers and neutrophils and decreasing lymphocytes over time. Monocytes and band neutrophils also increased during the corticosteroid stimulation, but only in the treated group. Both epinephrine and corticosteroids cause monocytosis and a decrease in eosinophils and basophils (Jain, 1993; Taylor, 2000). In our study, lower eosinophils values at time 1 in both groups were probably due to epinephrine, and then increased to decrease again due to corticosteroids, but this decrease took place later in the treated group. Basophils followed the same evolution in the control group, whereas no basophil rise was observed in the treated group.

Cortisol has been traditionally considered as a good stress indicator (Franzmann *et al*., 1975; Seal and Bush, 1987; Morton *et al*., 1995; Waas *et al*., 1999). However, serum cortisol concentration may be affected by a number of factors that must be considered, including its great interindividual variability, the existence of circadian, ultracircadian and seasonal secretory patterns and the effect of the sampling method (Turner, 1984; Moberg, 1985; Nilssen *et al*., 1985; Rushen, 1991; Ingram *et al*., 1999). Moreover, great interindividual differences exist in stress-induced corticosteroids concentrations, and it is difficult to relate physiological changes and animal welfare, so other authors consider cortisol a poor welfare indicator (Moberg, 1985 and 1987; Rushen, 1991). Serum cortisol values of Southern chamois were considerably higher than those reported previously for other wild

ungulates undergoing capture and handling operations (Kock *et al*., 1987b; Delgiudice *et al*., 1990a and b; Carragher *et al*., 1997; Waas *et al*., 1997; Marco *et al*., 1998; Waas *et al*., 1999; Read *et al*., 2000; Montané *et al*., 2002 and 2003) but similar to those described in stressed farmed red deer (Diverio *et al*., 1996a). The high cortisol values of Southern chamois could indicate a strong stress HPA response to pursuit, physical capture and restraint in this species. Moreover, high serum cortisol variability, like the one registered in Southern chamois, has already been described as one of the problems when using cortisol to assess stress (Moberg, 1985; Rushen, 1991). The elevated cortisol concentration of Southern chamois throughout the study period and the high variability of the values obtained difficult the interpretation of the data, and could explain the contradictory results found in the stress and transport study, at least partially.

Catecholamines and glucocorticoids increase serum glucose concentration by increasing glycogenolysis and glycogenesis (Brearley *et al*., 1990; Kaneko, 1997a; Ganong, 1998; Guyton and Hall, 2000), and serum glucose concentration has been reported to increase in stressed animals (Franzmann and Thorne, 1970; Bush *et al*., 1981; Hattingh *et al*., 1988; Carragher *et al*., 1997). In domestic ruminants, maximum glucose concentration is achieved two hours after ACTH injection, and then serum glucose decreases to basal values (Hartmann, 1988). This effect has already been reported in transported and physically captured captive roe deer (Montané, 2002), and agrees with data from Southern chamois.

Higher monocytes, band neutrophils, cortisol and glucose values in treated animals in our study are consistent in indicating lack of effect of acepromazine to modulate the hypothalamic-pituitary-adrenal cortex axis or even a higher degree of excitement in treated animals. An enhancing effect of acepromazine on stress response in cattle has been described, with higher cortisol (but no glucose) concentrations after the injection of acepromazine (Brearley *et al*., 1990). It has been suggested that chlorpromazine causes systemic release of epinephrine, which may increase adrenocorticotropic hormone (ACTH) and hence cortisol release (Bruss, 1980), and acepromazine may produce occasionally generalized CNS stimulation (Plumb, 1999), which could explain the higher excitement of treated animals in our study.

Serum lipids (cholesterol and triglycerides) concentration are considered to have little value as stress and welfare indicators (Broom and Johnson, 1993), and no changes in their concentration in stressed wild ungulates have been previously reported (Seal and Hoskinson, 1978; Kocan *et al*., 1981; Montané, 2002). However, catecholamines have a lipolytic effect on adipose tissue, stimulating fat mobilization and increasing circulating free fatty acids (Genuth, 1996; Guyton and Hall, 2000), but during stress cholesterol could be deviated to corticosteroid synthesis. Both increases and decreases in serum cholesterol and/or triglyceride concentrations concentration have been related to stress (Franzmann and

Thorne, 1970; Barrett and Chalmers, 1977; Locatelli *et al*., 1988; Saccon *et al*., 1992; Arnemo *et al*., 1994; Marco *et al*., 1997; Marco *et al*., 1998; Marco and Lavín, 1999). Southern chamois triglycerides concentration decreased over time from the initial sample, suggesting that precapture physical activity and catecholamines are more important than corticosteroids to increase serum triglyceride concentration in this species. Decreasing cholesterol values in treated Southern chamois in our study could be due either to a lesser degree of stress or to an increased cortisol synthesis, since they showed increasing cortisol values over time.

Lactate followed the same evolution of lipids, decreasing throughout the study from elevated initial values. Increased initial lactate concentration due to physical activity and catecholamines effect followed by a return to basal values when anaerobic metabolism is replaced by aerobic metabolism, as previously reported, can explain lactate dynamics (Verde and Gascón, 1987; Hattingh *et al*., 1988; Kaneko, 1997a; Montané, 2002).

It has been suggested that total bilirubin could increase in stress due to hepatocellular damage and/or release of haemoglobin during haemolysis, although it has not been proved (Bush, 1993). In ruminants, liver has a high capacity to excrete bilirubin, and hyperbilirubinemia is related to haemolytic conditions rather than to hepatic problems (Duncan *et al*., 1994; Tennant, 1997). Higher bilirubin values in control females than in control males could indicate a higher degree of stress-related haemolysis in females.

Serum creatinine concentration depends on protein catabolism and renal excretion. It is directly related to the muscular mass of the animal, and can be used to assess renal function, since its production is relatively constant (Finco, 1997). Physical capture and stress are supposed to increase creatinine and urea concentrations due to physical activity, catecholamine-induced renal vasoconstriction and corticosteroid-induced protein catabolism (Harthoorn, 1976; Gibert, 1991; Duncan *et al*., 1994; Finco, 1997). Increased creatinine concentrations resulting from muscular activity and vasospasm in the kidney produced by catecholamines have been reported in physically captured wild ungulates (Harthoorn, 1976). Sodium concentration varies along a relatively narrow interval, and vasoconstriction induced by catecholamine stimulates sodium reabsorption in the kidney (Carlson, 1997; DiBartola, 2000). However, controversy exists about sodium changes related to stress, since both increased and decreased sodium values have been described following a stress episode (Kocan *et al*., 1981; Kock *et al*., 1987a; Marco *et al*., 1998; Marco and Lavín, 1999). Creatinine decrease throughout all the study in the treated group and sodium concentration increase over time only in the control group indicate that the α-adrenergic blocking effect of acepromazine on renal arterioles promotes vasodilatation, avoiding catecholamines mediated vasoespasm and allowing normal filtration and excretion of both sodium and creatinine (Marco and Lavín, 1999; Montané *et al*., 2003). Renal vasodilatation reduces the

risk of renal hypoxia and ischemic necrosis associated to capture myopathy (Spraker, 1982) by ensuring oxygen supply to kidney cells. Serum urea concentration depends largely on the diet, although dehydration also can increase it (Duncan *et al*., 1994; Finco, 1997). Differences in diet and habitat could explain the higher values of urea in females in our study, and these sex differences in urea values should be considered when assessing biochemical parameters of Southern chamois.

Serum muscular enzymatic activity (ALT, AST, CK and LDH) increase in stressed animals during capture and handling operations due to increased cellular permeability and cellular damage, although some proteins increase in stressful situations without apparent lesions (Adams and Rinnie, 1982; Spraker, 1993; Duncan *et al*., 1994). Physical activity also increases serum enzymatic activity due to moderate transitory ischemia (Querengaesser *et al*., 1994). CK is a very sensitive indicator of muscle damage, and combined with AST (less specific but with a longer plasmatic half-life) are considered a good tool to assess muscle status and the most useful to make a diagnosis and a prognosis of capture myopathy (Chapple *et al*., 1991; Williams and Thorne, 1996; Kramer and Hofmann, 1997). LDH-5 isoenzyme is highly specific of skeletal muscle, and rises in LDH activity are highly correlated with CK, thus being a useful marker of muscle damage (Goddard and Grigor, 1997). Although in ruminants ALT activity less specific and has little diagnostic value (Kramer and Hoffmann, 1997), it has also been related to capture stress and may be useful to detect capture myopathy (Barrett and Chalmers, 1977; Vassart *et al*., 1992). Increases in these enzymes seem to be related to poor tissue perfusion, decreased heat dissipation and hypoxia, components of the pathophysiology of capture myopathy (Spraker, 1993; Williams and Thorne, 1996). ALT, AST, CK, and LDH increased over time from capture levels, indicating that their increases were related to stress rather than physical activity. These stress-related increases in serum enzymatic activity have been previously reported in wild ungulates physically restrained or not submitted to physical activity, which agree with results found in Southern chamois (Jones and Price, 1990; Montané *et al*., 2002 and 2003). Higher increases in ALT and LDH activities and statistically higher values of CK at time 4 in the control group indicate a protective effect of acepromazine on muscle against capture myopathy in Southern chamois. This effect has been previously reported in roe deer and horses (Beech, 1994; Montané *et al*., 2003), and it was attributed to vasodilatation in muscular arterioles by blocking α-adrenergic receptors or stimulating β_2-adrenergic receptors (Booth, 1982; Guyton and Hall, 2000). Muscle arterioles vasodilatation implies a decrease in anaerobic glycolysis and consequent systemic acidosis (Spraker, 1982) by increasing oxygen availability in the muscular tissue.

AP is found in intestine (intestinal isoenzyme), kidney, liver and bone (unspecific isoenzyme), and also there is a termostable corticosteroid induced alkaline phosphatase (Kramer and Hoffmann, 1997; Rijnberk and Mol, 1997). Decrease of AP in both groups is probably related to increases during physical exercise previous to capture, returning to basal values throughout the study period. However, it is difficult to find a precise explanation without knowing the exact distribution of AP isoenzymes in the Southern chamois and the isoenzymes participating in the variations found.

Chloride is the major extracellular ion (Carlson, 1997), and increases in its concentration are associated with a tendency towards acidosis (hyperchloremic acidosis), to compensate the decrease of bicarbonate (Autran de Morais, 2000). Potassium is mainly an intracellular ion (Carlson, 1997). Catecholamines cause an increase (α-adrenergic effect) followed by a decrease (β_2 effect) in serum potassium concentration, whereas acute administration of corticosteroids causes transitory hyperkalemia (Bia and DeFronzo, 1981). Exercise, muscular necrosis and acute metabolic acidosis cause a translocation of potassium from intercellular fluid to extracellular fluid which increases serum potassium concentration. Acute acidosis decreases urinary excretion of potassium, and renal failure induces hyperkalemia. Hyperkalemia induces changes in neuromuscular and heart excitability which alters the electrocardiogram and can lead to shock and death because of ventricular fibrillation (Spraker, 1982; Carlson, 1997; DiBartola and Autran de Morais, 2000). Both chloride and potassium increases have been related to physical capture or stress (Kock *et al*., 1987a; DeLiberto *et al*., 1989; DelGiudice *et al*., 1992; Peinado *et al*., 1993). In our study, chloride and potassium decreased over time in the control group and the treated group, respectively, and sodium increased significantly. Disproportionate chloride and sodium changes are related with acid-base unbalance. High chloride values are associated with a tendency towards acidosis (Carlson, 1997; Autran de Morais, 2000). Decreasing chloride and increasing sodium concentrations over time could be due to acidotic metabolism returning to normality after capture stress. Increased urinary potassium excretion is associated with high sodium and chloride intake in the kidney, since Na^+,K^+-ATPase excretes potassium to the tubular lumen and introduces sodium in the cell, with chloride diffusing passively to the intercellular space. Renal vasodilatation caused by the α-adrenergic effect of acepromazine could explain the decrease of serum potassium and the lack of changes in serum sodium concentration over time in the treated group. Potassium decreased over time in Southern chamois from initially elevated values due to the effect of physical exercise previous to capture and catecholamines (Van Beaumont *et al*., 1973; Bia and DeFronzo, 1981).

Table 7.1. Physiological parameters used to assess stress in Southern chamois.

Physical activity	SA axis	HPA axis	Biological consequences
Heart rate	Heart rate	Temperature	Muscular enzymes
Temperature	Temperature	WBC count	Bilirubin
RBC parameters	RBC parameters	WBC differential count	Lactate
Cholesterol	Platelet count	Cortisol	Creatinine
Tryglicerides	WBC count	Glucose	Urea
Lactate	WBC differential count	Creatinine	Sodium
AP	Glucose	Urea	Chloride
	Cholesterol		Potassium
	Tryglicerides		
	Lactate		
	Sodium		
	Chloride		
	Potassium		

7.3.3. Sex effect on stress response of physically restrained Southern chamois

Southern chamois males and females behaved differently in many of the physiological parameters analyzed to assess their stress response.

The later stabilization of heart rate in control females than in the other groups and the higher heart rate of control females than control males from minute 135 to minute 175 seem to point to a higher alarm degree of non treated females. Lower temperature of treated females when compared to treated males seems to indicate a stronger hypothermic effect of acepromazine in females.

Erythrocyte count, haemoglobin concentration and haematocrit decreased over time in males but did not change in females. Mean corpuscular volume and mean corpuscular haemoglobin concentration increased in females and males, respectively, but they did not change in the opposite sex. Decreases of erythrocytic parameters after catecholamine stimulation have been described, since their effects last 20-30 minutes (Wesson *et al*., 1979; Duncan *et al*., 1994). Lack of decrease of these parameters in Southern chamois females was probably due to a longer and stronger adrenalin secretion of females than males, either due to handling to obtain the sample or to physical restraint. Increasing MCV and lack of increase in MCHC in females could be due to continuing release of bigger erythrocytes from the spleen due to catecholamine stimulation (Cross *et al*., 1988).

Lymphocyte numbers decreased over time in males but remained stable in females. Since lymphocytosis in Southern chamois seems to be related to catecholamines secretion,

this fact further suggests that catecholamine secretion stayed longer in females. However, it could also be due to a more intense developing lymphopenia in males due to higher production and use of corticosteroids (Jain, 1993; Evans, 2000). Monocytes increased during the study only in males.

Cortisol values increased over time only in males, whereas females did not show statistical changes, indicating a higher HPA axis stimulation in males (Morton *et al*., 1995). Higher cholesterol values in treated females when compared to treated males could also be due to increasing cortisol concentration over time only in males, and a lesser stress level in treated females.

Higher values of glucose in treated females when compared with treated males seem to point to a higher stress response not controlled by acepromazine in females, since both catecholamines and corticosteroids have been reported to increase glucose concentration (Verde and Gascón, 1987).

Bilirubin increased more over time in females than in males, indicating increased haemolysis or hepatic compromise in females during the stress response (Duncan *et al*., 1994; Tennant, 1997).

Creatinine was higher and urea was lower in females than in males in all times, but since males and females were sampled in different seasons, these differences could be related to diet rather than to differences in the stress response (Finco, 1997). However, creatinine followed a different trend for each sex, decreasing in males and increasing in females, which suggests indicating a strong renal vasoconstriction due to catecholamines in females, due to a stronger response of the autonomic nervous system or a greater sensitization of chatecolamine α-adrenergic receptors in renal arterioles (Finco, 1997).

ALT levels increased over time in untreated males but not in females, suggesting a higher muscular damage due to stress in males. Statistically higher values of treated males when compared to treated females for all four muscular enzymes (ALT, AST, CK and LDH) at time 4 indicate that the protective effect of acepromazine is stronger on muscular arterioles α-adrenergic receptors of females. However, higher muscle enzymes values for males have been previously reported in different species of wild ungulate, indicating a stronger stress response in males (Kent *et al*., 1980; Kock *et al*., 1987a; Chapple *et al*., 1991). A stronger, more difficult to control with acepromazine stress response in males could also explain higher muscle enzymes values of males.

Chloride levels were higher in females at the end of the study period, whereas potassium was higher in females at the beginning of the study. Chloride and potassium dynamics indicate higher initial sympathetic stimulation and higher renal excretion of potassium and uptake of chloride over time in females than in males, probably related to higher catecholamines response in females (Bia and DeFronzo, 1981; Autran de Morais, 2000).

Table 7.2. Stress response differences in stress response between males and females.

Stronger catecholamine response in females	Stronger corticosteroid response in males	Stronger acepromazine effect in females
Erythrocyte count	Lymphocytes	Temperature
Haematocrit	Cortisol	ALT
Haemoglobin concentration	ALT	AST
Glucose		CK
Bilirubin		LDH
Creatinine		Chloride
Chloride		Potassium
Potassium		

7.4. TRANSPORT STRESS

7.4.1. Heart rate and rectal temperature

Heart rate is a widely used acute stress indicator, since it assesses the response of the autonomic nervous system to stressors in an objective way (Broom and Johnson, 1993; Hopster and Blockhuis, 1994; Waas *et al*., 1999). Statistically higher and increasing heart rate values in the control group only during transport, and not during previous physical restraint, indicate a higher stress level in control animals than in treated Southern chamois, and that transport was more stressful than physical restraint in the control group. Increases in heart rate during transport agree with previously reported data for red deer (Horalek and Jones, 1993) and roe deer (Montané *et al*., 2002), although decreases in heart rate during transport have also been described in red deer (Waas *et al*., 1997; Grigor *et al*., 1998). However, no effect on heart rate of the same tranquilizer (acepromazine) has been found in transported roe deer (Montané *et al*., 2002). Lack of statistical differences in heart rate during the pretransport period could be explained by reflex tachycardia secondary to acepromazine hypotension (Plumb, 1999). This effect would be overcome by stress-induced tachycardia in the control group during transport, appearing statistical differences due to the higher intensity of transport stress. The use of heart rate variability has been proposed as a better stress indicator than basic heart rate (Porges, 1985), but it was not different between control and treated Southern chamois. However, the tranquilizing effect of the drug could explain the lower interindividual variability of heart rate in the treated group, with control animals displaying a less uniform response than treated ones.

Body temperature increases in stress situations due to stress-induced hyperthermia (SIH) (Moe and Bakken, 1997; Bakken *et al*., 1999). SIH is time dependent, and temperature increases to reach a stable high level (1 to 1.5 ºC higher than basal values) in 10 minutes to decrease and return to baseline after 60 to 90 minutes (Zethof *et al*., 1994; Moe and Bakken, 1997). Temperature monitoring of Southern chamois in our study started 53 ± 13 minutes after capture, when capture related SIH started to decline, as seen in the decrease of temperature over time in both groups. Unlike heart rate, no increases in temperature during transport were observed. Acepromazine shortened capture related SIH, as demonstrated by earlier temperature decrease and stabilization in the treated group, and also decreased SIH intensity, since body temperature was lower in treated animals during pretransport. Acepromazine causes hypothermia due to depression of the reticular activating system and vasodilatation due to both central and α-adrenergic effects (Plumb, 1999), and both effect could participate to reduce body temperature in treated animals. Transport did not seem to cause an increase in temperature in the control group as it did in heart rate, which suggest that SIH is not directly mediated by the sympathetic-adrenal medulla axis, responsible of

heart rate increases, although correlations between SIH and both sympathetic-adrenal medulla and hypothalamic-pituitary-adrenal cortex axes have been found (Groenink *et al*., 1994). This relationship only demonstrates that SIH is a stress-mediated response, since it has been suggested that SIH is due to a change in the thermoregulatory set point due to prostaglandin E and interleukins 1 and 6 (Kluger *et al*., 1987; Briese and Cabanac, 1990; LeMay *et al*., 1990; Kent *et al*., 1993).

7.4.2. Haematology and serum biochemistry

Catecholamines are released from the adrenal medulla due to activation of the sympathetic nervous system during the stress response. Increases in RBC, haemoglobin concentration and haematocrit have been related to the effect of catecholamines on α-adrenergics receptors in the splenic capsule, inducing smooth muscle contraction and the release to circulation of erythrocytes stored in the spleen (Ganong, 1998; Jain, 1993), but also to a reduction in plasma volume (Wesson *et al*., 1979; Cross *et al*., 1988). The α-adrenergic blocking effect of acepromazine has been described to modulate the increase of red blood cell count, haematocrit and haemoglobin related to both transport and capture stress (Montané *et al*., 2002 and 2003). In our study, decreasing RBC, haematocrit and haemoglobin values during pretransport period and statistically higher values in control animals for RBC and haemoglobin at time 2 agree with previously reported data. Lack of statistical differences for haematocrit after transport has been also found in transported roe deer, and was attributed to the variability (2%) of the microhaematocrit method (Wintrobe, 1974; Montané *et al*., 2002). During transport, however, no statistical differences between treated and control groups and no statistical variation over time were found for these three parameters. These results indicate that catecholamine effects on spleen α-adrenergics receptors were present at capture but not during transport, since parameters influenced by the sympathetic-adrenal medulla axis effects on spleen (RBC, haemoglobin concentration and haematocrit) remained stable in both groups during transport.

After a stress episode, catecholamines induce lymphocytic leukocytosis during the first 20 to 30 minutes, followed by corticosteroid-induced leukocytosis and neutrophilia, which reach the maximum level 4 to 6 hours after the stress episode (Jain, 1993; Duncan *et al*., 1994). Both epinephrine and corticosteroids cause monocytosis and a decrease in eosinophils and basophils (Jain, 1993; Taylor, 2000). In our study, lymphocytes and neutrophils were the only leukocytes that decreased and increased, respectively, during the pretransport period, indicating that stress response was shifting from the catecholamines-dependent phase to the corticosteroid-dependent phase. However, total leukocyte count did not change in none of the groups during the pretransport period, but it increased in both groups during transport, indicating a higher stress response. Lymphocytes remained stable

whereas neutrophils increased during transport, suggesting that stress response was rather corticosteroid dependent. Monocytes and band neutrophils increases only during transport and only in the control group indicated a stronger stress monocytosis and neutrophil mobilization during transport which was inhibited by acepromazine in the treated group.

Cortisol has been traditionally considered as a good stress indicator (Franzmann *et al*., 1975; Seal and Bush, 1987; Morton *et al*., 1995; Waas *et al*., 1999), although great interindividual differences exists in stress-induced corticosteroids concentrations, cortisol is a poor welfare indicator and it is difficult to relate physiological changes and animal welfare (Moberg, 1985 and 1987; Rushen, 1991). Serum cortisol concentration increases only in the control group and statistical differences between treated and control groups after transport could be explained by the central tranquilizing effect of acepromazine. Cortisol concentration has been reported to increase during transport in wild ungulates (Kock *et al*., 1990)

Catecholamines and glucocorticoids increase serum glucose concentration by increasing glycogenolysis and glycogenesis, respectively (Ganong, 1998; Guyton and Hall, 2000), and both increases and decreases in serum glucose concentration have been reported in transported wild ungulates (Kock *et al*., 1990; Brelurut *et al*., 1991; Marco *et al*., 1997; Montané *et al*., 2002), although controversy exists about the usefulness of serum glucose concentration to measure stress (Sartorelli *et al*., 1992; Broom and Johnson, 1993; Waas *et al*., 1999). Decreases in serum glucose concentration in both groups only during the transport period and lack of differences between treated and control groups agree with data previously reported (Brearley *et al*., 1990: Marco *et al*., 1997; Montané *et al*., 2002). This decrease in serum glucose during transport have been attributed either to higher glucose concentration during capture returning to basal values during transport (Marco *et al*., 1997) or to food deprivation (Knowles and Warris, 2000; Montané *et al*., 2002). Transport in our study was too short to cause hypoglycaemia due to food deprivation, and the fact that pretransport blood sample was obtained more than 100 minutes after capture could be the responsible of not detecting capture-induced transient hyperglucemia.

Serum creatinine concentration is directly related to the muscular mass of the animal, and can be used to assess renal function, since its production is relatively constant (Finco, 1997). Increased creatinine concentrations resulting from muscular activity and vasospasm in the kidney produced by catecholamines have been reported in physically captured wild ungulates (Harthoorn, 1976). Sodium concentration varies along a relatively narrow interval, and vasoconstriction induced by catecholamine stimulates sodium reabsorption in the kidney (Carlson, 1997; DiBartola, 2000). An increase in protein catabolism will tend to produce an increase in serum urea concentration (Knowles and Warris, 2000). This increase may be due to physical exercise, diminished renal perfusion and to the catabolic effect of glucocorticoids

on catabolism (Finco, 1997). Decreases of serum creatinine concentration during transport in wild ungulates, with and without tranquilizer, have been previously reported, whereas no changes in sodium concentration were found in the same studies (Kock *et al*., 1990; Marco *et al*., 1997; Grigor *et al*., 1998; Montané *et al*., 2002). Increases of serum urea concentration related to stress after capture have been described (Gibert, 1993). In our study, both creatinine and sodium concentrations decreased before transport only in the treated group, remaining stable in both groups during transport. Thus, previously reported creatinine decreases could be related to creatinine concentration returning to basal values after capture stress rather than being an effect of transport. On the other hand, urea increased over time only in the control group. Decrease of both creatinine and sodium concentrations in the treated group during pretransport can be explained by the α-adrenergic blocking effect of acepromazine on renal arterioles, promoting vasodilatation, whereas increasing serum urea concentration in the control group indicates diminished renal perfusion due to the α-adrenergic effect of catecholamines. Statistically lower concentrations of creatinine before and after transport, decrease of sodium concentration and lack of increase of urea concentration in treated animals indicate that renal perfusion was improved by acepromazine, ensuring adequate oxygen supply to the kidney and reducing the risk of ischemic necrosis of the kidney (Jarvik, 1970; Montané *et al*., 2002).

It has been suggested that total bilirubin could increase in stress due to hepatocellular damage and/or release of haemoglobin during haemolysis, although it has not been proved (Bush, 1993). Acepromazine is metabolized in the liver and metabolites are eliminated in the urine (Plumb, 1999). However, in ruminants liver has a high capacity to excrete bilirubin, and hyperbilirubinemia is related to haemolytic conditions rather than to hepatic problems (Duncan *et al*., 1994; Tennant, 1997). An increase in serum bilirubin concentration during transport in animals treated with acepromazine has been previously described, although it was not statistically significant (Montané *et al*., 2002). Increase of serum bilirubin concentration in both groups of Southern chamois before transport could be due to stress-related haemolysis, whereas statistically higher values of treated animals after transport could be related as well to hepatic drug metabolism.

Catecholamines have a lipolytic effect on adipose tissue, stimulating fat mobilization and increasing circulating free fatty acids (Genuth, 1996; Guyton and Hall, 2000), but during stress cholesterol could be deviated to corticosteroid synthesis. Both increases and decreases in serum cholesterol concentration have been related to stress (Franzmann and Thorne, 1970; Barrett and Chalmers, 1977; Locatelli *et al*., 1988; Marco *et al*., 1997; Marco *et al*., 1998). Decreasing values in Southern chamois in our study could be due either to a decreasing degree of alarm or to the deviation of cholesterol to cortisol synthesis.

Potassium is mainly an intracellular ion (Carlson, 1997). Catecholamines may cause an increase (α-adrenergic effect) followed by a decrease (β_2 effect) in serum potassium concentration, whereas acute administration of corticosteroids causes transitory hyperkalemia (Bia and DeFronzo, 1981). Exercise, muscular necrosis and acute metabolic acidosis cause a translocation of potassium from intercellular fluid to extracellular fluid which increases serum potassium concentration. Acute acidosis decreases urinary excretion of potassium, and renal failure induces hyperkalemia. Hyperkalemia induces changes in neuromuscular and heart excitability which alters the electrocardiogram and can lead to shock and death because of ventricular fibrillation (Spraker, 1982; Carlson, 1997; DiBartola and Autran de Morais, 2000). Potassium decreases during transport have been previously described for roe deer, although this trend was not present in tranquilized animals (Montané *et al*., 2002). Decrease of potassium levels during transport could be due to catecholamines β_2 effect. Statistically lower values of potassium in treated animals before and after transport indicate that the α-adrenergic blocking effect of acepromazine in renal arterioles allows a more effective excretion of potassium in the kidney, thus decreasing the risk of shock, ventricular fibrillation and eventually death in treated Southern chamois during transport.

Serum enzymatic activity (ALT, AST, CK and LDH) increases during capture and handling operations due to increased muscle cell permeability or damage (Duncan *et al*., 1994). CK is a very sensitive indicator of muscle damage, and combined with AST (less specific but with a longer plasmatic half-life) are considered a good tool to assess muscle status and the most useful to make a diagnosis and a prognosis of capture myopathy (Chapple *et al*., 1991; Williams and Thorne, 1996; Kramer and Hofmann, 1997). LDH-5 isoenzyme is highly specific of skeletal muscle, and rises in LDH activity are highly correlated with CK, thus being a useful marker of muscle damage (Goddard and Grigor, 1997). Although in ruminants ALT activity less specific and has little diagnostic value (Kramer and Hoffmann, 1997), it has also been related to capture stress and may be useful to detect capture myopathy (Barrett and Chalmers, 1977; Vassart *et al*., 1992). Increases in these enzymes seem to be related to poor tissue perfusion, decreased heat dissipation and hypoxia, components of the pathophysiology of capture myopathy (Spraker, 1993; Williams and Thorne, 1996). All four enzymes have been reported to increase in transported wild ungulates (Kock *et al*., 1990; Brelurut *et al*., 1991; Marco *et al*., 1997; Montané *et al*., 2002). Statistically higher values for ALT, CK and LDH in the control group after transport, and increases before transport only in the control group for AST, CK and LDH, indicate that acepromazine had a protective effect against muscle damage. This effect could be related to vasodilatation of muscular arterioles by blocking α-adrenergic receptors or stimulating β_2-adrenergic receptors, as described previously (Booth, 1982; Beech, 1994; Guyton and Hall, 2000; Montané *et al*., 2002). The fact that all four enzymes increased in the treated group only during transport and that statistical differences between groups were found only after

transport suggest that transport supposed a higher vascular compromise due to stress than previous physical restraint.

AP is found in intestine (intestinal isoenzyme), kidney, liver and bone (unspecific isoenzyme), and also there is a termostable corticosteroid induced alkaline phosphatase (Kramer and Hoffmann, 1997; Rijnberk and Mol, 1997). It has been reported to decrease during transport in wild ungulates (Marco *et al*., 1997). Lactate is a metabolite of glucose produced by anaerobic glycogenolysis and muscular glycogenesis. In ruminants it can also be synthesized from lactic acid absorbed in the rumen (Kaneko, 1997a). Serum lactate concentration has been reported to increase during transport, although it varies greatly throughout the transport period and measured values depend on the duration of transport and timing of blood sampling (Brelurut *et al*., 1991; Waas *et al*., 1999). Serum AP activity and lactate concentration decreases before transport indicate that metabolism is returning to basal values after the capture event. However, the fact that during transport these two parameters decreased only in the treated group suggest that, while in treated animals return to basal values continued during transport, in control animals transport induced an excitement that activated metabolism again.

The results obtained show that acepromazine is capable of reducing the adverse consequences of transport stress in Southern chamois, not only due to its central sedative effect, as demonstrated by heart rate and cortisol concentration, but also due to its additional effects, specially its α-adrenergic blocking effects. These positive effects consist mainly in renal and muscular vasodilatation, preventing or reducing the development of renal and muscular hypoxia and consequent renal ischemic necrosis, muscular damage and metabolic acidosis, as indicated by creatinine, urea, sodium, potassium and muscular enzymes. Vasodilatation and depression of the reticular activating system also reduce stress-induced hyperthermia, preventing the development of capture myopathy. α-adrenergic blocking effects of acepromazine on smooth muscle of the spleen, however, did not seem to be so strong during transport as it was during previous physical restraint, which could be due to adaptation of spleen α-adrenergic receptors or to a higher degree of alarm during transport which could not be compensated by the drug. Transport was more stressful to Southern chamois than previous physical restraint, as demonstrated by higher increases in heart rate, monocytes, band neutrophils and muscular enzymes and stabilization of AP and lactate during transport.

7.4.3. Differences between transport and physical restraint stress in Southern chamois

Transport is a complex and more threatening stimulus than simple physical restraint, since it involves also a certain degree of physical restraint plus noise, movement, handling operations to load and unload the animals from the vehicle and usually previous capture. So,

during transport wild animals are submitted to a series of stressor which may have the same effect as a single higher intensity stimulus (Curtis, 1993; Montané, 2002). Effects of transport on Southern chamois were overall similar to those of physical restraint. However, some parameters showed higher changes during transport than during physical restraint.

Heart rate increased during transport, but remained stable during physical restraint. Increased heart rate due to stress during transport has been related to movement of the vehicle rather than noise of the engine (Horalek and Jones, 1993). Whatever the precise stimulus is, transport supposed a more intense stressor to cause a rise in heart rate than physical restraint.

In spite of decreasing after capture as previously reported (Wesson *et al*., 1979), erythrocyte count, haematocrit and haemoglobin concentration seemed to stabilize during transport, and haematocrit and haemoglobin concentration even were not statistically different from capture values after transport.

Creatinine did not change in transported Southern chamois, whereas it decreased in physically restrained animals, and urea increased throughout transport but remained stable during physical restraint. These results seem to point to a stronger renal vasoconstriction due to catecholamines, and therefore a stronger stress response, during transport than during physical restraint, since both creatinine and urea have been reported to increase due to catecholamine-induced renal vasoconstriction (Harthoorn, 1976; Finco, 1997).

Although they increased both in physically restrained and transported Southern chamois, higher serum levels attained for ALT, CK and LDH during transport seem to indicate that it caused a higher physiological compromise, probably because it supposed a stronger stressor than physical restraint (Spraker, 1993; Duncan et al., 1994).

Chloride decreased and sodium did not change over time in transported Southern chamois, which could be related to a higher stress level maintaining acidotic metabolism throughout transport (Spraker, 1982; Autran de Morais, 2000; DiBartola, 2000).

Overall, data from heart rate, erythrocyte count, haematocrit, haemoglobin concentration, creatinine, urea, ALT, CK, LDH, chloride and sodium indicate that transport was more stressful than physical restraint for Southern chamois, agreeing with results reported for other species of wild ungulates (Montané, 2002).

Table 7.3. Physiological parameters indicating higher stress during transport.

• Heart rate • Erythrocyte count • Haematocrit • Haemoglobin concentration • Creatinine • Urea	• ALT • CK • LDH • Chloride • Sodium

7.5. EFFECT OF ACEPROMAZINE ON STRESS RESPONSE OF SOUTHERN CHAMOIS

Vagal stimulation induced by acepromazine causes bradycardia (Plumb, 1999). Nevertheless, no differences in heart rate were observed in physically restrained animals, although untreated transported Southern chamois had higher heart rate than animals treated with acepromazine. Reflex tachycardia secondary to decreases in blood pressure has been described to negate bradycardia induced by acepromazine (Plumb, 1999). Heart rate data from Southern chamois suggest that tachycardia induced by physical restraint is similar to that caused by acepromazine, whereas transport-induced tachycardia in untreated animals overcomes the effect of acepromazine in sedated Southern chamois. However, earlier stabilization of heart rate in treated restrained animals when compared to controls may be attributed to the tranquilizing effect of the drug, agreeing with previously described results in different deer species (Diverio *et al*., 1996b; Montané *et al*., 2003).

Hypothermia is a side effect of acepromazine, preventing the development of halothane-induced malign hyperthermia in pigs (McGrath *et al*., 1981; Plumb, 1999), but neuroleptics do not avoid stress-induced hyperthermia (SIH), probably because this response is elicited before the drug can be administered (Olivier and Miczek, 1998; Montané, 2002). However, although probably SIH had already been elicited at the moment temperature monitoring began in Southern chamois, acepromazine decreased its intensity and duration, as shown by statistically lower temperature and earlier temperature stabilization in treated animals. This effect could be due either to the tranquilizer effect of the drug or to its hypothermic effect favouring temperature dispersal through vasodilatation. Acepromazine seemed then to control SIH better in Southern chamois than in roe deer (Montané, 2002). Hyperthermia is one of the components of capture myopathy, so acepromazine decreases the risk of developing this pathology.

Erythrocytic parameters (erythrocyte count, haemoglobin concentration and haematocrit) decreased when initial catecholamine stimulation ceased, but values were statistically lower in treated physically restrained animals due to the α-adrenergic blocking effects of acepromazine. Decrease of these parameters due to acepromazine has been already reported, although haematocrit decrease has not been a consistent finding in the different studies carried out with acepromazine (Booth, 1982; Plumb, 1999; Montané *et al*., 2002 and 2003). However, differences between treated and untreated Southern chamois were not present in transported animals, indicating that transport-related catecholamine stimulation overcame the α-adrenergic blocking effects of acepromazine. Although haemodilution due to vasodilatation has been also proposed as the cause of the acepromazine-induced haematocrit decrease (Turner and Hodgetts, 1960; Wesson *et al*.,

1979), no differences were observed in Southern chamois total protein concentration, confirming the main role of the α-adrenergic blocking effect.

Leukocyte differences between treated and untreated Southern chamois were only found in monocytes and band neutrophils, with treated animals showing higher values for both parameters during physical restraint and lower values during transport. Serum cortisol concentration followed the same trend. An exciting effect of phenotiazines has been described (Bruss, 1980; Brearley *et al*., 1990; Plumb, 1999), which could explain differences in restrained animals, whereas higher values of untreated transported animals could be due to higher stress level induced by transport overcoming acepromazine effects in treated Southern chamois.

Phenotiazine administration has been reported to produce a rise in serum glucose concentration (Booth, 1982), which could explain higher values of treated physically restrained Southern chamois. Lack of differences between treated and untreated transported domestic ruminants has also been reported (Brearley *et al*., 1990).

Creatinine decreased over time in treated but not in untreated Southern chamois, and urea increased during transport only in untreated animals, which could be explained by the α-adrenergic blocking effect of acepromazine on renal arterioles (Jarvik, 1970).

Bilirubin was significantly higher after transport in treated than in untreated Southern chamois, which could be related to interaction with hepatic metabolism of acepromazine (Plumb, 1999).

Serum ALT, AST, CK and LDH activities increased more consistently in untreated than in treated Southern chamois, although significant differences between treatments were mainly found in transported animals, since transport was a more stressful stimulus. The protective muscular vasodilatation induced by acepromazine, although not strong enough to prevent completely cellular damage and increase of serum enzyme activity, improves muscular blood flow, ensures oxygen supply to muscle cells, favours aerobic metabolism and decreases intracellular muscular acidosis due to the formation of lactic acid.

Serum sodium concentration decreased in treated animals during transport and increased in untreated animals during physical restraint, whereas potassium decreased more and showed lower values over time in treated animals. Potassium is released from muscular cells when their permeability is altered due to acidosis (Spraker, 1993), and is excreted through the kidney, where it is exchanged by sodium (DiBartola and Autran de Morais,

2000). Sodium and potassium concentrations indicate higher blood potassium levels in untreated animals, probably due to a more acidotic physiological status, exchanging by sodium. Potassium increases cardiac cells excitability, so lower potassium levels in treated animals indicate decreased risk of ventricular fibrillation. Acepromazine could then prevent shock and exercise rhabdomyolisis regulating potassium release from muscular cells (Freestone *et al*., 1991).

Overall, the use of acepromazine during capture, handling and transport operations improves Southern chamois welfare, and prevents the development of stress-associated pathologies, like capture myopathy. Acepromazine protective effects are due to its central tranquilizing effects, as indicated by heart rate, but also to its α-adrenergic blocking effects on spleen (erythrocytic parameters), renal arterioles (creatinine, urea, sodium and potassium), muscular vessels (muscular enzymes, potassium) and general circulation (temperature). These effects are more evident in more stressful operations, like transport, whereas in less stressful situations it may even cause a slight degree of excitement, although the physiological benefits of its use are still present.

Table 7.4. Protective effects of acepromazine on stressed Southern chamois.

Central tranquilization	**α-adrenergic blockage**			
	General	**Spleen**	**Kidney**	**Muscular**
Heart rate Temperature	Temperature	Erythrocyte count Haemoglobin Haematocrit	Creatinine Urea Sodium Potassium	ALT AST CK LDH Potassium

7.6. SALIVA CORTISOL

The finding of noninvasive techniques to assess stress is currently a matter of concern. Techniques to determine cortisol in fluids or tissues other than blood have been proposed to assess stress minimizing handling effect and improving animal welfare (Cooper *et al*., 1989; Palme and Möstl, 1997; Fritsche and Steinhart, 1998; Verkerk *et al*., 1998; Morrow, 2000; Palme *et al*., 2000; Chacón-Pérez *et al*., 2004). Cortisol has been determined in saliva either by enzyme immunoassay or by radio immunoassay (Cook *et al*., 1997; Chacón-Pérez *et al*., 2004). Individual, circadian, age, gender and stress related variations in salivary cortisol have been reported (Ekkel *et al*., 1996; Ruis *et al*., 1997; Kobelt *et al*., 2003). Moreover, whereas some stressors cause an increase in saliva cortisol, other stimuli do not seem to affect saliva cortisol concentration (Beerda *et al*., 1998). Saliva cortisol concentration has also been described as a non-invasive technique to assess stress in wild ungulates (Millspaugh *et al*., 2002).

Studies correlating salivary cortisol with plasma cortisol, total serum cortisol and free serum cortisol have been carried out (Cook *et al*., 1996; Anderson *et al*., 1999b; Chacón-Pérez *et al*., 2004). Highest correlation values (r=0.94) have been found between saliva and free plasma cortisol. Total and free cortisol are also related (r=0.97), so salivary and total serum cortisol are also highly correlated (r=0.7964-0.8813) (Fell *et al*., 1985; Greenwood and Shutt, 1992; Cook *et al*., 1996). Correlation between salivary and plasma cortisol is lower than between salivary and serum cortisol (Anderson *et al*., 1999b), although plasma and salivary cortisol also have a good correlation (r=0.75) (Chacón-Pérez *et al*., 2004). Southern chamois saliva and serum cortisol correlation was similar to those described previously, demonstrating that saliva cortisol concentration can be a useful indicator of serum cortisol concentration (Table 7.5).

Table 7.5. Reported correlation between saliva and serum or plasma cortisol.

Study	Cortisol concentrations compared	r
Fell *et al*., 1985	Saliva – Free serum/plasma	0.90 – 0.94
Cook *et al*., 1996	Saliva – Total serum	0.796 – 0.881
Chacón-Pérez *et al*., 2004	Saliva – Total plasma	0.75
Present study	Saliva – Total serum	0.7899

Mean values previously described for saliva cortisol concentration in stressed domestic species were similar to values of Southern chamois. Saliva:serum or plasma cortisol ratio has been described to oscillate around 2-15% (Fell *et al*., 1985; Greenwood and Shutt, 1992; Cook *et al*., 1996; Schönreiter *et al*., 1999; Chacón-Pérez *et al*., 2004). Saliva:serum cortisol ratio of analysed Southern chamois was as low as 2% (Table 7.6).

Saliva cortisol levels have been reported to increase 30-60 minutes after the injection of ACTH in wild ungulates (Millspaugh *et al*., 2002), and the ratio between serum and saliva cortisol varies with the excitement of the animal and its serum cortisol concentration (Fell *et al*., 1985). Elevated and increasing serum cortisol concentrations in captured Southern chamois still not detectable in saliva could explain the low ratio of Southern chamois, similar to domestic sheep after the application of a stressor (yarding). However, saliva and serum cortisol concentrations previous to capture and stress were not possible to assess, which could further clarify the dynamics of cortisol both in serum and saliva.

Table 7.6. Reported ratio of saliva and serum or plasma cortisol.

Study	Species	Saliva:blood cortisol (%)	Sample
Fell *et al*., 1985	Domestic sheep	2.6 – 10.43	Serum
Greenwood and Shutt, 1992	Domestic goat	11	Plasma
Cook *et al*., 1996	Domestic pig	8.6 – 13.3	Serum
Schönreiter *et al*., 1999	Domestic pig	5.9 – 7.5	Plasma
Chacón-Pérez *et al*., 2004	Cattle	10	Plasma
Present study	Southern chamois	2	Serum

The main problem found to determine saliva cortisol concentration was collecting enough good quality saliva from each animal, as shown by the low percentage of viable samples obtained. Currently, collecting devices specially designed to obtain saliva samples are available, which can help to solve this problem, altogether with increasing experience of the researchers.

To our knowledge, this is one of the first studies determining saliva cortisol concentration and comparing cortisol values in saliva and serum in wild ungulates. Further studies determining saliva and serum cortisol concentration in undisturbed animals, including ACTH stimulation as well as application of different stressors and studying recovery rates of exogenous cortisol added to randomly selected saliva samples of known cortisol concentration would be needed to definitely validate the technique.

8. CONCLUSIONS

1. Capture with drive-nets has proved to be a safe and useful method to capture Southern chamois, with a relatively high performance and maximum specificity.

2. Stress response of Southern chamois to physical capture and restraint followed a biphasic pattern related to consecutive effects of catecholamines and corticosteroids. Physical activity related to capture and catecholamines caused an increase in heart rate, rectal temperature, erythrocyte count, haemoglobin concentration, haematocrit, lymphocyte count, glucose, cholesterol, triglycerides, lactate, creatinine, alkaline phosphatase, chloride and potassium, which decreased over time after capture. Corticosteroids caused an increase in total leukocyte count, monocytes, mature and band neutrophils, serum cortisol concentration, total bilirubin, urea, serum muscular enzyme activity (ALT, AST, CK, and LDH) and sodium.

3. Female Southern chamois showed a more intense catecholamine response to stress, as indicated by erythrocytic parameters, glucose, bilirubin, creatinine, chloride and potassium, whereas corticosteroid response seemed to be stronger in males, according to lymphocytes, serum cortisol concentration and ALT.

4. Transport induced a stronger stress in Southern chamois, as indicated by higher values and lack of decrease over time in heart rate, erythrocytic parameters, creatinine, urea, ALT, CK, LDH, chloride and sodium.

5. Acepromazine improved animal welfare and decreased the risk for the life of captured and treated Southern chamois, which showed lack of increases in heart rate during transport, lower values and earlier stabilization of rectal temperature, higher decreases with significantly lower values for erythrocytic parameters, creatinine, urea, sodium and potassium and lower increases in serum enzymatic activity (ALT, AST, CK and LDH).

6. Acepromazine had a stronger tranquilizing and protective effect on females, as demonstrated by temperature, ALT, AST, CK, LDH, chloride and potassium.

7. Saliva cortisol concentration showed good correlation with serum total cortisol concentration, and is a potentially useful noninvasive tool to assess stress.

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