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Haematological and clinical biochemistry findings in captive juvenile guanacos (*Lama guanicoe* Müller 1776) in central Chile

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Abstract

The purpose of this study was to describe haematological and blood biochemistry findings of farmed guanacos in central Chile, in order to establish reference values for this species in captivity. Haematological and clinical biochemical measurements were performed on blood and plasma respectively, from 40 clinically healthy guanacos (20 females and 20 castrated males), aged between 2 and 3 years. The effects of gender and seasons of the year were studied. Gender affected the number of lymphocytes and the ratio of neutrophils:lymphocytes (N/L), with castrated males having a lower number of lymphocytes and higher N/L ratio than females. Seasons of the year affected most variables, presenting greater packed cell volume (PCV), haemoglobin (Hb), total protein (TP) and albumin values in winter than the rest of the seasons. White blood cells (WBCs) were not affected by season. Glucose decreased significantly over the year and creatine kinase (CK) activity, like glucose, had a tendency to decrease over the year, which may be related to habituation to sampling and handling. Haematological and clinical biochemistry values given in this study can serve as reference values for juvenile farmed guanacos in central Chile.

Keywords: Guanaco; Haematology; Clinical biochemistry; Health

1. Introduction

The evaluation of an animal's health status relies on the interpretation of present and past physical and clinical examination. The results of clinical laboratory testing are often critical in the evaluation of appropriate case management (Schalm et al., 1975; Garry, 1989; Kaneko et al., 1997). Haematological and biochemical measurements may vary depending on factors such as gender, age, pregnancy, physical exercise, weather, stress and season (Dougherty and Rosenblantt, 1965; Knill et al., 1969; Schalm et al., 1975; Kaneko et al., 1997).

There has been a recent increase in the use of wild species in farming systems (Hudson et al., 1989; Russel, 1991; Kyle, 1994). The guanaco is a wild South American camelid which in recent years has been farmed for meat and fine fibre in Chile and elsewhere (Fraser and Moorby, 1998; Bas and Gonzalez, 2000). Currently, there are approximately 900 individuals in captivity (Bas and Gonzalez, 2000). Little information is available about captive management including nutrition, assessment of health and diagnosis of diseases for this species (Hawkey and Gulland, 1988; Fraser and Moorby, 1998). However, there is some information about the clinical pathology of domesticated

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species of camelids such as the alpaca (*Lama pacos*) and the llama (*Lama glama*), which are currently used as reference values for the guanaco (Lewis, 1976; Montes et al., 1983; Lassen et al., 1986; Garry, 1989; Fowler, 1989; Vallenas, 1991; Hajduk, 1992).

The guanaco, being a new farm species, joins some other wild animals in captivity, for which little information is available about many of the haematological and biochemical values commonly used to evaluate the health status of traditional livestock species (Garry, 1989). Current available information on haematology and clinical biochemistry is scarce and comes from captured wild guanacos (Karesh et al., 1998; Gustafson et al., 1998), where stress is likely to have influenced measurements, or from small groups (Lewis, 1976; Hawkey and Gulland, 1988; González et al., 1998; Fraser and Moorby, 1998). In addition, comparative studies of the haematological variables and biochemical profiles of guanacos elsewhere, llamas and vicuñas have shown significant differences between the species in some of the parameters measured (Hawkey and Gulland, 1988; González et al., 1998). Although these species are very similar, it has been recommended that specific reference values should be obtained for each species (Fowler, 1989; Zapata, 1999).

The purpose of this study was to describe haematological and biochemical values in a healthy guanaco population under farming conditions. Differences due to gender (females versus castrated males) and seasons (summer, autumn, winter and spring) were studied in order to obtain reference values for this species in captivity. No intact males were studied, because farming conditions only allow a small number to be kept for mating.

2. Materials and methods

2.1. Animals

Forty, clinically healthy, 2 and 3 years old guanacos (20 females and 20 castrated males), each weighing approximately 95 kg were randomly chosen from 71 guanacos reared in captivity. The guanacos graze on 3 ha of naturalised pasture, and their diet is supplemented with alfalfa hay. This study was carried out between May 1998 and March 1999.

The guanacos were weighed once a month and vaccinated in spring and autumn against clostridial diseases. A drop-floor restraint chute was used to restrain guanacos for veterinary procedures and blood sampling before the study began. Animals were fed daily by a stockperson. All these handling and training procedures were performed, not just to prevent and control diseases, but to familiarise the animals with people and handling in order to minimise the stress response to blood sampling.

2.2. Blood sampling

Blood samples were taken from the jugular vein and collected into ethylene-diamine-tetraacetic-acid (EDTA) and heparinized tubes (Vacutainer®, Becton Dickson). These samples were used for haematology and clinical biochemistry, respectively (Villouta et al., 1997).

Heparinized tubes were centrifuged immediately. The plasma obtained was subsequently separated into 1.5 ml microtubes and frozen at −70 °C for further analysis, which was carried out around 2 weeks later. EDTA tubes were kept at room temperature for a maximum of 4 h before being analysed.

Blood samples were taken from each guanaco every morning on the following dates: autumn, between 28 May and 25 June; winter, between 12 and 21 August; spring, between 15 October and 5 November and summer, between 4 and 26 March.

2.3. Measurements

Packed cell volume (PCV) and haemoglobin (Hb) concentration were determined by the microhaematocrit and cyanmethaemoglobin method, respectively. Mean corpuscular haemoglobin concentration (MCHC) was calculated using the standard formula (Schalm et al., 1975). Total white blood cell (WBC) counts were carried out in a Neubauer counting chamber using the haemacytometer technique. Blood films on coverslips were fixed in absolute methanol and stained with Giemsa and differential WBC counts were performed according to Schalm et al. (1975). In order to minimise the source of error, the total white blood cell and differential cell counts were performed by the same experienced person (Dr. Gladys Villouta, Professor of Clinical Pathology, in charge of

the laboratory). Manual counts were used instead of automated cell counts because in Chile few veterinary clinical laboratories use this technique.

Total plasma protein (TP) and albumin were measured using the biuret and bromocresol green methods, respectively, and globulin was estimated by difference. The activity of aspartate aminotransferase (AST, EC 2.6.1.1, AST®) and creatine kinase (CK, E.C. 2.7.3.2, CK Nac-activated®) were measured at 30 °C by the UV test (Laboratory Boehringer Mannheim); blood urea nitrogen (BUN) and glucose levels were determined by Berthelot and glucose oxidase methods, respectively (Merckotest®). Calcium (Ca) was determined using *o*-cresolphthalein complexone and inorganic phosphorous (P) was determined by a colourimetric method, using the phosphomolybdate technique (Merckotest®).

2.4. Statistics

Descriptive statistics (mean, standard deviation and range) were used to summarise the data for each season and gender, and a repeated measurement ANOVA was conducted. Significant differences were considered at $\alpha = 0.05$. Data normality was confirmed by the Kolmogorov–Smirnov test. PCV (l/l) was arcsine square root transformed and CK was log transformed (Dytham, 1999). Repeated and simple contrasts were used for analysing the effect of seasons on haematological and biochemical indicators.

3. Results

Red blood cell values and total and differential white blood cell counts for guanacos are shown in Tables 1 and 2. There was no significant difference in the RBC values between the genders ($P > 0.05$), however, there were some differences between seasons. The highest PCV and Hb values were observed in winter ($P < 0.05$) and the lowest value of MCHC was found in autumn ($P < 0.05$).

Effect of gender on white cells is shown in Table 3. The number of lymphocytes ($F(1, 35) = 25.44$, $P < 0.05$) and the ratio of neutrophils and lymphocytes (N/L) ($F(1, 34) = 9.22$, $P < 0.05$) were affected by gender. Overall, males presented a lower number of lymphocytes than females, and consequently had a higher N/L ratio. By season, males had significantly lower number of lymphocytes than females in all the seasons, except spring ($P > 0.05$), and the N/L ratio was significantly higher in males than females in all the seasons, except autumn ($P > 0.05$).

Table 4 shows the plasma biochemistry values obtained from farmed guanacos. There was no significant effect of gender on plasma biochemistry variables ($P > 0.05$). Nevertheless, most variables showed significant differences between seasons ($P < 0.05$), except CK which presented a wide observed range within each season. Except for the AST levels between spring and summer ($P > 0.05$), AST and

Table 1
Red blood cell values in farmed guanacos (*Lama guanicoe*) in four seasons of the year

Variable	Autumn ($N = 41$)	Winter ($N = 39$)	Spring ($N = 38$)	Summer ($N = 38$)
PCV[a] (l/l)				
Mean ± S.D.	0.34 ± 0.03 a	0.37 ± 0.02 b	0.36 ± 0.0 3 ac	0.33 ± 0.02 ad
Range	0.28–0.42	0.29–0.42	0.30–0.43	0.28–0.37
Hb[b] (g/dl)				
Mean ± S.D.	15.5 ± 1.64 a	17.4 ± 1.23 b	16.7 ± 1.74 c	15.6 ± 1.22 d
Range	11.9–19	13.8–19.5	14–21.2	13.1–17.9
MCHC[c] (g/dl)				
Mean ± S.D.	45.3 ± 3.64 a	47.0 ± 2.09 b	46.5 ± 1.98 b	47.0 ± 2.84 b
Range	36–54	40.5–52	42.3–53	41.1–55.4

Letters a–d indicate significant differences among columns at 0.05 level.
[a] Packed cell volume.
[b] Haemoglobin.
[c] Mean corpuscular haemoglobin concentration.

Table 2
White blood cell values in farmed guanacos (*Lama guanicoe*) in four seasons of the year

Variable	Autumn ($N = 41$)	Winter ($N = 39$)	Spring ($N = 38$)	Summer ($N = 38$)
Leukocytes ($10^9\,l^{-1}$)				
Mean ± S.D.	9.5 ± 3.10	10.7 ± 2.90	10.2 ± 3.06	10.1 ± 2.66
Range	3.1–15.8	5.1–20.2	4.7–15.7	5.1–20.0
Band cells ($10^9\,l^{-1}$)				
Mean ± S.D.	0.22 ± 0.16	0.20 ± 0.08	0.25 ± 0.20	0.20 ± 0.10
Range	0.03–0.63	0.08–0.39	0.07–0.92	0.08–0.40
Neutrophils ($10^9\,l^{-1}$)				
Mean ± S.D.	6.3 ±2.61	6.94 ± 2.70	6.74 ± 2.42	6.56 ± 2.43
Range	0.90–12.10	3.03–17.33	3.35–12.25	3.41–17.20
Lymphocytes ($10^9\,l^{-1}$)				
Mean ± S.D.	2.83 ± 1.19	3.20 ± 1.19	2.83 ± 1.15	2.98 ± 1.28
Range	0.69–6.05	1.42–6.04	0.89–5.57	0.57–6.57
Monocytes ($10^9\,l^{-1}$)				
Mean ± S.D.	0.27 ± 0.13	0.35 ± 0.19	0.28 ± 0.17	0.28 ± 0.19
Range	0.06–0.53	0.09–0.87	0.05–0.89	0.08–0.80
Eosinophils ($10^9\,l^{-1}$)				
Mean ± S.D.	0.13 ± 0.08	0.23 ± 0.12	0.28 ± 0.18	0.17 ± 0.13
Range	0.06–0.31	0.09–0.47	0.08–0.62	0.08–0.55
Basophils ($10^9\,l^{-1}$)				
Mean ± S.D.	0.21 ± 0.22	0.12 ± 0.06	0.13 ± 0.04	0.32 ± 0.14
Range	0.06–0.76	0.07–0.24	0.08–0.21	0.08–0.44

Table 3
The effect of gender on lymphocytes and N/L ratio in farmed guanacos in central Chile during different seasons of the year

Season	Lymphocytes ($10^9\,l^{-1}$) (mean ± S.D.)		N/L (mean ± S.D.)	
	Females	Males	Females	Males
Autumn	3.4 ± 1.17 b ($n = 20$)	2.3 ± 0.92 aA ($n = 21$)	2.3 ± 1.20 ($n = 20$)	2.9 ± 1.95 ($n = 21$)
Winter	3.7 ± 1.16 b ($n = 19$)	2.7 ± 1.01 aB ($n = 20$)	1.9 ± 0.69 b ($n = 19$)	3.1 ± 1.92 a ($n = 20$)
Spring	3.2 ± 1.20 ($n = 19$)	2.5 ± 1.00 AB ($n = 19$)	2.3 ± 1.14 b ($n = 19$)	3.2 ± 1.62 a ($n = 19$)
Summer	3.7 ± 1.30 b ($n = 19$)	2.2 ± 0.77 aAB ($n = 19$)	2.0 ± 0.99 b ($n = 19$)	3.6 ± 2.81 a ($n = 19$)

Letters a, b indicate significant differences between genders at the 0.05 level and A, B indicate significant differences between seasons at the 0.05 level.

glucose were always statistically different ($P < 0.05$). Ca had the highest value in autumn, whereas P had the lowest in this season ($P < 0.05$). TP and albumin values peaked in winter ($P < 0.05$).

Glucose levels exhibited a significant decreasing trend between consecutive seasons ($P < 0.05$). CK activity also showed a possible tendency to decrease, though it was not significant ($P > 0.05$).

4. Discussion

4.1. Red blood cells

PCV concentrations were similar to those reported by other researchers. Karesh et al. (1998) reported values between 0.30 and 0.48 l/l in wild darted guanacos. In captive guanacos in zoos, PCV ranges between

Table 4
Plasma biochemistry findings in farmed female and male guanacos (*Lama guanicoe* Müller) in central Chile in different seasons of the year

	Autumn	Winter	Spring	Summer
BUN[a] (mmol/l)				
Mean ± S.D.	4.6 ± 1.39 a	5.3 ± 1.27 b	5.6 ± 1.09 bc	5.8 ± 0.81 c
Range	1.5–6.5	3.3–7.6	3.6–7.8	4.3–7.8
AST[b] (U/L)				
Mean ± S.D.	127.7 ± 21.74 a	147.6 ± 35.20 b	135.7 ± 26.99 ac	104.9 ± 23.29 d
Range	86–179	99.0–227	80.0–196	68–166
CK[c] (U/L)				
Mean ± S.D.	71.6 ± 93.53 a	79.8 ± 96.41 a	57.6 ± 56.81 a	43.3 ± 28.41 a
Range	24–540	18.0–411	12.0–310	23–198
Ca (mmol/l)				
Mean ± S.D.	2.2 ± 0.33 a	2.0 ± 0.38 b	2.0 ± 0.31 b	1.8 ± 0.23 b
Range	1.4–2.9	1.2–2.8	1.5–2.9	1.5–2.4
P (mmol/l)				
Mean ± S.D.	2.0 ± 0.31 a	2.4 ± 0.61 b	2.5 ± 0.45 bc	2.6 ± 0.36 c
Range	1.3–2.6	1.3–4.6	1.7–3.8	1.8–3.3
Glucose (mmol/l)				
Mean ± S.D.	6.2 ± 1.44 a	6.2 ± 1.06 a	4.2 ± 0.69 b	5.0 ± 0.68 c
Range	4.3–10.6	4.4–9.3	2.7–5.5	3.5–6.6
TP[d] (g/l)				
Mean ± S.D.	54.0 ± 5.10 a	63.0 ± 4.0 b	57.0 ± 7.0 c	51.0 ± 4.0 d
Range	46.0–65.0 a	54.0–71.0	44.0–69.0	45.0–61.0
Albumin (g/l)				
Mean ± S.D.	35.0 ± 5.0 a	41.0 ± 4.0 b	38.0 ± 4.0 c	38.0 ± 4.0 c
Range	26.0–46.0	30.0–50.0	30.0–45.0	30.0–44.0
Globulin (g/l)				
Mean ± S.D.	19.0 ± 6.0 a	22.0 ± 6.0 a	20.0 ± 6.0 a	14.0 ± 6.0 b
Range	7.0–32.0	10.0–39.0	10.0–35.0	5.0–28.0

Letters a–d indicate statistical difference among seasons at the 0.05 level.
[a] Blood urea nitrogen.
[b] Aspartate aminotransferase.
[c] Creatine kinase.
[d] Total protein.

0.36 and 0.42 l/l (González et al., 1998; Hawkey and Gulland, 1988). These values are similar to alpacas (Hajduk, 1992). In the majority of previous reports of domesticated camelids, PCV level differed between sexes (Montes et al., 1983; Garry, 1989). In this study, PCV values were not significantly different between genders.

PCV, Hb, TP and albumin concentrations were significantly higher in winter than the other seasons. This increase may be interpreted as haemoconcentration probably due to slight dehydration (Meyer et al., 1992), caused probably by decreased access to water, which was temporarily frozen in the morning during winter.

4.2. White blood cells

The number of leukocytes were within the range that has been reported for captive zoo guanacos by González et al. (1998) ((8.6–11.2) × $10^9\,l^{-1}$) and by Hawkey and Gulland (1988) ((2.7–14.7) × $10^9\,l^{-1}$), but lower than values reported from wild caught guanacos by Karesh et al. (1998) ((14.2–30.3) × $10^9\,l^{-1}$). This increased

value of leukocytes was speculated to be a trapping and handling stress response (Karesh et al., 1998).

In general, WBC differential counts were similar to values reported by González et al. (1998) and Hawkey and Gulland (1988), but lymphocyte and eosinophil numbers were slightly lower in this study than was reported by Hawkey and Gulland (1988).

Except for the number of lymphocytes and N/L ratio, WBC were not affected by gender, as was reported for alpacas (Montes et al., 1983). Males presented overall a lower number of lymphocytes and higher N/L ratio than females.

It is well known that physiological stress response, induced by an increase of endogenous corticosteroids in the bloodstream, is accompanied by neutrophilia, lymphopenia, monocytosis and eosinopenia and an increased N/L, however there are variations between species, e.g. horses usually show no monocyte change (Meyer et al., 1992). The mechanism of lymphopenia is associated with lympholysis in blood and lymphoid tissues and/or altered distribution of lymphocytes out of the vascular pool into other body compartments, such as the marrow (Jain, 1986). In South American camelids, leukocyte kinetics seem to be different from those of other ungulate species. Although no studies have documented the degree of change expected in the WBC count in response to stress, clinical research in llamas suggests that in adult llamas, a lymphocyte count less than $1 \times 10^9\,l^{-1}$ has been described as indicative of stress (Garry, 1989). In the current study, the lower limit of the observed range for the number of lymphocytes was similar to the figure described as indicating a stress response. Thus, a stress response may be masking the real values in juvenile guanaco males.

In this study, the finding of reduced lymphocyte count observed in castrated males, compared with females, and the interpretation of this as a sign of stress response is consistent with the social behaviour of the males in the wild, which consists of permanent hierarchical fights most common among guanacos aged 3–4 years old (Wilson and Franklin, 1985). Despite the fact that the studied males were castrated and their hierarchical fights are less frequent than intact males, they do, in practice, fight in captivity. Thus, their social behaviour may contribute to elevated stress, which the male guanacos have to cope with.

4.3. Clinical biochemistry

In this study, most biochemical variables were similar to values reported for llamas (Fowler and Zinkl, 1989; Lassen et al., 1986) and for wild guanacos (Fraser and Moorby, 1998; González et al., 1998; Karesh et al., 1998). However, this study reports lower concentrations of blood glucose than these earlier studies. This might be because the guanacos were familiarised to handling, presence of people and the blood sampling procedure, and therefore less stressed than animals in the other studies.

There were no differences in clinical biochemistry between genders, but there were significant differences between seasons. The BUN levels showed changes over seasons; the lowest values were observed in autumn and then increased over seasons until spring and summer. These findings might be explained by the quality of ingested diet, particularly the protein intake. Alfalfa hay supplementation was higher in winter, because of diminished availability of pasture during winter. Pasture starts to grow slowly from August up to November when there is both best quality and quantity of grasses (Avendaño, 1988).

CK activity and glucose level had a tendency to decrease over seasons. Both are related to the response of the animals to blood sampling. Glucose increases as part of the stress response and CK activity also increases with muscular exertion produced by attempts to escape during sampling (Meyer et al., 1992). The CK activity showed a wide range of values, indicating that CK varied considerably between animals, probably due to individual temperament. Nevertheless, the CK activity had a tendency to decrease over time, with a wide range of variation. The fall in the level of both glucose and CK may indicate a gradual habituation to handling over time.

5. Conclusions

In farmed guanacos living in central Chile, gender affected only the number of lymphocytes and the ratio of neutrophils: lymphocytes; castrated males had a lower number of lymphocytes and a higher N/L ratio. Most variables were affected by season. PCV, Hb, TP and albumin were increased in winter, indicating haemoconcentration, probably due to dehydration.

Except for the number of lymphocytes in males, WBC did not change with season. Plasma biochemistry was not affected by gender, but was influenced by season. Most differences between seasons may be explained by differences in availability of forage and the stress response to handling.

Differences found between genders and seasons did not have clinical relevance because animals were clinically healthy, therefore the values presented in this study can serve as reference values for juvenile farmed guanacos in central Chile.

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