ADRENOCORTICOTROPHIN-INDUCED STRESS RESPONSE IN CAPTIVE VICUNAS (*VICUGNA VICUGNA*) IN THE ANDES OF CHILE

C Bonacic*‡, D W Macdonald* and G Villouta†

* Wildlife Conservation Research Unit, University of Oxford, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK

‡ Departamento de Ciencias Animales, Facultad de Agronomia e Ingenieria Forestal, Pontificia Universidad Católica de Chile, Casilla 306, Correo 22, Santiago, Chile

† Facultad de Ciencias Veterinarias, Universidad de Chile, Santa Rosa 11735, Santiago, Chile

‡ Contact for correspondence and requests for reprints: bonacic@puc.cl

Abstract *Animal Welfare 2003, **12**: 369-385*

The vicuna is mainly used in two ways: wild captured, shorn and returned to the wild; or wild captured and maintained in captivity as part of a programme of sustainable use in the Andes of South America. Farming of wild vicunas has hitherto involved no assessment of their welfare. In this study we measured a set of basic blood parameters in order to characterise baseline values in captivity, and we then characterised adrenal cortical responsiveness using an ACTH challenge. The ACTH challenge is widely used for assessing neuroendocrine responses to stress and is now increasingly being applied to studies of wild animals' welfare. Five male vicunas were injected with exogenous ACTH and their responses compared with those of a control group injected with placebo. Behavioural and haematological changes were monitored. Injection of ACTH produced a 4.5-fold increase in cortisol concentration within 1 h. Total white blood cell count almost doubled in less than 5 h. The neutrophil:lymphocyte ratio also changed, with a decrease in lymphocytes and an increase in neutrophils, suggesting that the neutrophil:lymphocyte ratio was affected by the ACTH challenge. Packed cell volume increased from 40% to 44%. Observations of individual vicunas during sampling revealed no discernible behavioural differences between treated and control animals; however, animals that had higher initial baseline cortisol concentration made more attempts to escape, and vocalised more during handling, regardless of whether they were treated with ACTH or placebo. The results reveal the different blood parameter levels associated with stress in different species and highlight the hazard of interpreting stress levels in one species on the basis of measures calibrated in another. We provide calibrated reference values for future studies of stress in vicunas.

Keywords: *animal welfare, Camelid, Chile, guanaco, stress, vicuna*

Introduction

The sustainable use of wildlife is an internationally important topical issue, which involves the balance of diverse, sometimes incommensurable, considerations (Moreira & Macdonald 1996; Prescott-Allen & Prescott-Allen 1996; Taylor & Dunstone 1996). Among the disciplines widely recognised as relevant to this topic are biology, economics and development (Eltringham 1988; Robinson & Bolen 1989). Another consideration less

† Died in 2002

frequently given adequate scientific consideration, but which we believe to be highly relevant, is animal welfare (Taylor & Dunstone 1996; Bonacic & Gimpel 2000). This relevance is illustrated by the case of the vicuna (or vicuña; *Vicugna vicugna*), a camelid that is currently used in two main ways: wild captured, shorn and returned to the wild; and wild captured and maintained in captivity as part of a programme of sustainable use in Argentina (Rebuffi 1993), Peru (Wheeler & Hoces 1997), Chile (Galaz 1998) and Bolivia (Oryx 1999). The product that it is hoped can be used sustainably is the vicuna's wool, which is highly commercially valuable for fine textile commodities (Koford 1957; Wheeler & Hoces 1997). However, the capture, handling and, most dramatically, shearing, of a wild animal is likely to be stressful. The obvious question is, how stressful? The answer is relevant not only in so far as ethical concerns may dictate that there is some level of stress beyond which the value of the harvest is outweighed by this dimension of the cost of securing it; in addition, and pragmatically, the sales of a product whose value lies in a sophisticated, western, luxury market may depend on potential consumers being reassured that the harvest is not unacceptably stressful. However, answering this question is not easy technically. Little is known of the stress response in wild South American Camelids (SACs) (Fowler 1989; Johnson 1994), although lessons might be learnt from studies on the effects of transport, isolation, cold and heat that have been carried out on domestic SACs in the USA and Europe (Jurgens *et al* 1988; Jurgens 1993; Fowler 1998; Anderson *et al* 1999). Clearly, aspects of management for sustainable use, such as pursuit, capture, handling and shearing, are likely to trigger similar stress responses in wild vicunas as those reported in domestic SACs (Bonacic & Gimpel 1995). Although lessons learnt from domestic camelids may be helpful, they do not substitute for data on vicunas themselves, in their natural environment — the Andes of South America (Baunmann *et al* 1975; Fowler & Zinkl 1989; Smith *et al* 1999). Emotional and physical stressors may trigger a series of changes that are broadly recognised as part of the physiological stress response (Selye & Ogilvie 1957; Nelson 1995; Hofer & East 1998), of which the adrenocortical response is a major component (Eckert & Randall 1983; Schmidt-Nielsen 1990). This involves release of corticotrophin-releasing hormone (CRH) from the hypothalamus, adrenocorticotrophic hormone (ACTH) from the pituitary gland, and glucocorticoids from the adrenal cortex (Harlow *et al* 1987; Becker *et al* 1992; Norris 1997).

As uniform stress conditions are difficult to reproduce, a standard procedure used to measure the consequences of a given level of stress in a species is to induce a cortisol elevation that is similar to that produced during an acute stress response in other species. That is, animals are injected with ACTH and the resulting corticosteroid release is measured (Goddard *et al* 1994; Ferre *et al* 1998; Bubenik & Bartos 1993). Depending on the dose and route of administration, ACTH causes a peak of cortisol (in most mammalian species) or corticosterone (in rodents and birds) concentration within 30–60 min, followed by a decline over 180–240 min (Harlow *et al* 1987; Ludders *et al* 1998). This method provides a measure of adrenal cortex activity that has been used as a quantitative indicator of the effect of potential stressors (Harlow *et al* 1987; Barnett & Hemsworth 1990; Goddard *et al* 1994; Kraabel & Miller 1997; Ludders *et al* 1998).

The ACTH challenge test has been used in ungulates including various species of deer, mountain sheep and domestic animals (Vanmourik & Stelmasiak 1984; Smith & Bubenik 1990; Bubenik & Reyestoledo 1994; Ferre *et al* 1998). The induced stress response is characterised by an increase in circulating neutrophils and a decrease in lymphocytes and eosinophils (eg bighorn sheep; Harlow *et al* 1987; Ferre *et al* 1998). The usefulness of this measure of the stress response is enhanced when it is combined with other physiological and behavioural observations (Goddard *et al* 1994; Goddard *et al* 1996).

Therefore, as a step toward tackling the question of how stressful to wild vicunas are different aspects of the wool harvest, we sought to quantify the species' ACTH response under semi-natural conditions. One single dose of exogenous ACTH was injected into captive vicunas to characterise the adrenal cortical response. Behavioural (vocalisations, and body movements during sampling), haematological (packed cell volume, total and differential white blood cell count), and biochemical (blood glucose concentration) alterations associated with the rise in plasma cortisol concentration were monitored. ACTH challenge by injection of a single dose is used in human medicine and has been broadly utilised in domestic animals, zoo animals and some other wild species with no major animal welfare concerns. The aim of the present study was to provide reference/calibrated values for assessing stress in vicunas, which can then be used in the assessment of the stress response in captive vicunas (see Bonacic & Macdonald, pp 387–402, this issue). This experiment was approved by an ethical committee in the Department of Zoology, University of Oxford, UK.

Methods

Animals and sampling procedures

Ten male vicunas (body weight = 31.5 ± 2.6 kg, aged 1–3 years old) captured from the wild herd of 18 animals were studied in captivity in the altiplano area of Las Vicunas Natural Reserve (4400 m above sea level) between October and November 1998 (study duration approximately 40 days). The Reserve (South 18°16'–19°00'; West 68°57'–69°27') lies within the Surire basin in the Parinacota Province of Chile (490 401 ha). This is a typical altiplano or *puna* habitat, surrounded by high mountains and volcanic peaks. The minimum temperature in spring fluctuates from night to day between –11°C and 3°C.

Humane handling and research protocols

Although the study was undertaken in the natural habitat of the wild vicuna, we sought to follow welfare standards recommended in the UK (Wolfensohn & Lloyd 1994) particularly with reference to the code of refinement, reduction and re-assessment during experimental design and execution (Dawkins & Gosling 1992). Chilean research regulations on wild species were followed throughout and the study was licensed by the Ministry of Agriculture of Chile (Cuchacovich 1997). The experimental design followed the powerful model described by Krebs (1999) as 'BACI' (before–after, control–interference). We selected juvenile males (1.5–3 years old) from bachelor groups for the study, as these individuals were peripheral to the social structure of the vicuna population, were unlikely to reproduce, and, being of sub-adult size, were relatively easy to handle.

The animals were penned in corrals of 6.2 m × 6.2 m with bare soil and 2.1 m high wire-mesh fencing, and were given alfalfa hay and water *ad libitum*. They were assigned randomly to either a treatment or a control group. Habituation consisted of daily human contact by physical examination of every vicuna for 40 days before the ACTH experiment (ie handling, blindfolding, disinfecting of the skin in the jugular vein region and, on four occasions, blood sampling; see below). Food and water intake was measured (see Appendices 1 and 2), and four blood samples were taken on the day of capture and on days 1, 4 and 12 after capture. Within 12 days, behavioural observations indicated increased habituation, with a decreased resistance to handling, and blood profiles became closer to reference values reported for other South American Camelids (see Bonacic & Macdonald, pp 387–402, this issue). At the end of the habituation period, the study was conducted on two consecutive mornings in November 1998. In order to eliminate any effect of circadian variation in cortisol

concentration, all of the animals were sampled between 0900h and 1000h (Bubenik *et al* 1983; Bubenik & Bartos 1993). No food or water was available for approximately 12 h overnight before sampling (standard veterinary procedure to avoid blood changes due to ingestion of food and to reduce risks of regurgitation during handling).

Each animal was approached and restrained by two experienced handlers. A third person carried out the injections and blood collection. A fourth person observed the whole procedure from outside each corral (2–3 m away) and recorded the time taken for the procedure and any behavioural response from the animals during handling. The procedure was double-blind in that none of the team knew, at the time of handling, which individuals were assigned to which treatment. In practice, handling and venepuncture involved minimal restraint. Two blood samples were taken before ACTH or placebo injection and used as the pre-treatment control (ie baseline) values. In the first baseline sampling (30 min before ACTH injection), heart rate and rectal temperature were also recorded during clinical examination. Following the second baseline sampling (immediately before ACTH injection), ACTH or a saline solution (placebo) was injected in 5 ml dilution (see below for sampling times). Further samplings consisted only of blood collection. Blood samples were taken from the jugular vein; 5 ml samples were collected in one EDTA (ethylene diamine tetra-acetic acid) tube and one heparin tube. These samples were used for haematology and clinical biochemistry, respectively.

Five treatment animals were injected with 0.25 mg intravenous cosyntropin B (Cortrosyn®, Organon Inc, Bedford, Ohio USA). Cosyntropin B is an open-chain polypeptide that contains the first 24 of the 39 amino acids of naturally occurring ACTH, a feature that reduces its antigenicity while preserving its corticosteroidogenic activity (Organon Inc, Bedford, Ohio USA). Five control animals were injected with a sterile saline solution (placebo). Blood cortisol concentrations were measured for both baseline samples (at times –30 min and 0 min). Five post-ACTH samples were obtained at times 30 min, 60 min, 90 min, 180 min and 300 min. The same schedule was used for sampling of blood glucose concentration. Total and differential white blood cell counts (WBC) and packed cell volume (PCV) were sampled at 0 min, 180 min and 300 min.

Storage and analysis of blood samples

Blood samples (5 ml) were obtained by jugular venepuncture. Standard blood tubes (Vacutainer®) were used containing EDTA for haematological assays and heparin for plasma cortisol. WBC and blood glucose concentration were measured in the field immediately after sampling and blood smears were prepared for further differential white blood cell counts in the Veterinary Laboratory in Santiago (Coles 1980; Schalm & Jain 1986). Plasma (1 ml) was extracted for cortisol concentration analysis by centrifugation in the field and stored in liquid nitrogen within 2 h of collection of the sample. Heparin tubes were centrifuged immediately at approximately 1500×g for 15 min at 4°C. The plasma was subsequently decanted into separate 1.5 ml microtubes and frozen at –18°C until analysis. EDTA tubes were kept refrigerated in a portable cooler for a maximum of 4 h before being analysed. Cortisol concentration was measured from blood plasma using a validated radioimmunoassay for the species (Brandeys 1988; Hall 1978; Zekan & Ezcurra 1998). Mean inter-assay and intra-assay coefficients of variation were 10.8% and 7.5%, respectively. Blood glucose concentration was measured using a portable glucometer (Elite®, Bayer) after validating its accuracy using the hexoquinase test in the Haematology Laboratory of the School of Veterinary Medicine, University of Chile.

Behavioural observations during sampling

Each vicuna was observed from a distance of 2–3 m and its reaction to handling and sampling was noted. This included the number of vocalisations and body movements such as leg kicks, head movements and attempts to stand during handling and blood sampling.

Statistical analysis

Repeated measures analysis of variance was used to compare ACTH and control groups (between-subject effect) in six repeated samples (within-subject effect) for cortisol concentration and glucose concentration, and three samples for WBC (total and differential) and PCV (Winer & Michels 1991; Gurevitch & Scheiner 1993). The neutrophil:lymphocyte ratio response was analysed using non-parametric regression analysis. The response to ACTH injection was compared between consecutive samples and the first sample (ie the baseline sample) by contrast analysis (method: SIMPLE in SPSS) when the interaction 'treatment × time' was not significant (SPSS 1997). Otherwise, UNIANOVA analysis for specific times was used to compare between treatments.

Behavioural reactions (number of body movements and number of vocalisations) were pooled before and after ACTH treatment and analysed using non-parametric tests (Wilcoxon and Mann-Whitney tests). Unless otherwise stated, mean, standard deviation of the mean and sample size are reported beside *P*-values when statistical analyses are significant ($P < 0.05$) for parametric tests. Median and interquartile ranges are presented for non-parametric tests. Each parameter was checked for normality and homoscedasticity (Norušis & SPSS Inc 1998). The data were analysed using SPSS version 7.5 (SPSS 1997).

Results

Sampling time

Mean restraint time was 3 min 36 s (data pooled for pre- and post-ACTH samplings). Mean pre-ACTH blood-sampling time was 3 min longer than mean post-ACTH blood-sampling time (5 min: 41 ± 45 s, and 2 min: 52 ± 18 s; paired *t*-test = 10.6, $P < 0.001$, respectively) because of the extra time needed for baseline sampling. The main changes in blood parameters are summarised in Table 1. Because there was no significant difference between the values for time –30 min and time 0 min, we present only the latter.

Cortisol concentration response to ACTH

There was a significant overall treatment effect on mean plasma cortisol concentration (control: 50.38 ± 8.2 nmol l^{-1} versus treated: 96.59 ± 8.2 nmol l^{-1}; $F_{1,8} = 16$, $P < 0.004$). Whereas the mean plasma cortisol concentration in the control group approximated the pre-treatment level (mean 48.9 ± 9.7 nmol l^{-1}, considered the baseline for this experiment) and exhibited no significant variation throughout the study (Figure 1a), cortisol concentration peaked (mean 161.5 ± 13 nmol l^{-1}) 60 min after ACTH injection and remained high (mean 158 ± 14 nmol l^{-1}) for up to 90 min thereafter. In short, the cortisol concentration measured for the ACTH-treated group rose 4.5-fold relative to the mean pre-treatment baseline. Thereafter, it declined by 180 min after treatment, and had returned to baseline by the 300 min reading. Individual variation showed that maximum cortisol concentration levels of 215.6 and 219.4 nmol l^{-1} were reached by one animal in two samples taken at 60 and 90 min, respectively (animal ID = 43), whereas the highest value reached in the control group individuals was only 70.6 nmol l^{-1}, 90 min after the sampling started.

White blood cell response to ACTH

The total number of circulating leukocytes was significantly affected by ACTH injection ($F_{1,8} = 7.0$, $P = 0.03$; Figure 1b). The baseline WBC count of 7450 ± 521 cells μl^{-1} increased to 11340 ± 1517 cells μl^{-1} after ACTH treatment (Table 1). WBC composition also changed, with an increase in neutrophils and a decrease in lymphocytes causing a significant rise in the neutrophil:lymphocyte ratio in ACTH-treated animals (Figure 1c). Cortisol concentration and the neutrophil:lymphocyte ratio were highly correlated in ACTH-treated animals (Spearman correlation = 0.76, $P = 0.003$, n = 13), but we detected no change in monocyte or eosinophil levels.

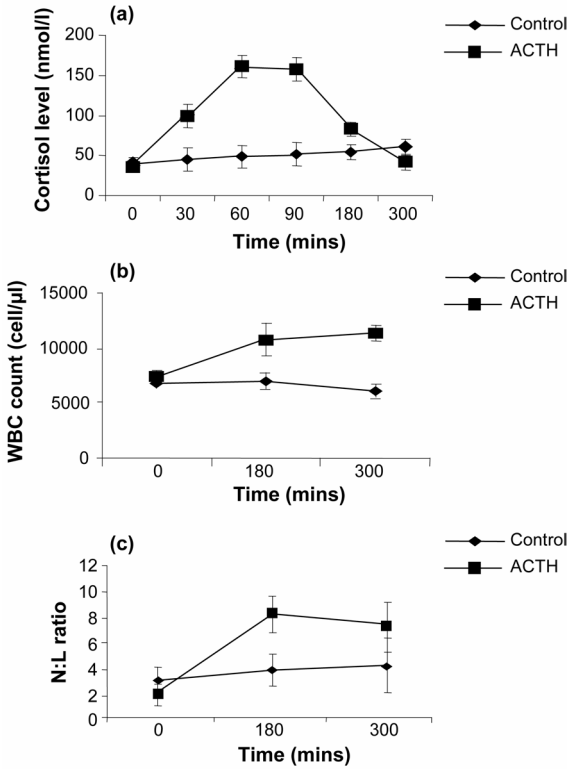

Figure 1 **(a) Cortisol concentration after ACTH injection; (b) Total white blood cell count after ACTH injection; (c) Neutrophil:lymphocyte ratio after ACTH injection (means ± standard errors).**

Effect of ACTH on glucose concentration

There were no significant differences in blood glucose concentration between ACTH-injected and placebo-injected animals ($F_{1,8} = 0.01$, $P = 0.89$). However, the pooled values of glucose concentration showed an increase from baseline level to 98.5 ± 14.4 mg dl^{-1} after 30 min, reaching a peak of 122 ± 19 mg dl^{-1} at 90 min (pooled data from both groups in the third sampling; Figure 2a). The difference in blood glucose concentration between pre-treatment samples and the three subsequent samples was statistically significant regardless of group (control or treated), and glucose concentration returned to baseline level (96 ± 12 mg dl^{-1}) by the reading at 180 min. Although not related to the experimental

treatment, blood glucose concentrations during the experiment were related to baseline cortisol concentration (Table 2).

Effect of ACTH on packed cell volume

Changes in PCV counts did not parallel patterns in other measured parameters and showed no overall treatment effect ($F_{1,8} = 2.7$, $P = 0.13$). Control animals remained close to the original baseline value (39 ± 1.8%), while treated animals showed a significant increase of PCV to 44 ± 1.26% only at 30 min, subsequently returning to the baseline value (mean difference = –6.2 ± 1.4%, $t = –4.2$, $df = 8$, $P = 0.003$; Figure 2b).

Effect of ACTH on behaviour

Overall, vicunas injected with ACTH vocalised less (Table 3), although the tendency of control animals to vocalise more frequently than treated animals was not statistically significant ($U = 10.5$, $P = 0.6$). However, there was a significant correlation between absolute values of cortisol in the control group and total number of vocalisations ($r^2 = 0.97$, $F_{1,4} = 48.2$, $P = 0.006$). In terms of movements during handling, one individual (ID = 43) in the ACTH-treated group consistently struggled more than any other. In the overall sample there was no relationship between number of movements in the two treatment groups ($r^2 = 0.03$, $F_{1,4} = 0.008$, $P = 0.9$), but when the above outlier was excluded there was a tendency towards less movement at higher values of cortisol.

Table 1 Response in blood parameters to ACTH in vicunas.

Time (min)	**0**	**30**	**60**	**90**	**180**	**300**
Cortisol concentration (nmol l^{-1})						
Control mean	42.0	45.2	48.9	51.4	54.4	60.6
Standard error	4.8	5.6	9.7	8.5	6.5	11.3
ACTH mean	35.5	99.3	161.5	158.0	83.0	42.1
Standard error	5.8	19.1	17.1	18.5	10.8	8.9
Alpaca	20.6	Range	17–23			
Guanaco	30.3	Range	16–37			
White blood cell count (cells μl^{-1})						
Control mean	6790				7000	6090
Standard error	445				774	584
ACTH mean	7450				10730	11340
Standard error	521				1462	678
*Reference**	12200					
Range	8000–22000					
Packed cell volume (%)						
Control mean	39.0	37.8			39.8	37.4
Standard error	1.8	0.9			0.7	0.4
ACTH mean	40.0	44.0			39.0	37.6
Standard error	0.8	1.2			1.1	0.8
*Reference**	37.2					
Range	31–43					
Glucose concentration (mg dl^{-1})						
Control mean	102.1	115.2	125.2	126.6	106.8	89.2
Standard error	6.5	9.8	9.9	11.0	9.9	5.0
ACTH mean	98.5	103.8	119.2	118.8	113.4	103.2
Standard error	4.8	6.6	10.8	6.5	6.7	5.2
*Reference**	125.0					
Range	75–154					

*Reference values from Fowler (1998) and Karesh (1998).

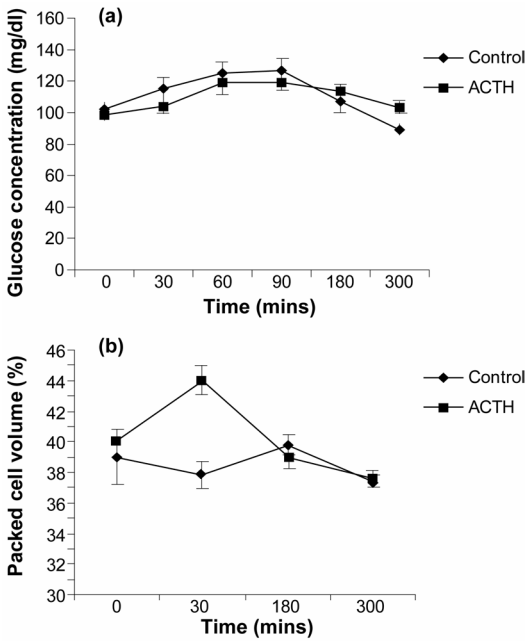

Figure 2 **(a) Blood glucose concentration after ACTH injection; (b) Packed cell volume after ACTH injection (means ± standard errors).**

Table 2 **Correlation between baseline cortisol concentration (nmol l^{-1}) and blood glucose concentration (mg dl^{-1}) in the entire sample (treated and control pooled).**

Cortisol (baseline)	Glucose (baseline)	Glucose (30 min)	Glucose (60 min)	Glucose (90 min)	Glucose (180 min)	Glucose (300 min)
Sample size	10	10	10	10	10	10
Pearson correlation	0.86	0.67	0.85	0.67	0.56	0
Significance (2-tailed)	0.001	0.035	0.002	0.035	0.092	0.995

Table 3 **Behavioural changes associated with handling during blood sampling before and after ACTH injection. There was a significant difference only between total number of vocalisations during sampling before and after ACTH injection ($Z = -2.07$; $P = 0.038$). ACTH-treated animals vocalised less after the ACTH injection.**

Mean behavioural records per animal	n	Percentiles		
		25th	50th (Median)	75th
Total movements (baseline)	10	0.5	1	1.0
Total movements (after ACTH)	10	0.4	0.8	1.7
Total number of vocalisations (baseline)	10	0.5	1	2.5
Total number of vocalisations (after ACTH)	10	0.2	0.5	1.7

Discussion

Experimental considerations

This study was designed to evaluate changes in cortisol, glucose, packed cell volume and white blood cell count associated with ACTH-injection in wild vicunas held captive in their natural environment. This response is compared with baseline values gathered from the same animals before ACTH and after repeated handling during 40 days of habituation. The resulting baseline is an approximation of that of free-ranging vicunas to the extent that the captive animals habituated to handling and sampling. We assume that if control animals showed little variation in their blood profiles after placebo injection, despite the handling involved in every sampling, then the ACTH challenge was the cause of any effect observed in the treated animals. In that case, control group blood values can be considered as the baseline against which treatment values are compared (Ludders *et al* 1998). Although we cannot know whether the baseline values pre-ACTH are the same as those of undisturbed, free-ranging animals, we believe that these are currently the closest estimates available. Indeed, there are ethical and conservation reasons to be hesitant about killing wild vicunas to obtain blood samples, although remote telemetric methods could provide an alternative for the future (Goddard *et al* 1998).

Cortrosyn® was used because it exhibits the full corticosteroidogenic activity of natural ACTH and its pharmacological properties are similar to those of purified natural ACTH (Ludders *et al* 1998). The choice of the dose was based on evidence that 0.25 mg of Cortrosyn® will stimulate maximum adrenal cortex activity and has the same effect as 25 IU of natural ACTH in humans and other mammals (Ludders *et al* 1998; LeRoy 1999). A dosage range of 10–50 IU of ACTH is frequently reported in the literature as being suitable in similar experiments involving small and medium sized ungulates (Bubenik *et al* 1991; Bubenik & Bartos 1993; Goddard *et al* 1994; Ferre *et al* 1998; Bubenik *et al* 1999). Indeed, among red deer there was apparently no dose effect on the peak cortisol levels attained within this dosage range (Goddard *et al* 1994), although the duration of the response was positively dose-dependent (Goddard *et al* 1994; Ingram *et al* 1997). Our goal here, however, was simply to measure the maximum cortisol concentration response. LeRoy (1999) demonstrated that 25 IU of ACTH effectively triggered adrenocortical activity in guanaco (*Lama guanicoe*), the vicuna's closest relative. The increase in cortisol concentration in guanaco was 5.6-fold, from 16.3 nmol l^{-1} to 92 nmol l^{-1} (LeRoy 1999).

Endogenous adrenocortical response to ACTH in vicunas

We undertook these studies under the logistically difficult circumstances of the vicuna's remote, high-altitude habitat because this is the environment in which any plans for their sustainable use will be implemented. The reference values we sought are a prerequisite for quantifying the magnitude of the vicuna stress response under management practices such as capture and shearing (see Bonacic & Macdonald, pp 387–402, this issue).

The cortisol released by the ACTH-treated animals reached a peak concentration 3–4 times that of the baseline. From this response curve, we conclude that when evaluating the stress of alternative handling and shearing protocols, one could expect to detect peak plasma cortisol concentrations within 30–60 min (a result in accordance with findings for other species, eg Ingram *et al* 1997; Bubenik & Bartos 1993; Goddard *et al* 1994).

South American Camelids are known in general to have a significantly higher leukocyte count than other domestic ungulates (Wernery *et al* 1999), and our results confirm that this generalisation applies to the vicuna. The predominant white blood cell type in SACs is the

neutrophil, with a neutrophil:lymphocyte ratio close to 1:2 ± 0.41 (Fowler 1989). Our results revealed that within 180 min of plasma cortisol concentration rise, the neutrophil:lymphocyte ratio increased (as in other species; Wernery *et al* 1999), a shift mainly attributable to the absolute increase in neutrophils (neutrophilia) detected in peripheral blood (Figure 3). The same response has been reported for bighorn sheep (Kraabel & Miller 1997).

Garry (1989) reports that leukocyte kinetics in domestic llamas are different to those of other ruminant species, and there is a suggestion that the camelid response to stress may be closer to that of perisodactyls, such as the horse (Fowler 1998). Adrenalin-induced excitement neutrophilia appears to be a common occurrence and may, in part, explain the wide variability in 'normal' neutrophil counts described in the literature (Wernery *et al* 1999; Garry 1989). Stress neutrophilia, resulting from endogenous corticosteroid release and identified by concurrent lymphopenia (a decrease in lymphocyte numbers), also appears to be common in ill llamas (Garry 1989). Previously, it has been considered that sustained lymphocyte counts below 2000 cells μl^{-1} in young llamas and 1000 cells μl^{-1} in adults indicate chronic stress (Garry 1989; Fowler 1998). In this study, ACTH-treated animals demonstrated a sharp fall from 3000 cells μl^{-1} to less than 2000 cells μl^{-1}. Therefore, cortisol concentration did change the neutrophil:lymphocyte ratio in treated animals following the pattern of a stress response.

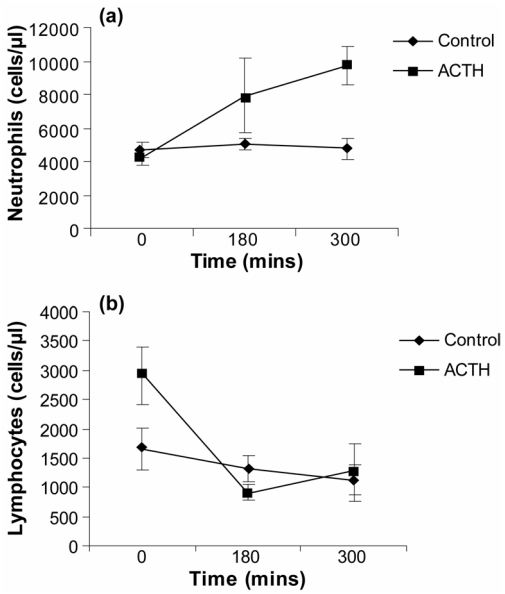

Figure 3 **(a) Neutrophil count after ACTH injection; (b) Lymphocyte count after ACTH injection (means ± standard errors). The initial difference of lymphocyte count between groups is not significant ($Z = -1.9$, $P = 0.063$).**

The resting blood glucose concentration of the camelids is remarkably high compared to values for other farm animals such as sheep and goats and better equates with the high range typical of monogastric species (Johnson 1994; Kaneko *et al* 1997). We expected an increase in blood glucose concentration after handling and sampling. Not only is a basic mammalian

response to cortisol (along with glucagon and adrenalin) to liberate glucose into the bloodstream, but this has also been specifically reported for llamas (Garry 1989). However, while glucose concentration did increase from baseline values of around 98–102 mg dl^{-1} to 126–118 mg dl^{-1} after 90 min, there was no difference in this between the two treatment groups. That is, the response could be attributed to handling alone. Indeed, handling and the associated release of adrenalin are known to provoke a rise in blood glucose from 100–120 mg dl^{-1} to 180–200 mg dl^{-1} in llamas (Garry 1989). Perhaps the absence of a strong glucose concentration response in our vicunas is further evidence of their advanced habituation and thus adds support to our proposal that the baseline cortisol levels reported here approximate those in the wild. A pilot study using an ACTH challenge with a similar dose and experimental protocols conducted on farmed guanacos also failed to provide a clear glucose response (LeRoy 1999). The physiological mechanisms of glucose response to stress remain to be investigated in more detail in SACs (Fowler 1998; Smith *et al* 1999).

Packed cell volume (PCV) increased within 30 min after ACTH administration and then returned to baseline levels, a phenomenon reported in South American domestic camelids after a stress episode but with no known interpretation (D Anderson, personal communication 2000). In so far as neither glucose concentration nor PCV response followed the more classical stress response exhibited by cortisol concentration and white blood cell changes, we suggest that ACTH injection may not completely simulate the changes produced by 'natural' stressors. It does, however, provide a standard quantitative measure of an animal's response to stress. Furthermore, we may expect inter-specific variation in stress responses; in this case, our results would be explicable if, in vicunas, packed cell volume and glucose were more responsive to exercise-related stressors than to ACTH challenge.

Although the vicunas always tried to dodge the handler, once restrained they appeared inexpressive and this made it difficult to identify ethological measures that might indicate their stress. Acknowledging this shortage of suitable measures, we nonetheless found no correlation between cortisol concentration and reactions during handling. Of course, we cannot know whether the rather tranquil appearance, to the human eye, of these vicunas reflects genuine calm or the inadequacy of humans at discerning vicuna consternation, or a catatonic withdrawal response as an alternative to fight and flight. However, there were clearly individual variations in response to ACTH; it may be noteworthy that the one animal that was atypically skittish had the highest cortisol concentration.

Inter-species response to ACTH challenge

The ACTH response exhibited by the vicuna — specifically, cortisol concentration increase and white blood cell changes — was broadly similar to that described in other non-domestic captive species of South American Camelids. However, and importantly, this study provides further evidence of differences in stress response between taxa. A comparison between two sub-orders of Artiodactyla (the Tylopoda, represented by SACs, and the Ruminantia, represented by Cervids) reveals substantial differences in the magnitude of their responses to ACTH (Table 4). The fold-increase in cortisol concentration from baseline values to a maximum physiological response is 4.7 ± 0.8 ($n = 4$ species) for SACs, while in deer the fold-increase is 12.6 ± 4.9 ($n = 5$). This difference is significant ($t = -3.0$, $df = 7$, $P = 0.01$) and suggests that the assumption that cortisol levels indicative of stress in one species indicate the same level of stress in another species may be unjustified. The biological basis for these differences is unknown, and their implications for studies of animal welfare are considerable. Indeed, even within taxa, there is evidence of variation in corticosteroid responses.

Table 4 **Ratio of increase in cortisol concentration after ACTH treatment in different ungulate species.**

Species/status	Mean cortisol concentration	Standard error	Ratio of increase	n	Reference
Vicuna (captive)					
Basal	35.5 nmol l^{-1}	5.3		5	Present study
ACTH	161.64 nmol l^{-1}	13.9	4.55	5	
Guanaco (captive)					
Basal	16.3 nmol l^{-1}			4	LeRoy 1999
ACTH	92 nmol l^{-1}		5.6	4	
Guanaco (captive)	102.2 nmol l^{-1}		6.27		Bustos 1998
Guanaco (calves wild)					
Hand-captured	55.18 nmol l^{-1}		3.39		Gustafson 1998
Alpaca (domestic)					
Basal	0.75 µg dl^{-1}	0.09		6	Anderson *et al* 1999
After transport	1.95 µg dl^{-1}	0.07	2.6	6	
Pudu (captive)					
Basal	0.2–0.7 µg dl^{-1}	—		7	Bubenik & Reyestoledo 1994
ACTH	2.6 µg dl^{-1}	—	13	7	
Red deer (captive hinds)					
Basal	5.75 nmol l^{-1}	—		20	Ferre *et al* 1998
ACTH	120.23 nmol l^{-1}	—	20.9	20	
Red deer (captive calves)					
Basal	63.1 nmol l^{-1}	7.5		20	Goddard *et al* 1994
ACTH	142.4 nmol l^{-1}	14.4	2.26	20	
Red deer (captive)					
Basal	1–2 µg/100 ml	—		7	Bubenik & Bartos 1993
ACTH	10 µg/100 ml	—	10.0	7	
Fallow deer (captive)					
Basal	1–2 µg/100 ml	—		8	Bubenik & Bartos 1993
ACTH	8 µg/100 ml	—	8.0	8	
Sheep (domestic)					
Basal	22.39 nmol l^{-1}	—		20	Ferre *et al* 1998
ACTH	259.04 nmol l^{-1}	—	11.57	20	

Bubenik and Bartos (1993) described that there was great consistency in the sensitivity of the adrenal cortex to exogenous administration of ACTH between several species of deer (where 10 IU elicits a maximal response), but these species differed markedly from domestic bovids (for which 80 IU were necessary). This particular comparison of the readiness of the pituitary–adrenal cortical system to respond to external stressors by raising cortisol concentrations was made between wild and domestic species. However, comparisons of our data for vicunas with those for deer (both being wild species) demonstrate that domestication

cannot be the only determinant of such inter-specific differences. Nonetheless, domestication (both in terms of genetic selection and behavioural habituation) is clearly one relevant factor: Harlow *et al* (1987) and Goddard *et al* (1994), comparing wild and tamed individuals of, respectively, bighorn sheep and red deer, reported lesser responses to ACTH treatment in the tamed individuals (ie those that were regularly handled). We might therefore expect the levels recorded in our vicunas to be lower than those for non-habituated animals. Goddard *et al* (1994) also found that red deer calves responded less to ACTH than did adults (perhaps because of differences in cortical:medullary ratio and age-related differences in receptor sensitivity). Since the interpretation of cortisol levels can have crucial welfare, and indeed political (Bateson & Wise 1998; Bradshaw & Bateson 2000), consequences, understanding the bases of these inter- and intra-specific variations is a priority.

Animal welfare implications

Animal welfare is one of the many strands of science that can inform the judgements necessary in conservation biology. The quest for reliable and easily measured indicators of stress is urgent. The non-consumptive, sustainable use of vicunas has had a long and interesting history (Wheeler & Hoces 1997) and now, in terms of both wildlife management and local community development, it is imperative to decide whether it is feasible. Many of the relevant questions are ecological (Bonacic *et al* 2002), others are concerned with economics, development and politics (Wheeler & Hoces 1997), but an additional, and important, consideration is the impact of the procedures on the welfare of the vicunas (Bonacic 2000; Bonacic & Gimpel 2000). A quantitative measure of stress is required, against which alternative procedures can be calibrated. Our comparisons (Table 4) illustrate that these calibrations cannot safely be drawn from the literature on other species. A first step, therefore, was to produce baseline levels of a measure that might be used for comparisons of stress levels of vicunas. For this purpose, we opted for the ACTH response, and our results, presented here as reference values, provide a platform for subsequent studies of the welfare of managed vicunas (see Bonacic & Macdonald, pp 387–402, this issue).

Acknowledgements

This paper was completed while C Bonacic was an HSUS Fellow in the WildCRU Programme for Studies of Animal Welfare in Wildlife Conservation, and is also an output of the South American Wild Camelids Sustainable Use Project funded by the European Union (ICA4-2000-10229). We are grateful to Ruth Cox and Eleny Montero for help in the field, to Ruth Feber and Paul Johnson for substantial input to the preparation and analysis of the paper and for helpful comments on the manuscript by Professor George Kollias, Pete Goddard, Jessica Gimpel and Rubina Mian.

References

Anderson D, Grubb T and Silveira F 1999 The effect of short duration transportation on serum cortisol in alpacas (*Lama pacos*). *The Veterinary Journal 157*: 189-191

Barnett J L and Hemsworth P H 1990 The validity of physiological and behavioral measures of animal-welfare. *Applied Animal Behaviour Science 25*: 177-187

Bateson P and Wise D 1998 Welfare of hunted red deer. *Veterinary Record 142*: 95

Baunmann I, Bligh J and Vallenas P 1975 Temperature regulation in the alpaca (*Lama pacos*). *Comparative Biochemistry and Physiology 50C(C)*: 105-109

Becker J B, Breedlove S M and Crews D 1992 *Behavioral Endocrinology*. MIT Press: Cambridge, Massachusetts, USA

Bonacic C 2000 *Sustainable use of the vicuna (*Vicugna vicugna*)*: *a physiological and ecological assessment*. DPhil Thesis, University of Oxford, Oxford, UK

Bonacic C and Gimpel J 1995 Sustainable use of South American wild camelids: theory and practice. *Newsletter of the European Fine Fibre Network 5*: 23-26

Bonacic C and Gimpel J 2000 Current advances on research and management of the vicuna (*Vicugna vicugna*) in Chile. *Ciencia e Investigacion Agraria 27(Enero-Abril)*: 39-50

Bonacic C and Macdonald D 2003 The physiological impact of wool-harvesting procedures in vicunas (*Vicugna vicugna*). *Animal Welfare 12*: 387-402

Bonacic C, Macdonald D, Galaz J and Sibly R 2002 Vicuna (*Vicugna vicugna*) density-dependence and sustainable use: the recovery of a protected population in Chile, 1975-1992. *Oryx 36(2)*: 118-125

Bradshaw E and Bateson P 2000 Welfare implications of culling red deer (*Cervus elaphus*). *Animal Welfare 9*: 3-24

Brandeys A 1988 *Radioimmunoassay test for plasmatic cortisol in South American Camelids*. Unpublished internal report, Endocrinology Laboratory, Faculty of Biological Sciences, Pontificia Universidad Catolica de Chile

Bubenik G A and Bartos L 1993 Cortisol levels in red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) after an acute ACTH administration. *Canadian Journal of Zoology 71*: 2258-2261

Bubenik G A, Brown R D and Schams D 1991 Antler cycle and endocrine parameters in male axis deer (*Axis axis*): seasonal levels of LH, FSH, testosterone, and prolactin and results of GnRH and ACTH challenge tests. *Comparative Biochemistry and Physiology (Part A — Physiology) 99*: 645-650

Bubenik G A, Brown R D, Schams D and Bartos L 1999 The effect of ACTH on the GnRH-induced release of LH and testosterone in male white-tailed deer. *Comparative Biochemistry and Physiology (Part C — Pharmacology, Toxicology & Endocrinology) 122*: 173-179

Bubenik G A, Bubenik A B, Schams D and Leatherland J F 1983 Circadian and circannual rhythms of LH, FSH, testosterone (T), prolactin, cortisol, T3 and T4 in plasma of mature, male white-tailed deer. *Comparative Biochemistry and Physiology (Part A — Physiology) 76*: 37-45

Bubenik G A and Reyestoledo E 1994 Plasma levels of cortisol, testosterone and growth-hormone in Pudu (*Pudu puda molina*) after ACTH administration. *Comparative Biochemistry and Physiology (Part A — Physiology) 107*: 523-527

Bustos P 1998 *Estudio de algunas variables fisiológicas del guanaco (*Lama guanicoe guanicoe*) durante la lactancia artificial en cautiverio.* Veterinary Medical Thesis, Universidad de Chile, Santiago, USA [Title translation: The study of some physiological parameters of the guanaco (*Lama guanicoe guanicoe*) during artificial lactation during captivity]

Coles E H 1980 *Veterinary Clinical Pathology*. Saunders: Philadelphia, USA

Cuchacovich J 1997 Scientific capture of vicunas. Permit number 3233. Resolucion de captura. Santiago, Ministry of Agriculture: 2

Dawkins M S and Gosling M 1992 *Ethics on Research on Animal Behaviour*. Academic Press: London, UK

Eckert R and Randall D J 1983 *Animal Physiology: Mechanisms and Adaptations*. W H Freeman: San Francisco, USA

Eltringham S K 1988 *Wildlife Resources and Economic Development*. International Book Distributors: Dehra Dun, India

Ferre I, Goddard P J, Macdonald A J, Littlewood C A and Duff E I 1998 Effect of method of blood sample collection on adrenal activity in farmed red deer and sheep following administration of ACTH. *Animal Science 67*: 157-164

Fowler M 1998 *Medicine and Surgery of South American Camelids, 2nd Edn*. Iowa State University Press: Ames, Iowa, USA

Fowler M E 1989 *Medicine and Surgery of South American Camelids*. Iowa State University Press: Ames, Iowa, USA

Fowler M E and Zinkl J G 1989 Reference ranges for hematologic and serum biochemical values in llamas (*Lama glama*). *American Journal of Veterinary Research 50*: 2049-2053

Galaz J 1998 El manejo de la vicuna en Chile. In: Valverde V (ed) *La Conservacion de la Fauna Nativa Chilena*: *Logros y Perspectivas* p 178. Corporacion Nacional Forestal: Santiago, Chile [Title translation: The management of vicunas in Chile]

Garry F 1989 Clinical pathology of llamas. *Veterinary Clinics of North America: Food Animal Section 5(1)*: 55-70

Goddard P J, Gaskin G J and Macdonald A J 1998 Automatic blood sampling equipment for use in studies of animal physiology. *Animal Science 66*: 769-775

Goddard P J, Gordon I J and Hamilton W J 1996 The effect of post-capture management strategy on the welfare and productivity of wild red deer (*Cervus elaphus*) hinds introduced to farming systems. *Animal Science 63*: 315-327

Goddard P J, Rhind S M, Hamilton W J, Macdonald A J, Fawcett A R, Soanes C and McMillen S R 1994 The adrenocorticotropic hormone stimulation test — its potential use and limitations in red deer (*Cervus elaphus*). *Canadian Journal of Zoology 72*: 1826-1830

Gurevitch J and Scheiner S M 1993 *Design and Analysis of Ecological Experiments*. Chapman & Hall: London, UK

Hall P 1978 The World Health Organisation's Programme for the standardization and quality control of radioimmunoassay of hormones in reproductive physiology. *Hormone Research 9*: 440-449

Harlow H J, Thorne E T, Williams E S, Belden E L and Gern W A 1987 Adrenal responsiveness in domestic sheep (*Ovis aries*) to acute and chronic stressors as predicted by remote monitoring of cardiac frequency. *Canadian Journal of Zoology 65*: 2021-2027

Hofer H and East M 1998 Stress and behavior. In: Møller A P, Milinski M and Slater P J B (eds) *Advances in the Study of Behavior, Vol 27* pp 405-497. Academic Press: London, UK

Ingram J R, Matthews L R, Carragher J F and Schaare P R 1997 Plasma cortisol responses to remote adrenocorticotropic hormone (ACTH) infusion in free-ranging red deer (*Cervus elaphus*). *Domestic Animal Endocrinology 14*: 63-71

Johnson L 1994 Update on lama medicine. *The Veterinary Clinics of North America 10*: 196-199

Jurgens K 1993 High altitude adaptation of oxygen transport properties of blood and circulation in South American Camelids. In: Gerken M and Renieri C (eds) *Proceedings of the European Symposium on South American Camelids* pp 219-222. Universita' degli studi de Camerino: Camerino, Italy

Jurgens K D, Pietschmann M, Yamaguchi K and Kleinschmidt T 1988 Oxygen binding-properties, capillary densities and heart weights in high-altitude Camelids. *Journal of Comparative Physiology (Part B — Biochemical Systemic and Environmental Physiology) 158*: 469-477

Kaneko J J, Harvey J W and Bruss M 1997 *Clinical Biochemistry of Domestic Animals*. Academic Press: London, UK

Koford C 1957 The vicuna and the puna. *Ecological Monographs 27*: 152-219

Kraabel B J and Miller M W 1997 Effect of simulated stress on susceptibility of bighorn sheep neutrophils to Pasteurella haemolytica leukotoxin. *Journal of Wildlife Diseases 33*: 558-566

LeRoy A 1999 *Nivel de cortisol maximo en guanacos (*Lama guanicoe*) para su utilizacion como indicador de estres*. Undergraduate Thesis, Pontificia Universidad Catolica de Chile, Santiago, Chile [Title translation: Maximum cortisol levels in guanacos (*Lama guanicoe*) as stress indicators]

Ludders J W, Langenberg J A, Czekala N M, Erb H N and McCormick H 1998 Serum corticosterone response to adrenocorticotropic hormone stimulation in Florida sandhill cranes. *Journal of Wildlife Diseases 34*: 715-721

Moreira J and Macdonald D 1996 Capybara use and conservation in South America. In: Taylor V and Dunstone N (eds) *The Exploitation of Mammal Populations* p 415. Chapman & Hall: London, UK

Nelson R J 1995 *An Introduction to Behavioral Endocrinology*. Sinauer Associates: Sunderland, Massachusetts, USA

Norris D O 1997 *Vertebrate Endocrinology*. Academic Press: London, UK

Norušis M J and SPSS Inc 1998 *SPSS 8.0 Guide to Data Analysis*. Prentice Hall: New Jersey, USA

Oryx 1999 Sustainable use of vicuna. *Oryx 33*: 208

Prescott-Allen R and Prescott-Allen C 1996 Assessing the impacts of uses of mammals: the good, the bad and the neutral. In: Taylor V and Dunstone N (eds) *The Exploitation of Mammal Populations* pp 45-61. Chapman & Hall: London, UK

Rebuffi G 1993 *Captura de vicunas en semicautiverio*. In: *Proceedings of the Third International Symposium on South American Camelids* pp 73-84. La Paz, Bolivia [Title translation: Vicuna captures in semi-captivity]

Robinson W L and Bolen E G 1989 *Wildlife Ecology and Management*. Macmillan: London, UK

Schalm O W and Jain N C 1986 *Schalm's Veterinary Hematology*. Lea & Febiger: Philadelphia, USA

Schmidt-Nielsen K 1990 *Animal Physiology*: *Adaptation and Environment*. Cambridge University Press: Cambridge, UK

Selye H and Ogilvie W H 1957 *The Stress of Life*. Longmans Green: London, UK

Smith B, Timm K and Long P 1999 *Llama and Alpaca Neonatal Care*. Clay Press: Jackson, USA

Smith J H and Bubenik G A 1990 Plasma-concentrations of glucocorticoids in white-tailed deer — the effect of acute ACTH and dexamethasone administration. *Canadian Journal of Zoology 68*: 2123-2129

SPSS 1997 *SPSS Advanced Statistics 7.5*. SPSS: Chicago, USA

Taylor V and Dunstone N 1996 *The Exploitation of Mammal Populations*. Chapman & Hall: London, UK

Vanmourik S and Stelmasiak T 1984 Adrenal-response to ACTH stimulation in rusa deer (*Cervus rusa timorensis*). *Comparative Biochemistry and Physiology (Part A — Physiology) 79*: 581-584

Wernery U, Fowler M and Wernery R 1999 *A Color Atlas of Camelid Hematology*. Book News Inc: Portland, USA

Wheeler J and Hoces D 1997 Community participation, sustainable use, and vicuna conservation in Peru. *Mountain Research and Development 17(3)*: 283-287

Winer B and Michels K 1991 *Statistical Principles in Experimental Design*. McGraw-Hill: New York, USA

Wolfensohn S and Lloyd M 1994 *Handbook of Laboratory Animal Management and Welfare*. Oxford University Press: Oxford, UK

Zekan S and Ezcurra E 1998 *Standardization and Quality Control of Laboratory Procedures*. World Health Organization: London, UK

Appendix 1 Food intake of captive vicuna in November 1998. The animals were given alfalfa hay and water *ad libitum*. Individual mean intake.

Date	n	Mean (kg)	Standard error of mean
10 Nov 98	1	0.6000	—
11 Nov 98	1	0.5500	—
13 Nov 98	3	0.6903	0.1545
14 Nov 98	5	1.2433	0.2444
15 Nov 98	4	0.8281	5.916×10^{-2}
16 Nov 98	4	1.0927	0.2814
17 Nov 98	4	0.8938	3.733×10^{-2}
18 Nov 98	1	0.6250	—
20 Nov 98	3	0.9028	9.722×10^{-2}
21 Nov 98	4	0.9135	9.369×10^{-2}
22 Nov 98	3	1.0208	0.1267
24 Nov 98	1	1.0000	—
26 Nov 98	2	0.9375	6.250×10^{-2}
Total	36	0.9339	5.497×10^{-2}

Appendix 2 Food intake as percentage of body weight. Reference values for SACs 1.8% body weight as fed (Fowler 1998).

Date	Statistics	Individual intake (kg)	Food intake/bodyweight/day (%)
10 Nov 98	n	1	1
	Mean	0.6000	1.7561
	Standard error of mean	—	—
11 Nov 98	n	1	1
	Mean	0.5500	1.8605
	Standard error of mean	—	—
13 Nov 98	n	3	3
	Mean	0.6903	2.2797
	Standard error of mean	0.1545	0.5791
14 Nov 98	n	5	5
	Mean	1.2433	3.9753
	Standard error of mean	0.2444	0.6329
15 Nov 98	n	4	4
	Mean	0.8281	2.7205
	Standard error of mean	5.916×10^{-2}	0.2206
16 Nov 98	n	4	4
	Mean	1.0927	3.4782
	Standard error of mean	0.2814	0.7308
17 Nov 98	n	4	4
	Mean	0.8938	2.8879
	Standard error of mean	3.733×10^{-2}	2.542×10^{-2}
18 Nov 98	n	1	1
	Mean	0.6250	2.2222
	Standard error of mean	—	—
20 Nov 98	n	3	3
	Mean	0.9028	2.8193
	Standard error of mean	9.722×10^{-2}	0.2166
21 Nov 98	n	4	4
	Mean	0.9135	2.9351
	Standard error of mean	9.369×10^{-2}	0.3514
22 Nov 98	n	3	3
	Mean	1.0208	3.3100
	Standard error of mean	0.1267	0.2251
24 Nov 98	n	1	1
	Mean	1.0000	3.3827
	Standard error of mean	—	—
26 Nov 98	n	2	2
	Mean	0.9375	2.8949
	Standard error of mean	6.250×10^{-2}	3.192×10^{-2}
Total	n	36	36
	Mean	0.9339	3.0056
	Standard error of mean	5.497×10^{-2}	0.1561