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The Stress Response to Repeated Capture in Mouflon (*Ovis ammon*): Physiological, Haematological and Biochemical Parameters

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With 6 tables

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Summary

Two groups of mouflons (*Ovis ammon*) were held in captivity to study the effects of repeated capture on physiological, haematological and biochemical parameters. The first one (Group I) was of 6 mouflons captured in the wild, while the second (Group II) was also of 6 mouflons, but which had been in captivity for 3 years. In Group I, body temperature, mature neutrophil count and lactate increased during activity, while red blood cells, haemoglobin, ALT, AST, total lipid, phospholipids, cholesterol, BUN, creatinine, phosphorus and zinc decreased at different times during the study period. In Group II, few statistical differences were observed. Most of these changes were related to stress and reflected a lack of adaptation to repeated handling.

Introduction

Wild ungulates are probably the group of animals which have been subjected to the greatest capture and handling operations as a result of the increasing concern about their conservation, and also due to the increase in game ranching management. Thus, there are many studies in the literature dealing with the capture and handling stress response in these species and its measurement using biological parameters (WESSON et al., 1979 a,b; KOCK et al., 1987 a,b, 1990; CROSS et al., 1988; HATTINGH et al., 1988; DELGIUDICE et al., 1990; KNOX et al., 1992; PEINADO et al., 1993, 1995; MORTON et al., 1995).

Repeated handling of animals may allow adaptation to restraint which can be evidenced by biological parameters. In laboratory animals repeatedly subjected to the same stressors, a change in the physiological response in the hypothalamic-adrenal and sympathetic-adrenal axes has been noted. Thus, learning and conditioned responses determine an animal's response to a repeated stressor (HATTINGH et al., 1988). It has been observed not only in laboratory and domestic animals, but also in wild animals, that repeated handling produces changes in the physiological responses, which lead to an animal's adaptation (FRANZMANN and THORNE, 1970; HATTINGH et al., 1988; CHAPPLE et al., 1991). However, some of these reports were about captive-born animals, so results may be different when free-ranging animals are investigated.

Despite being a species which has been subjected to intensive management in Europe, very few reports on the biological parameters and on alterations due to stress in mouflon exist (HAWKEY, 1975; HAWKEY et al., 1984; MARCO et al., 1997). Also, only a few studies have investigated blood values in the same animals under both condition, free-living and captivity,

using the same methods and being performed by the same authors (PEINADO et al., 1995). The present study investigates the effects of repeated handling upon biological parameters during 1 year in two groups of mouflons, one recently captured and kept in captivity, and the other living in captivity for many years.

Material and Methods

Six adult mouflons (Group I), between 2 and 7 years old (5 females and 1 male), were captured using an enclosure-trap in the National Hunting Reserve of Freser-Setcases in the Eastern Pyrenees (NE Spain) in the spring of 1993. Thereafter, they were transported in wooden boxes to the Wildlife Rescue Centre in Valcalent (Lleida), 300 km south of the capture site, where they were kept in captivity for a 1 year period. The other group of six mouflons (Group II) was of 3 females and 3 males between 3 and 6 years old. They were captured in the same area 3 years ago and translocated to the Wildlife Rescue Centre. They were kept in a big pen of 10 000 m² and adapted to captivity, showing normal behaviour. No pathological problems were observed before the beginning of the study. They were fed with alfalfa and grass hay, water, salt *ad libitum* and occasionally cereals. during that time they became used to human presence and were handled once a year for veterinary control, but did not adapt to handling and restraint.

Both groups were kept in different, fenced pens for the study, each one of 300 m², fed with alfalfa hay, grass hay, water, salt *ad libitum* and occasionally cereals. Each group was repeatedly handled the same day at the same time by physical means for determination of physiological parameters and blood sampling. They were yarded in a small pen and each animal was physically restrained for no more than 10 minutes. Time elapsed from yarding to release of all animals after handling was about 2 hours and took place at the same time of day, between 11.00 and 13.00 h. Samples were obtained at the same time in both groups, after the capture of Group I, at 5, 10, 20, 30, 40, 50, 60 days and 1 year.

Blood samples of 10 ml were obtained from the jugular vein, using disposable syringes and 0.9 gauge needles. After removing the needle, 2.5 ml of blood was placed in commercial tubes with anticoagulant (EDTA K₃, Aulabor, Barcelona, Spain). The remaining blood was placed in a tube without any additive and left to clot at room temperature. Serum was then harvested after 2 hours and frozen at -28°C until biochemical analysis was performed, 3 weeks after collection.

Physiological parameters included recording rectal temperatures, pulse rates and respiratory rates.

Red blood cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC) and platelet count (PLT) were measured with a semi-automatic haematology analyser (Sysmex F-800, Toa Medical Electronics, Hamburg, Germany), adapted by adjusting its sensitivity to work with animal blood. PCV value was also carried out, according to the standard microhaematocrit method, using a haematocrit centrifuge (Micro-Haematocrit Centrifuge, Hawksley, England, UK) at 11 000 rpm 9072 *g* for 5 minutes, in order to adjust the values obtained with the analyser. Differential leukocyte count was performed after blood smears had been made and stained with commercial Diff-Quick stain (Química Clínica Aplicada S.A., Amposta, Tarragona, Spain). Cells were observed under × 1000 magnification and classified out of a total of 100 leukocytes.

Values of some of the biochemical parameters were analysed using a Kovas Bio autoanalyser (Roche, Nutley, NJ, USA) at the Servei de Bioquímica Clínica of the Universitat Autònoma de Barcelona (Barcelona, Spain). Levels of alanine amino transferase (ALT), aspartate amino transferase (AST), creatine phosphokinase (CK), gamma glutamyl transferase (GGT), lactic dehydrogenase (LDH), alkaline phosphatase (AP), total lipid, phospholipids, triglycerides, cholesterol, lactate, urea, direct bilirubin, total bilirubin, creatinine and glucose were determined.

Total serum protein concentration (TP) was determined by the Biuret method, using a 4010 photometer (Boehringer Mannheim, Hamburg, Germany). Serum protein electrophoresis was performed on cellulose acetate membranes in a high-resolution buffer at 250 V for 30 min. Membranes were then stained and cleared before reading in a photodensitometer Digiscan Atom 434 (Biotron Scientific Instruments, Barcelona, Spain).

Serum cortisol was analysed by radioimmunoassay (RIA) at the Universidad de León (León, Spain) with the ImmuChemTM Cortisol ¹²⁵I kit (ICN Biomedical, Costa Mesa, CA, USA).

Sodium, potassium, chloride, calcium, phosphorus, magnesium, copper, iron and zinc were determined by atomic emission spectroscopy, with the ICP technique (Inductive Coupled Plasma), using a Thermo Jarrell Ash ICP Polyscan 61E (Thermo Jarrell Ash Co., Franklin, MA, USA) with autosampler at the Servicio Científico Técnico of the Universidad de Barcelona.

In the statistical study, a repeated measures analysis of variance, considering samples from Day 5 to 1 year, was used to compare parameters at day 5 with measurements during the captivity period for each group (I and II). Parameters were compared between groups at each measurement using a Student's *t*-test.

Results

Tables 1–6 show the values of physiological, haematological and biochemical parameters in Groups I and II during the study period.

Body temperature increased during captivity in Group I, with statistical differences at 20, 40 and 60 days. In Group II, only the respiration rate increased occasionally.

The main haematological changes in Group I were a decrease of RBC and Hb at 10 days and Hb at 20 days, and an increase in mean numbers of the mature neutrophil count after 1 year. In Group II, there was also an increase in the mature neutrophil count at 40 days and 1 year.

Biochemical changes in Group I were a decrease in the values of ALT, AST, total lipid, phospholipids, cholesterol, BUN, creatinine, phosphorus and zinc, while lactate increased. The changes in Group II were a decrease in AST and zinc, and an increase in lactate, BUN, total bilirubin and cortisol.

Discussion

Baseline data on physiological values are necessary if such values are to be used in the study and management of wild animal populations. Physiological measurements are being made routinely as the methods of capture and handling of free-living animals improve. However, the effects of different capture and handling techniques on biological parameters, as well as the species, the type of stress and the individual's previous experience, produce detectable physiological changes which must be considered (MORTON et al., 1995).

Physiological parameters

The increase in body temperature in some periods of captivity in Group I was probably due to muscular activity during physical exertion (SMITH, 1990), but in wild animals, pain and fear have been suggested as another cause (KREEGER et al., 1990). Similar values were found in Group II, even higher at some times, but there were no significant differences along the study period. Also, the respiratory rate in this group was higher and increased significantly at 40 days, probably because of increased exertion during handling. In bighorn sheep (*Ovis canadensis*), if animals reach a critical capture stress threshold, organ system damage and myopathy are likely to follow. Thus, if body temperature exceeds 42.2°C, and/or the respiration rate is above 69 rpm, or the heart rate is above 140 bpm, emergency treatment should be established (FOWLER, 1993). Heart and respiratory rate values were increased occasionally in our study, but no emergency treatment was administered and no mortality was observed. Values of physiological parameters of both groups are in agreement with those of other wild sheep species, although higher values in respiratory rate have been described, possibly due to capture stress (MCDONALD et al., 1981; KOCK et al., 1987a).

Haematological parameters

RBC and Hb decreased after 10 and 20 days of captivity, but didn't change significantly during the rest of the study period. In chital deer (*Axis axis*) subjected to repeated handling for blood sampling, CHAPPLE et al. (1991) described a decrease of these parameters in successive samples due to a reduction in stress with adaptation to restraint and handling. No tendency towards a decrease was observed, possibly because the mouflons in our study were free-ranging animals which had recently been captured, and chital deer were farmed animals which were born in captivity. Also, no tendency was observed in Group II. In general, sympathetic responses, such as elevation of RBC induced by catecholamines during stress, have been suggested as being difficult to extinguish due to their role in vigilance (HARGREAVES and HUTSON, 1990). Values of RBC, PCV, Hb and erythrocyte indexes are similar to those described in mouflon (HAWKEY et al., 1984), Dall's sheep (*Ovis dalli*) (FOREYT et al., 1983) and bighorn sheep (KOCK et al., 1987b).

The mature neutrophil count increased over time, but it was only statistically significant

Table 1. Physiological parameters of mouflon (Group I) at 5, 10, 20, 30, 40, 50, 60 days and 1 year of captivity

Measurement	5 days Mean \pm SD	10 days Mean \pm SD	20 days Mean \pm SD	30 days Mean \pm SD	40 days Mean \pm SD	50 days Mean \pm SD	60 days Mean \pm SD	1 year Mean \pm SD
Temperature ($^{\circ}$ C)	39.4 \pm 0.2	40.5 \pm 0.4	40.7 \pm 0.4*	40.5 \pm 0.5	40.7 \pm 0.8*	40.6 \pm 0.5	40.7 \pm 0.8*	40.8 \pm 0.3
Heart rate (bpm)	108 \pm 31	142 \pm 20	136 \pm 20	129 \pm 19	124 \pm 35	157 \pm 45	133 \pm 26	121 \pm 13
Respiratory rate (rpm)	33 \pm 8	45 \pm 13	41 \pm 13	43 \pm 11	54 \pm 16	49 \pm 16	49 \pm 11	47 \pm 23

* P < 0.05 significantly different from value at 5 days of captivity.

Table 2. Physiological parameters of mouflon (Group II) at 5, 10, 20, 30, 40, 50, 60 days and 1 year of captivity

Measurement	5 days Mean \pm SD	10 days Mean \pm SD	20 days Mean \pm SD	30 days Mean \pm SD	40 days Mean \pm SD	50 days Mean \pm SD	60 days Mean \pm SD	1 year Mean \pm SD
Temperature ($^{\circ}$ C)	40.3 \pm 1.0	41.1 \pm 0.7	41.1 \pm 0.9	40.4 \pm 0.3	41.4 \pm 0.8	40.5 \pm 0.3	40.4 \pm 0.5	40.8 \pm 0.2
Heart rate (bpm)	145 \pm 23	146 \pm 45	126 \pm 15	118 \pm 24	162 \pm 47	126 \pm 16	139 \pm 20	135 \pm 29
Respiratory rate (rpm)	42 \pm 12	68 \pm 26	57 \pm 15	53 \pm 20	99 \pm 40*	54 \pm 14	63 \pm 25	66 \pm 15

* P < 0.05 significantly different from value at 5 days of captivity.

Table 3. Haematological parameters of mouflon (Group I) at 5, 10, 20, 30, 40, 50, 60 days and 1 year of captivity

Measurement	5 days Mean \pm SD	10 days Mean \pm SD	20 days Mean \pm SD	30 days Mean \pm SD	40 days Mean \pm SD	50 days Mean \pm SD	60 days Mean \pm SD	1 year Mean \pm SD
Red blood cells ($\times 10^{12}/l$)	11.5 \pm 1.2	9.6 \pm 1.1*	11.8 \pm 2.5	12.1 \pm 1.2	11.8 \pm 0.7	12.0 \pm 0.8	12.2 \pm 1.1	11.4 \pm 1.2
Packed cell volume (%)	41.8 \pm 5.3	34.3 \pm 2.7*	33.9 \pm 3.4*	39.3 \pm 2.4	38.7 \pm 1.6	39.2 \pm 2.9	38.1 \pm 2.9	38.0 \pm 4.2
Haemoglobin (g/dl)	15.6 \pm 2.2	12.2 \pm 0.9	11.8 \pm 1.4	14.0 \pm 0.9	14.0 \pm 0.5	14.3 \pm 0.9	13.9 \pm 0.9	13.6 \pm 1.6
MCV (fl)	31.7 \pm 1.2	35.8 \pm 2.2	29.1 \pm 4.5	32.6 \pm 1.8	32.7 \pm 1.0	32.8 \pm 1.7	31.3 \pm 1.0	33.4 \pm 1.2
MCH (pg)	11.8 \pm 0.3	12.7 \pm 0.7	10.3 \pm 1.7	11.6 \pm 0.6	11.8 \pm 0.5	12.0 \pm 0.5	11.4 \pm 0.4	12.0 \pm 0.7
MCHC (g/dl)	37.1 \pm 0.6	35.6 \pm 0.8	35.4 \pm 1.2	35.6 \pm 0.7	36.2 \pm 0.05	36.6 \pm 0.9	36.5 \pm 0.6	35.9 \pm 0.8
White blood cells ($\times 10^9/l$)	3.97 \pm 1.86	6.18 \pm 1.67	6.12 \pm 0.97	5.62 \pm 1.47	5.65 \pm 1.05	6.23 \pm 1.50	6.72 \pm 2.41	9.75 \pm 3.40
Lymphocytes ($\times 10^9/l$)	2.37 \pm 0.94	4.13 \pm 1.05	3.58 \pm 1.00	3.64 \pm 1.16	3.78 \pm 1.10	3.47 \pm 1.21	3.41 \pm 0.56	3.20 \pm 0.62
Monocytes ($\times 10^9/l$)	0.07 \pm 0.08	0.09 \pm 0.09	0.02 \pm 0.03	0.02 \pm 0.03	0.03 \pm 0.05	0.10 \pm 0.08	0.05 \pm 0.04	0.15 \pm 0.10
Neutrophils ($\times 10^9/l$)	1.49 \pm 1.11	1.96 \pm 0.80	2.46 \pm 0.23	1.94 \pm 0.72	1.80 \pm 0.56	2.52 \pm 0.67	3.16 \pm 2.23	6.34 \pm 2.98*
Neut. Band ($\times 10^9/l$)	0.04 \pm 0.08	0.01 \pm 0.02	0.06 \pm 0.04	0 \pm 0	0.01 \pm 0.02	0 \pm 0	0.05 \pm 0.04	0.04 \pm 0.07
Eosinophils ($\times 10^9/l$)	0.002 \pm 0.005	0 \pm 0	0.013 \pm 0.028	0.015 \pm 0.024	0.016 \pm 0.025	0.029 \pm 0.045	0.067 \pm 0.084	0.020 \pm 0.040
Basophils ($\times 10^9/l$)	0 \pm 0	0 \pm 0	0 \pm 0	0.008 \pm 0.019	0.040 \pm 0.054	0.078 \pm 0.019	0 \pm 0	0 \pm 0
Platelet count ($\times 10^9/l$)	561.2 \pm 148.4	511.2 \pm 147.1	693.8 \pm 44.1	469.3 \pm 89.0	548.5 \pm 72.9	565.3 \pm 158.1	521.0 \pm 136.9	308.8 \pm 121.8

* P < 0.05 significantly different from value at 5 days of captivity.

Table 4. Haematological parameters of mouflon (Group II) at 5, 10, 20, 30, 40, 50, 60 days and 1 year of captivity

Measurement	5 days Mean \pm SD	10 days Mean \pm SD	20 days Mean \pm SD	30 days Mean \pm SD	40 days Mean \pm SD	50 days Mean \pm SD	60 days Mean \pm SD	1 year Mean \pm SD
Red blood cells ($\times 10^{12}/l$)	11.6 \pm 0.6	11.1 \pm 0.8	12.8 \pm 2.0	11.5 \pm 0.8	12.2 \pm 1.0	11.9 \pm 1.3	12.1 \pm 1.2	12.6 \pm 0.7
Packed cell volume (%)	37.0 \pm 2.7	35.0 \pm 2.8	36.6 \pm 3.7	35.8 \pm 3.4	37.0 \pm 3.8	39.0 \pm 4.3	37.1 \pm 4.8	39.8 \pm 3.2
Haemoglobin (g/dl)	13.4 \pm 1.0	12.7 \pm 1.1	12.9 \pm 1.2	13.0 \pm 1.0	13.5 \pm 1.3	14.2 \pm 1.3	13.5 \pm 1.4	14.5 \pm 0.9
MCV (fl)	31.8 \pm 1.4	31.6 \pm 1.0	29.2 \pm 4.6	31.1 \pm 1.5	30.1 \pm 2.4	32.9 \pm 2.0	30.7 \pm 1.7	31.4 \pm 1.1
MCH (pg)	11.5 \pm 0.5	11.4 \pm 0.4	11.1 \pm 0.5	11.3 \pm 0.4	11.1 \pm 1.0	12.0 \pm 0.8	11.2 \pm 0.3	11.5 \pm 0.2
MCHC (g/dl)	36.1 \pm 0.5	36.1 \pm 0.8	35.3 \pm 1.3	36.4 \pm 0.5	36.5 \pm 0.9	36.5 \pm 0.9	36.4 \pm 1.3	36.6 \pm 0.8
White blood cells ($\times 10^9/l$)	6.13 \pm 1.49	8.25 \pm 1.18	6.38 \pm 2.03	7.23 \pm 2.04	8.90 \pm 1.68	6.57 \pm 2.09	7.63 \pm 2.38	10.45 \pm 2.47
Lymphocytes ($\times 10^9/l$)	4.79 \pm 1.59	4.49 \pm 0.72	4.57 \pm 1.07	4.15 \pm 0.73	4.10 \pm 0.35	3.96 \pm 1.04	4.25 \pm 1.05	4.27 \pm 1.00
Monocytes ($\times 10^9/l$)	0.06 \pm 0.04	0.15 \pm 0.11	0.07 \pm 0.03	0.04 \pm 0.05	0.04 \pm 0.06	0.20 \pm 0.18	0.07 \pm 0.04	0.17 \pm 0.18
Neutrophils ($\times 10^9/l$)	1.08 \pm 0.25	3.03 \pm 0.93	1.53 \pm 0.99	2.82 \pm 1.38	1.44 \pm 1.75*	2.27 \pm 1.21	3.16 \pm 2.09	5.91 \pm 1.61*
Neut. Band ($\times 10^9/l$)	0.01 \pm 0.02	0.02 \pm 0.04	0.01 \pm 0.02	0.03 \pm 0.05	0.02 \pm 0.04	0 \pm 0	0.02 \pm 0.05	0.02 \pm 0.04
Eosinophils ($\times 10^9/l$)	0.197 \pm 0.184	0.555 \pm 0.619	0.202 \pm 0.158	0.161 \pm 0.125	0.272 \pm 0.311	0.136 \pm 0.114	0.131 \pm 0.107	0.089 \pm 0.050
Basophils ($\times 10^9/l$)	0 \pm 0	0 \pm 0	0 \pm 0	0.009 \pm 0.022	0.029 \pm 0.045	0 \pm 0	0 \pm 0	0 \pm 0
Platelet count ($\times 10^9/l$)	402.3 \pm 145.2	347.7 \pm 121.1	472.7 \pm 170.6	450.7 \pm 104.9	292.0 \pm 164.4	555.2 \pm 148.0	425.7 \pm 114.0	319.0 \pm 141.9

P < 0.05 significantly different from value at 5 days of captivity.

Table 5. Biochemical parameters of mouflon (Group I) at 5, 10, 20, 30, 40, 50, 60 days and 1 year of captivity

Measurement	5 days Mean \pm SD	10 days Mean \pm SD	20 days Mean \pm SD	30 days Mean \pm SD	40 days Mean \pm SD	50 days Mean \pm SD	60 days Mean \pm SD	1 year Mean \pm SD
ALT (IU/l)	47.3 \pm 27.1	28.8 \pm 23.0	14.4 \pm 4.6*	8.8 \pm 6.0*	10.5 \pm 5.7*	12.0 \pm 3.7*	16.2 \pm 6.1	15.1 \pm 9.2
AST (IU/l)	1001.9 \pm 537.2	176.8 \pm 20.9*	64.3 \pm 20.9*	114.9 \pm 38.9*	105.9 \pm 29.4*	103.7 \pm 17.6*	137.6 \pm 20.3*	159.9 \pm 42.8*
CK (IU/l)	186.6 \pm 98.1	66.35 \pm 13.74	49.17 \pm 26.29	85.8 \pm 19.5	170.2 \pm 29.8	134.7 \pm 24.1	178.6 \pm 46.9	1640.4 \pm 2913.6
GGT (IU/l)	72.7 \pm 30.5	66.2 \pm 20.3	64.1 \pm 11.8	71.9 \pm 18.6	64.9 \pm 14.5	54.6 \pm 10.8	68.7 \pm 20.6	71.8 \pm 45.5
LDH (IU/l)	2364.2 \pm 617.7	1030.9 \pm 187.8	846.4 \pm 174.5	969.3 \pm 274.7	918.4 \pm 164.2	852.1 \pm 154.7	1038.1 \pm 120.1	1092.8 \pm 166.9
AP (IU/l)	228.2 \pm 47.5	201.4 \pm 83.8	198.1 \pm 74.7	169.0 \pm 76.7	272.3 \pm 95.0	190.0 \pm 61.7	171.6 \pm 65.4	202.2 \pm 83.7
Albumin (g/l)	28.3 \pm 2.5	27.8 \pm 3.0	29.5 \pm 3.4	32.3 \pm 5.5	26.9 \pm 3.1	30.2 \pm 5.8	29.9 \pm 5.3	26.3 \pm 7.3
Alfa-1 globulines (g/l)	6.7 \pm 1.0	5.7 \pm 2.0	4.6 \pm 1.9	5.9 \pm 1.0	3.7 \pm 1.1	3.8 \pm 0.6	2.3 \pm 1.0	4.3 \pm 1.3
Alfa-2 globulines (g/l)	7.2 \pm 1.6	5.4 \pm 1.3	5.7 \pm 2.1	5.9 \pm 0.8	6.5 \pm 2.2	6.4 \pm 1.2	6.1 \pm 2.1	5.8 \pm 0.6
Beta globulines (g/l)	5.3 \pm 1.6	4.5 \pm 1.5	4.7 \pm 1.3	3.9 \pm 0.7	5.3 \pm 1.9	4.7 \pm 1.7	5.4 \pm 1.3	3.7 \pm 1.4
Gamma-1 globulines (g/l)	19.0 \pm 3.7	16.3 \pm 2.6	17.2 \pm 5.2	22.0 \pm 3.5	20.9 \pm 3.5	19.4 \pm 2.3	19.2 \pm 3.9	23.4 \pm 6.4
Gamma-2 globulines (g/l)	6.4 \pm 2.8	4.8 \pm 3.1	4.6 \pm 2.5	4.6 \pm 2.2	2.9 \pm 1.2	2.5 \pm 1.7	3.0 \pm 1.4	5.5 \pm 3.7
Albumin/globuline	0.68 \pm 0.13	0.79 \pm 0.21	0.87 \pm 0.24	0.80 \pm 0.24	0.69 \pm 0.12	0.83 \pm 0.23	0.86 \pm 0.22	0.79 \pm 0.07
Total protein (g/l)	70.9 \pm 6.9	64.0 \pm 3.2	65.8 \pm 7.8	73.8 \pm 4.5	66.3 \pm 4.2	67.2 \pm 3.9	65.3 \pm 4.5	68.5 \pm 6.4
Total lipid (g/l)	5.93 \pm 1.72	4.30 \pm 0.96	2.86 \pm 0.73*	3.36 \pm 0.89	5.19 \pm 0.96	5.58 \pm 0.74	3.62 \pm 0.86	1.89 \pm 0.38*
Phospholipids (mmol/l)	36.13 \pm 5.67	21.83 \pm 2.74	23.47 \pm 10.63	20.74 \pm 6.92	19.22 \pm 5.92*	25.53 \pm 5.15	20.03 \pm 6.44	19.38 \pm 7.02
Triglycerides (mmol/l)	0.22 \pm 0.08	0.26 \pm 0.07	0.37 \pm 0.03	0.30 \pm 0.12	0.24 \pm 0.14	0.13 \pm 0.08	0.36 \pm 0.15	0.39 \pm 0.08
Cholesterol (mmol/l)	4.34 \pm 0.35	1.78 \pm 0.40*	1.16 \pm 0.21*	1.20 \pm 0.43*	1.49 \pm 0.36*	1.36 \pm 0.18*	1.53 \pm 0.26*	0.95 \pm 0.29*
Lactate (mmol/l)	4.74 \pm 1.40	7.25 \pm 1.00	7.17 \pm 1.02	7.18 \pm 0.52	6.56 \pm 0.75	6.30 \pm 0.66	8.99 \pm 1.62*	10.78 \pm 1.05*
BUN (mmol/l)	23.4 \pm 11.4	7.7 \pm 0.9*	7.6 \pm 0.8*	9.7 \pm 1.0*	10.1 \pm 1.7*	7.4 \pm 0.9*	8.0 \pm 2.0*	10.7 \pm 3.2
Direct bilirubin (μ mol/l)	0.34 \pm 0.17	1.54 \pm 0.0	0 \pm 0	0.17 \pm 0	1.37 \pm 0	0 \pm 0	0 \pm 0	0.86 \pm 0
Total bilirubin (μ mol/l)	4.10 \pm 0.34	2.91 \pm 0.68	3.42 \pm 0.34	3.42 \pm 0.68	4.10 \pm 0.86	2.917 \pm 0.68	2.05 \pm 0.68	6.67 \pm 1.54
Creatinine (μ mol/l)	155.6 \pm 48.6	106.1 \pm 13.3	99.9 \pm 1.40	115.8 \pm 4.42	122.0 \pm 25.6	98.1 \pm 11.5*	123.8 \pm 17.7	84.0 \pm 14.1*
Glucose (mmol/l)	3.87 \pm 1.98	8.06 \pm 1.93	7.28 \pm 1.58	6.35 \pm 1.58	7.79 \pm 2.04	5.69 \pm 1.52	7.39 \pm 2.87	3.69 \pm 1.66
Cortisol (nmol/l)	154.2 \pm 123.6	93.5 \pm 33.1	104.6 \pm 25.9	107.6 \pm 34.2	128.3 \pm 9.4	128.6 \pm 22.6	134.1 \pm 36.4	139.1 \pm 89.7
Sodium (mmol/l)	155.4 \pm 6.9	149.3 \pm 11.1	158.0 \pm 7.1	146.4 \pm 13.2	138.8 \pm 3.7	145.2 \pm 8.4	150.4 \pm 6.7	156.2 \pm 12.0
Potassium (mmol/l)	4.2 \pm 0.5	5.2 \pm 0.7	5.0 \pm 0.4	4.0 \pm 0.4	4.6 \pm 0.40	4.7 \pm 0.4	4.0 \pm 0.4	4.4 \pm 0.8
Chloride (mmol/l)	122.8 \pm 16.2	103.0 \pm 12.8	113.2 \pm 5.5	97.7 \pm 14.5	126.5 \pm 13.0	121.3 \pm 17.8	121.0 \pm 12.0	98.5 \pm 4.65
Calcium (mmol/l)	2.07 \pm 0.51	2.60 \pm 0.29	2.62 \pm 0.18	2.29 \pm 0.19	2.21 \pm 0.20	2.31 \pm 0.13	2.29 \pm 0.13	2.27 \pm 0.36
Phosphorus (mmol/l)	4.93 \pm 1.93	4.12 \pm 0.87	3.48 \pm 0.42	3.09 \pm 0.52	3.25 \pm 0.52	3.19 \pm 0.71	2.80 \pm 0.64	2.35 \pm 0.55*
Magnesium (mmol/l)	0.82 \pm 0.08	1.07 \pm 0.16	1.15 \pm 0.12	1.07 \pm 0.12	1.07 \pm 0.08	1.07 \pm 0.08	1.07 \pm 0.08	1.15 \pm 0.29
Copper (μ mol/l)	15.86 \pm 3.58	14.76 \pm 3.20	15.23 \pm 3.16	12.76 \pm 1.30	12.09 \pm 1.00	12.40 \pm 2.32	12.07 \pm 3.33	17.03 \pm 10.35
Iron (μ mol/l)	50.71 \pm 30.66	23.27 \pm 6.37	36.28 \pm 11.12	26.80 \pm 6.03	24.58 \pm 4.24	27.73 \pm 2.45	34.42 \pm 5.30	30.16 \pm 9.89
Zinc (μ mol/l)	14.09 \pm 2.33	9.58 \pm 2.77	8.21 \pm 1.87*	10.38 \pm 2.11	7.25 \pm 1.05*	9.01 \pm 1.05	8.10 \pm 1.57*	6.84 \pm 1.06*

* P < 0.05 significantly different from value at 5 days of captivity.

Table 6. Biochemical parameters of mouflon (Group II) at 5, 10, 20, 30, 40, 50, 60 days and 1 year of captivity

Measurement	5 days Mean \pm SD	10 days Mean \pm SD	20 days Mean \pm SD	30 days Mean \pm SD	40 days Mean \pm SD	50 days Mean \pm SD	60 days Mean \pm SD	1 year Mean \pm SD
ALT (IU/l)	9.0 \pm 4.1	16.3 \pm 3.0	12.8 \pm 4.7	12.6 \pm 2.7	46.8 \pm 80.3	55.3 \pm 94.0	33.8 \pm 40.1	18.3 \pm 2.8
AST (IU/l)	144.4 \pm 19.9	87.9 \pm 32.3	73.2 \pm 24.2*	146.2 \pm 16.1	111.9 \pm 27.7	100.0 \pm 11.0	93.6 \pm 44.0	127.2 \pm 26.7
CK (IU/l)	82.2 \pm 69.7	95.5 \pm 84.5	68.8 \pm 29.9	104.4 \pm 53.5	1294.6 \pm 792.1	689.1 \pm 1230.5	441.7 \pm 725.9	332.7 \pm 174.3
GGT (IU/l)	56.9 \pm 10.2	50.8 \pm 6.7	50.7 \pm 8.1	57.8 \pm 14.5	46.4 \pm 5.0	45.5 \pm 8.2	51.3 \pm 13.3	63.5 \pm 16.5
LDH (IU/l)	1259.3 \pm 176.6	1020.6 \pm 245.8	1006.7 \pm 180.4	1212.2 \pm 193.3	1277.1 \pm 720.1	1062.3 \pm 504.4	940.4 \pm 189.5	1008.2 \pm 68.5
AP (IU/l)	216.2 \pm 116.0	171.4 \pm 112.9	235.0 \pm 143.6	302.4 \pm 117.3	235.0 \pm 106.1	214.7 \pm 96.7	185.4 \pm 121.1	253.9 \pm 155.3
Albumin (g/l)	28.1 \pm 4.3	28.1 \pm 3.8	31.9 \pm 4.7	29.9 \pm 3.8	30.5 \pm 2.5	29.5 \pm 1.4	28.4 \pm 3.9	30.0 \pm 2.6
Alfa-1 globulines (g/l)	3.9 \pm 1.4	4.8 \pm 0.9	3.8 \pm 1.1	4.2 \pm 1.2	4.2 \pm 0.8	4.9 \pm 2.1	3.1 \pm 0.9	4.6 \pm 1.1
Alfa-2 globulines (g/l)	5.7 \pm 0.5	5.7 \pm 1.6	5.1 \pm 2.2	6.4 \pm 0.9	5.7 \pm 1.5	6.3 \pm 1.9	6.5 \pm 1.8	6.1 \pm 0.6
Beta globulines (g/l)	5.4 \pm 2.0	3.6 \pm 1.7	5.1 \pm 1.9	4.3 \pm 1.6	2.9 \pm 0.9	4.6 \pm 2.0	5.5 \pm 2.0	3.3 \pm 1.5
Gamma-1 globulines (g/l)	21.7 \pm 9.0	28.2 \pm 7.9	25.6 \pm 4.7	27.1 \pm 4.2	28.4 \pm 6.0	24.9 \pm 2.6	21.2 \pm 5.1	27.9 \pm 4.4
Gamma-2 globulines (g/l)	5.0 \pm 1.0	5.7 \pm 1.6	4.2 \pm 2.1	3.6 \pm 1.9	1.3 \pm 0.9	3.6 \pm 3.6	2.7 \pm 2.0	2.0 \pm 1.1
Albumin/globuline	0.69 \pm 0.06	0.87 \pm 0.13	0.86 \pm 0.12	0.74 \pm 0.08	0.80 \pm 0.06	0.71 \pm 0.07	0.81 \pm 0.15	0.72 \pm 0.05
Total protein (g/l)	69.8 \pm 8.2	71.2 \pm 6.1	73.0 \pm 2.7	73.8 \pm 5.9	73.3 \pm 4.8	71.5 \pm 3.4	66.7 \pm 6.1	74.2 \pm 6.5
Total lipid (g/l)	3.17 \pm 0.82	3.92 \pm 1.25	3.01 \pm 0.58	2.66 \pm 0.90	5.29 \pm 1.44	5.18 \pm 1.56	2.94 \pm 0.80	2.56 \pm 0.44
Phospholipids (mmol/l)	17.42 \pm 5.15	18.19 \pm 6.92	16.45 \pm 6.67	15.87 \pm 7.28	18.93 \pm 8.21	16.62 \pm 3.28	16.42 \pm 2.45	23.44 \pm 6.54
Triglycerides (mmol/l)	0.19 \pm 0.10	0.20 \pm 0.08	0.30 \pm 0.06	0.20 \pm 0.07	0.16 \pm 0.07	0.11 \pm 0.08	0.17 \pm 0.05	0.34 \pm 0.09
Cholesterol (mmol/l)	1.00 \pm 0.18	1.42 \pm 0.36	0.96 \pm 0.14	1.16 \pm 0.40	1.21 \pm 0.42	1.23 \pm 0.20	1.14 \pm 0.17	1.21 \pm 0.30
Lactate (mmol/l)	7.35 \pm 1.05	7.92 \pm 1.14	6.85 \pm 0.72	6.99 \pm 0.72	9.88 \pm 1.16*	5.89 \pm 0.34	8.43 \pm 0.55	10.33 \pm 2.13
BUN (mmol/l)	8.7 \pm 0.5	7.6 \pm 1.4	9.5 \pm 1.3	10.4 \pm 0.9	10.0 \pm 1.5	9.1 \pm 1.2	9.9 \pm 2.7	12.8 \pm 0.9*
Direct bilirubin (μ mol/l)	0 \pm 0	0 \pm 0	0 \pm 0	0.17 \pm 0.17	0.34 \pm 0.51	0.34 \pm 0.51	0.51 \pm 0	0.34 \pm 0.17
Total bilirubin (μ mol/l)	2.91 \pm 1.03	2.39 \pm 0.51	2.57 \pm 1.03	2.74 \pm 0.68	2.22 \pm 0.86	3.76 \pm 1.20	2.22 \pm 1.03	5.81 \pm 1.20*
Creatinine (μ mol/l)	103.4 \pm 12.4	116.7 \pm 8.84	105.2 \pm 12.4	104.3 \pm 9.7	115.8 \pm 15.9	108.7 \pm 12.4	104.3 \pm 18.6	89.3 \pm 9.7
Glucose (mmol/l)	4.91 \pm 1.02	8.48 \pm 3.91	5.63 \pm 0.79	5.48 \pm 1.34	6.40 \pm 1.24	5.80 \pm 1.06	4.59 \pm 0.69	5.49 \pm 1.25
Cortisol (nmol/l)	63.7 \pm 28.7	107.9 \pm 39.7	95.2 \pm 15.7	97.7 \pm 27.3	109.8 \pm 29.5	192.0 \pm 53.8*	134.6 \pm 17.9	101.8 \pm 30.1
Sodium (mmol/l)	139.9 \pm 3.0	142.5 \pm 3.8	154.4 \pm 7.9	155.3 \pm 11.9	154.9 \pm 5.5	144.1 \pm 5.7	151.3 \pm 7.4	152.0 \pm 5.1
Potassium (mmol/l)	4.6 \pm 0.9	4.9 \pm 0.8	4.9 \pm 0.7	4.6 \pm 0.7	4.5 \pm 0.7	4.5 \pm 0.6	4.5 \pm 0.7	4.0 \pm 0.7
Chloride (mmol/l)	108.8 \pm 3.5	110.2 \pm 10.4	114.0 \pm 16.8	92.3 \pm 17.2	132.0 \pm 13.0	113.2 \pm 12.5	112.3 \pm 13.0	88.7 \pm 1.9
Calcium (mmol/l)	2.21 \pm 0.06	2.28 \pm 0.07	2.53 \pm 0.18	2.52 \pm 0.29	2.36 \pm 0.14	2.26 \pm 0.14	2.43 \pm 0.13	2.31 \pm 0.15
Phosphorus (mmol/l)	3.09 \pm 0.45	3.22 \pm 0.61	3.25 \pm 0.58	2.96 \pm 0.48	3.03 \pm 0.77	2.90 \pm 0.26	2.93 \pm 0.42	2.70 \pm 0.81
Magnesium (mmol/l)	1.03 \pm 0.082	0.82 \pm 0.12	1.19 \pm 0.08	0.99 \pm 0.16	1.19 \pm 0.08	1.07 \pm 0.08	1.07 \pm 0.08	1.15 \pm 0.12
Copper (μ mol/l)	7.96 \pm 2.12	9.00 \pm 1.38	12.09 \pm 1.73	11.10 \pm 2.34	12.04 \pm 1.84	11.67 \pm 2.14	11.19 \pm 2.79	11.19 \pm 4.21
Iron (μ mol/l)	37.59 \pm 2.94	23.86 \pm 6.27	37.71 \pm 7.25	22.20 \pm 7.39	34.78 \pm 6.07	27.15 \pm 7.54	44.45 \pm 24.95	27.03 \pm 4.12
Zinc (μ mol/l)	9.17 \pm 0.90	5.67 \pm 0.71*	8.77 \pm 1.32	7.75 \pm 1.61	7.71 \pm 1.06	8.47 \pm 1.31	7.55 \pm 1.52	7.90 \pm 1.72

* P < 0.05 significantly different from value at 5 days of captivity.

after 1 year in Group I and at 40 days and 1 year in Group II. There are two possible causes: first, a greater exposure to disease, although it is unlikely that it occurred, because veterinary control performed on the animals in captivity may have reduced the probability of infections; second, the stress of repeated handling could have been the cause. Stress leukocytosis is caused primarily by a mature neutrophilia, although a slight increase in band neutrophils may be detected. However, this increase lasts for only a few hours, becoming normal within approximately 24 hours (JAIN, 1986).

WBC, lymphocyte, monocyte, mature and band neutrophil, basophil and PLT values were similar to those described in mouflon (HAWKEY et al., 1984) and in other wild sheep species (FOREYT et al., 1983; KOCK et al., 1987b). The eosinophil count was similar to that described by HAWKEY et al. (1984). Nevertheless, greater values appeared in other species of wild sheep (FOREYT et al., 1983), probably because of endoparasitism.

Biochemical parameters

In wild and domestic ungulates, AST and CK are the most sensitive enzymes to detect muscular alterations during stress (CHAPPLE et al., 1991; DUNCAN and PRASSE, 1986), although some authors think that ALT measurement could be useful in cases of capture myopathy (VASSART et al., 1992). In the present study, values of these enzymes were higher in Group I during the first several days, probably because of the effects of capture and transport five days before (MARCO et al., 1997).

ALT activity was very high after the capture of Group I and then decreased to lower values, although it seemed to increase slightly after 40 days. AST showed the same behaviour as ALT in Group I, with very high activity at 5 days after capture and a decrease after that. No statistical differences were observed in the levels of CK, possibly because they stabilised earlier than ALT and AST, as has been observed in studies of paralytic myoglobinuria of the horse in which, after an increase in AST and CK activities, the restoration of normal values was observed earlier for CK than for AST (KANEKO, 1989). This fact is useful for diagnosis and prognosis, since high levels of AST and normal concentrations of CK after muscular aggression are indicative of no further muscular damage. ALT, AST and CK values of the mouflons of our study are in agreement with those of some species from the genus *Ovis* (FOREYT et al., 1983), but were higher in others (FRANZMAN and THORNE, 1970; McDONALD et al., 1981; KOCK et al., 1987b), possibly due to capture stress. Also, there were no statistical differences in the activity of LDH, probably because it has been very increased during the sampling time period, when comparing these with values described in other wild ovids (McDONALD et al., 1981; FOREYT et al., 1983; KOCK et al., 1987b).

Total lipid, phospholipids and cholesterol decreased during the captivity period in Group I, but not in Group II. In domestic ruminants and in the mountain hare (*Lepus timidus*), an increase of these parameters in stressful situations due to the induced lipolysis has been observed (SACCON et al., 1992; SOVERI et al., 1992; PALTRINIERI et al., 1994), and this could have happened in the recently captured group, which had higher levels at 5 days than Group II.

The differences observed in lactate levels during the study may be in relation with the intensity of physical exertion in the different samples. Lactate production largely depends upon the intensity of muscular activity and anaerobiosis (KANEKO, 1989). The highest values were recorded during the last sampling period in both groups. The fact that mouflons were physically untrained after 1 year of captivity and that they had to run to try to escape when they were restrained for sampling may have influenced the results. These values were higher than those considered normal in domestic sheep (KANEKO, 1989).

BUN was lower during sampling periods in Group I. A deficiency in water intake during the first few days in this group may have had an influence, because the animals had difficulty in finding water in the pens, before they were adapted. Creatinine values were also higher at 5 days, compared to values of Group II. Thus, a compensated oliguria due to a relative dehydration could have happened. Values obtained during the study period were similar to those of other wild ovids (FOREYT et al., 1983). However, FRANZMANN and THORNE (1970) and KOCK et al. (1987b) registered lower values in physically captured bighorn sheep.

Few statistical differences were found in cortisol levels. In bighorn sheep, KOCK et al. (1987b) considered pathologic cortisol levels higher than 5 mg/dl, which was observed in our study in Group I at 5 days and 1 year, and in Group II at 50 days, but, as was stated before, no emergency treatment was administered and no mortality was observed.

The understanding of biological parameters is complex because many sources of variation exist (HAWKEY, 1975; PEINADO et al., 1995). In this study, most of the observed changes were related to stress, as discussed previously, and did not reflect adaptation of mouflons to repeated handling. During that time, no mortality or infectious diseases were observed. Some of the parameters indicative of stress did not manifest any marked trend, and others, such as mature neutrophil count and lactate, increased over successive samples, suggesting that adaptation was not achieved. Other parameters, like ALT and AST, decreased after capture, but increased slightly at the end of the study period although no statistical differences were found.

The effects of handling stress must therefore be considered when measuring biological parameters. Values given in this study may be useful as reference, but one must consider the conditions of the animals used and the sampling method mentioned.

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