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Hematological profile of wild vicuñas (*Vicugna vicugna*) from the Apolobamba National Natural Integrated Management Area, Bolivia

Perfil hematológico en vicuñas silvestres (*Vicugna vicugna*) en el Área Natural de Manejo Integrado Nacional Apolobamba, Bolivia

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This study established the hematological profile of vicuñas (*Vicugna vicugna*) in the wild. This species represents a sustainable natural resource for local communities, and considering management practices and animal welfare is fundamental for one of the most successful species conservation experiences in the country and the region. Blood samples were taken from 78 vicuñas in 14 communities during capture and shearing efforts in the 2021 season in the Apolobamba National Natural Integrated Management Area (ANMIN), located in the Franz Tamayo and Bautista Saavedra provinces of the department of La Paz, Bolivia. The results obtained from the hematological profile were: total erythrocyte count 15.2 X 10^{12} L, hematocrit 42 L/L, total solids 5.6 g/dl, hemoglobin 11.8 g/L, mean corpuscular volume 28.1 fL, mean corpuscular hemoglobin 9.2 pg, mean corpuscular hemoglobin concentration 33 g/L, total leukocyte count 10.3 x 10^{9} L, neutrophils 62 %, fallen neutrophils 5 %, basophils 0 %, eosinophils 0.09 %, lymphocytes 31 %, monocytes 2 % and platelet count 65 x 10^{3} L. The parameters obtained are within the values recorded for the species. Certain changes in a few communities were observed, such as elevated hematocrit, leukocytosis and the ratio of neutrophils and lymphocytes associated with physiological changes due to the stress of the vicuñas, which might be related to the inadequate implementation of animal welfare practices.

Key words: Harvest; hematology; La Paz; management; vicuñas.

En el presente estudio se estableció el perfil hematológico de vicuñas (*Vicugna vicugna*) silvestres. Esta especie representa para las comunidades locales un recurso natural sostenible y considerar las prácticas de manejo y bienestar animal es fundamental para una de las experiencias de conservación de una especie con mayor éxito a nivel país y la región. Se tomaron muestras sanguíneas de 78 vicuñas en 14 comunidades aprovechando el arreo, captura y esquila de la temporada 2021, en el Área Natural de Manejo Integrado Nacional (ANMIN) Apolobamba, ubicada en las provincias Franz Tamayo y Bautista Saavedra del departamento de La Paz, Bolivia. Los resultados obtenidos del perfil hematológico fueron: recuento total de eritrocitos 15.2 x 10^{12} L, hematocrito 42 L/L, sólidos totales 5.6 g/dl, hemoglobina 11.8 g/L, volumen corpuscular media 28.1 fL, hemoglobina corpuscular media 9.2 pg, concentración de hemoglobina corpuscular media 33 g/L, recuento total de leucocitos 10.3 x 10^{9}/L, neutrófilos 62 %, neutrófilos cayados 5 %, basófilos 0 %, eosinófilos 0.09 %, linfocitos 31 %, monocitos 2 % y recuento plaquetario 65 x 10^{3} L. Los parámetros obtenidos se encuentran dentro de los valores conocidos para la especie, aunque se observan ciertas alteraciones en pocas comunidades, como la elevación del hematocrito, la leucocitosis y la relación de neutrófilos y linfocitos asociados a cambios fisiológicos por la deshidratación y el esfuerzo muscular y el estrés de las vicuñas, los cuales estarían relacionados con una inadecuada implementación de las prácticas de bienestar animal.

Palabras clave: Aprovechamiento; cosecha; hematología; La Paz; vicuñas.

The vicuña (*Vicugna vicugna*), along with the guanaco (*Lama guanicoe*), alpaca (*Vicugna pacos*) and llama (*Lama glama*), play a key role as the herbivorous camelids with the highest biomass "in the Andean region of South America" (Quispe 2011), and have a cultural value in the cosmovision of Andean peoples (Rojo *et al.* 2012). The vicuña inhabits the Andean steppes of Ecuador, Perú, Bolivia, Chile, and Argentina, between 3,800 and 5,000 m, and produces one of the finest and most sought-after fibers in the world (Vilá *et al.* 2010; Concha *et al.* 2013). In Bolivia, the conservation

and use of the vicuña is a valuable resource for the communities where this species is present, which, through protection and monitoring efforts together with the government sector, have managed to increase their populations (MMAyA *et al.* 2021).

According to the International Union for Conservation of Nature (IUCN), globally the vicuña is currently categorized as Least Concern (Acebes *et al.* 2018), and in Bolivia it has a similar categorization (Tarifa and Aguirre 2009). One of the places with the highest number of vicuñas in Bolivia is the Apolobamba National Natural Integrated Management Area (ANMIN) in the department of La Paz with a population of 15,278 vicuñas (SERNAP 2023), where the main threats to the species are mining and forage competition between wild animals and domestic livestock, which impacts overgrazing for prairies and high altitude peatlands or *bofedales* (Alberto 2020; Alberto and Barrera 2022).

Studies conducted on vicuñas in Apolobamba have focused on monitoring pathogens and exposure to diseases such as bovine viral diarrhea, bluetongue, pseudotuberculosis, vesicular stomatitis, leptospirosis, foot-and-mouth disease and brucellosis, in all cases with negative results (Nallar *et al.* 2005). In another study, hematological parameters were recorded, as well as sero-exposure to brucellosis and foot-and-mouth disease with negative results (Beltrán-Saavedra *et al.* 2011). Evaluations of vicuña ectoparasites and endoparasites were also conducted (Beltrán-Saavedra *et al.* 2011; Ruiz 2016) in Apolobamba.

Currently, vicuña protection, management and use are carried out by the communities, framed within a set of organizational, technical and operational activities according to the Technical Guidelines for the Management and Conservation of the Vicuña (MDRyT-MMAyA 2013) established at the national level. Vicuñas are captured, sheared, and released to obtain the fiber following established protocols, which have improved fiber production yields, generated knowledge on the status of the population, and developed sustainable use with good animal welfare practices according to the IUCN South American Camelid Specialist Group (GECS 2012) and the World Organization for Animal Health (OMSA 2018).

The handling, capture and transport of ungulates, carnivores and birds can cause stress to the animals (Grigor *et al.* 1998). In the case of the vicuña, its exploitation (herding, capture, shearing) affects animal welfare criteria regarding nutrition, environment, health, behavior and mental state (Zapata 2023). These alterations can be measured by changes in hematological and biochemical parameters in the blood (Grigor *et al.* 1998; Bonacic and Macdonald 2003). Studies on the effects of vicuña herding methods, herding distance, and restraint, showed that vicuñas increase cortisol concentration when herded with vehicles, and increase blood creatine kinase when under restraint (Bonacic *et al.* 2006).

Sustainable management of vicuñas includes a series of components (MDRyT-MMAyA 2013), and during the harvesting season they are herded, captured, and handled to harvest fiber under established protocols, but in some communities these protocols are not fully complied with. In this study, we compare the management carried out by the communities and report the hematological parameters of wild vicuñas managed in Apolobamba and evaluate the physiological changes with respect to animal welfare practices.

The Apolobamba ANMIN is located to the west of the department of La Paz and includes the municipalities of Curva, Charazani, Pelechuco and Mapiri, with an altitudinal range of 800 to 6,200 m, including a low or humid *yungas* zone, an intermediate zone of inter-Andean valleys and a high Andes zone including extensive prairies known as the *altiplano*. Apolobamba covers 483,743.8 ha, bordered to the west by Perú, to the north by Madidi National Park and Natural Integrated Management Area (PNANMI), to the east by the municipality of Apolo and to the south by the Moco Moco, Ayata and Tacacoma municipalities (MMAyA-SERNAP 2016). The present study was conducted in 14 communities that make up the regional association of vicuña handlers of Apolobamba (Figure 1), who harvest vicuña independently and have implemented the protocol for good animal welfare practices and sanitary measures in the sustainable use of vicuña fiber (MMAyA *et al.* 2021).

Taking advantage of the vicuña shearing season between September and December following Bolivia's national vicuña management plan (MDRyT-MMAyA 2013), when local communities schedule herding, capture and shearing days approved by the national authority, the General Directorate of Biodiversity and Protected Areas (DGBAP) and coordinated with the Regional Association of Vicuña Management Communities, we collected biological samples from wild vicuñas (blood).

Once vicuñas were captured in the corral, data was taken on each vicuña selected for shearing, including biometric measurements, as well as an external examination, and a hood placed on the head to obstruct its visibility. Subsequently, they were transferred one by one to the shearing area, where they were held in a latero-ventral position for sampling. Sampling was random and occurred between 10:00 and 12:00 hr.

Between September and November 2021, blood samples (5 ml) were collected in tubes with ethylenediaminetetraacetic acid (EDTA) from 78 vicuñas (49 females and 29 males) by puncture of the jugular vein, under permits issued by the national authority (N°717/09 MMAyA-VMABCC-DGBAP). The samples were stored in preservers and kept at a temperature of 0 to 4 °C with ice packs until they were sent to the laboratory. Whole blood was processed by manual hemogram (Reagan *et al.* 1999; Alvarez 2021) and parameters such as total erythrocyte and leukocyte count, hematocrit, total solids, platelet count, differential neutrophil count, increased neutrophils, basophils, eosinophils, lymphocytes and monocytes were established in the labo-

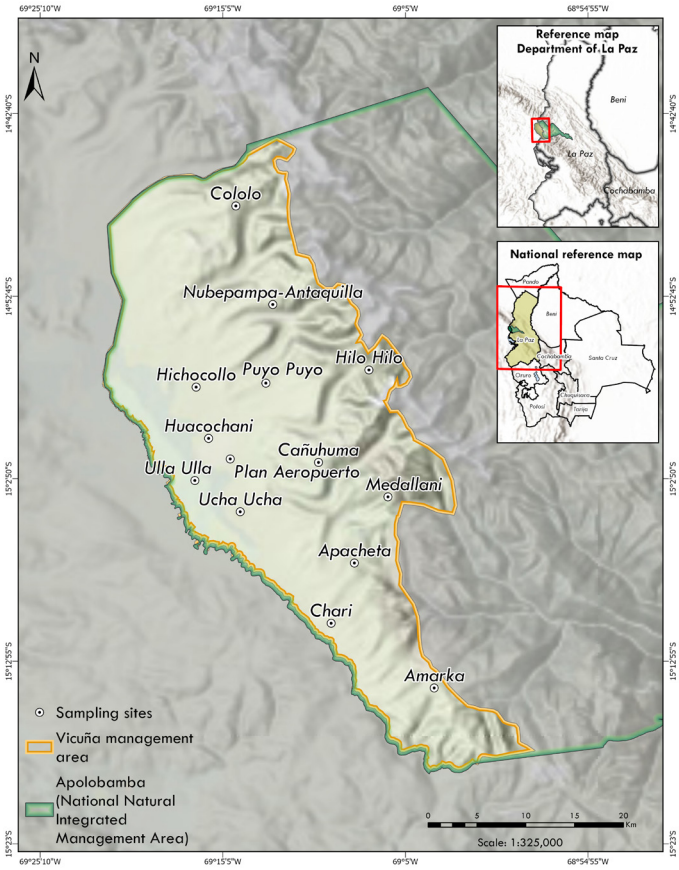

Figure 1. Map of communities and vicuña (*Vicugna vicugna*) sampling sites in the Apolobamba National Natural Integrated Management Area, La Paz, Bolivia.

ratory of Wildlife Conservation Society (WCS) and Specialized Laboratories (LabGenetics) in La Paz, Bolivia.

The significance of differences in the parameters evaluated was analyzed using a one-way ANOVA, with community as a fixed factor. All graphs and statistical analyses were performed in the R programming environment (R Development Core Team 2022).

The hematological parameters of wild vicuñas from Apolobamba are presented in Table 1 including values from previous studies. Changes were observed in hematocrit levels, as well as in the differential neutrophil and lymphocyte counts and total leukocyte count. There were significant differences in the percentage of hematocrit across communities ($F_{13,63} = 3.094$, $P = 0.0013$), with vicuñas from the Chari and Cololo communities having a higher hematocrit percentage than the Puyo Puyo and Puyo Puyo Japu communities (Puyo Puyo-Chari $P = 0.048$, Puyo Puyo Japu-Cololo $P = 0.0042$, Puyo Puyo-Cololo $P = 0.010$; Figure 2a). The result suggests that the vicuñas of the Chari and Cololo communities were more dehydrated due to increased muscle activity, possibly related to the activity of herding and capture.

According to the differential white cell count, the neutrophil/lymphocyte ratio showed a significant difference ($F_{13,63} = 5.365$, $P < 0.0001$). This parameter shows that the vicuñas of the Cañuhuma, Hilo Hilo and Cololo communities were the most stressed by having higher values (neutrophilia and lymphocytosis).

The Hilo Hilo community had significantly higher values than Amarka ($P = 0.0071$), Apacheta ($P < 0.0001$), Chari ($P < 0.0001$), Hichocollo ($P = 0.0001$), Huacochani ($P = 0.0006$), Plan Aeropuerto ($P = 0.0068$), Puyo Puyo ($P = 0.0008$) and Ulla Ulla ($P = 0.0011$). Similarly, Cololo has higher values than Apacheta ($P = 0.0197$) and Chari ($P = 0.00962$). Finally, Cañuhuma was significantly higher than Chari ($P = 0.0356$; Figure 2b).

Total leukocyte count, which is also a stress-related parameter, showed a significant difference between communities ($F_{13,63} = 3.067$, $P = 0.0015$). Vicuñas from the Huacochani community showed significantly higher leukocyte counts than Puyo Puyo Japu ($P = 0.002$) and Hichocollo ($P = 0.0345$; Figure 2c).

The average hematological values for most parameters in this study are similar to those previously recorded for vicuñas in Perú (Table 1: Copaira 1949; Fowler 1998; Quispe 2011; Titi-Pacosoncco *et al.* 2017; Esteban 2019). However, for hematocrits and the neutrophil-lymphocyte ratio, higher values of the recorded range may indicate certain physiological consequences (Bonacic and Macdonald 2003).

A previous study in Apolobamba on hematology in vicuñas recorded similar values to the present study (Beltrán-Saavedra *et al.* 2011). However, the values for total erythrocyte and leukocyte counts were lower than those recorded in this study possibly due to the Unopette cell counting method for mammals and the marked morphological difference in camelid blood cells. This technique may have marginalized a significant proportion of red and white blood cells, so these values are not reliable for comparison.

Other studies also determined differing hematocrit values, such as Copaira (1949) from 31 to 43 L/L, and Fowler (1998) who recorded 36 L/L, Quispe (2011) recorded 35.5 to 38 L/L, and Titi-Pacosoncco *et al.* (2017), who recorded 39 to 41 L/L. The values recorded in our study (32 to 53 L/L), are high in some cases, mainly in the vicuñas of the Chari and Cololo communities, which may indicate that during the activity of herding, capture and shearing, the vicuñas register physiological effects, such as dehydration due to intense exercise, fear, or stress.

The recorded effects are consistent with vicuña management in the Chari and Cololo communities, where deficiencies in the application of good animal welfare practices during herding such as use of motorcycles in Chari, as well as the capture, handling and restraint of the vicuñas was observed, for example, the lack of use of hoods to protect eyes and limit awareness, causing the vicuñas to suffer greater stress. In addition, the vicuñas were herded over longer distances, between 7 to 10 km, which could lead to dehydration.

Similar results were obtained by Bonacic *et al.* (2006), who evaluated the capture of vicuñas and measured factors such as herding methods, herding distance and restraint,

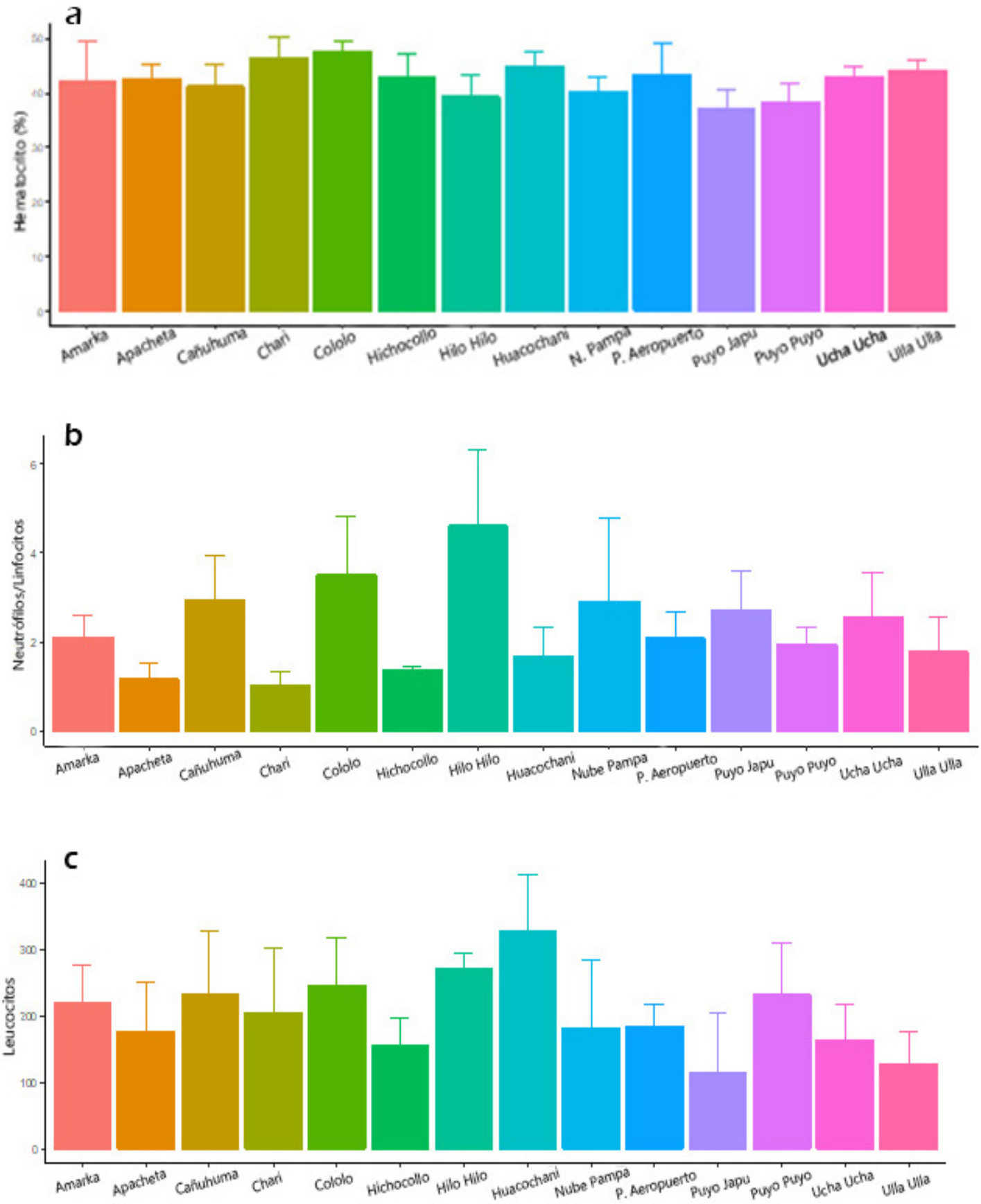

Figure 2. a) Percentage of hematocrit; b) ratio of neutrophil/lymphocyte count; c) total leukocyte count in vicuñas (*Vicugna vicugna*) from the Apolobamba National Natural Integrated Management Area, La Paz, Bolivia, according to the communities sampled, the error bars represent the standard deviation of the average.

Table 1. Hematological values found in 78 vicuñas (*Vicugna vicugna*) from the Apolobamba National Natural Integrated Management Area, La Paz, Bolivia.

Hematological parameter	Units	Mean	Range	Other studies	Source
Total erythrocyte count (RBC)	($x10^{12}$ L)	15.2	11.1 – 21.1	15.42	Copaira 1949; Fowler 1998
Hematocrit (Ht)	(L/L)	42	32 - 53	37	Copaira 1949; Fowler 1998; Quispe 2011; Titi-Pacosoncco *et al*. 2017
Total solids	(g/dl)	5.6	4.5 - 6.5		
Hemoglobin (Hb)	(g/L)	11.8	10.6 – 12	13.07	Fowler 1998; Quispe 2011
Mean Corpuscular Volume (MCV)	(fL)	28.1	18 – 39	27.4	Fowler 1998
Mean Corpuscular Hemoglobin (MCH)	(pg)	9.2	6 – 12	10.2	Fowler 1998
Mean Corpuscular Hemoglobin Concentration (MCHC)	(g/L)	33	33	37.5	Fowler 1998
Total white blood cell count (WBC)	($x10^{9}$ L)	10.3	2.05 - 20.9	15.42	Copaira 1949; Fowler 1998
Neutrophils	%	62	40 - 82	53.5	Copaira 1949; Fowler 1998; Esteban 2019
Increased neutrophils	%	5	1 - 9		
Basophils	%	0		0.75	
Eosinophils	%	0.09	0 - 1	11.25	Copaira 1949; Fowler 1998; Esteban 2019
Lymphocytes	%	31	12 - 56	34	Copaira 1949; Fowler 1998; Esteban 2019
Monocytes	%	2	0 - 6	13.5	Copaira 1949; Fowler 1998; Esteban 2019
Platelet count (PLT)	($x10^{3}$ L)	65	45 - 81	116	

and their results showed that vicuñas increase cortisol concentration when herded with vehicles, compared to those herded on foot; herding distance did not show significant changes in physiological parameters; and restraint after which vicuñas showed increases in creatine kinase in the blood.

Neutrophil and lymphocyte parameters previously recorded for the species are 41 to 67 % and 17.5 to 42.5 %, respectively (Copaira 1949). Fowler (1998) recorded 46.8 % for neutrophils and 33.8 % for lymphocytes, and Ruiz *et al.* (2019) recorded 40 to 67 % for neutrophils and 27 to 51 % for lymphocytes. Our records are higher, 40 to 82 % for neutrophils and 12 to 56 % for lymphocytes. The differential white cell count showed a significant difference in the neutrophil/lymphocyte ratio in vicuñas from the Cañuhuma, Hilo Hilo and Cololo communities. These values could indicate that the vicuñas are more stressed. The herding, capture and shearing in these communities was delayed until the afternoon (4 to 6 pm) due to climatic factors (snowfall), which also affected the time of rest before shearing.

Hurtado (2020) indicates that acute stress in vicuñas is related to cases of neutrophilia and lymphocytosis, as well as biochemical changes such as increased glucose, alkaline phosphatase (ALP), alanine transaminase (ALT), and severe infections. Previous studies (Bonacic and Macdonald 2003; Bonacic *et al.* 2006) evaluated blood glucose parameters, cortisol, neutrophil-lymphocyte ratio in vicuñas sheared immediately at the capture site versus vicuñas transferred to another site and sheared 12 days after capture, showing an increase in creatine kinase levels in vicuñas sheared immediately as compared to those sheared later.

The leukocytosis observed in the vicuñas of the Hichocollo community may also be associated with acute stress. Our results agree with previous studies where increased values in the total leukocyte count and the neutrophil/lymphocyte ratio were observed (Bonacic *et al.* 2003). In addition, they evaluated the neuroendocrine response to stress, through the application of adrenocorticotropic hormone (ACTH) in groups of vicuñas during shearing (Bonacic *et al.* 2003). Their results indicate a 4.5-fold increase of cortisol in blood after 1 hour of application, as well as leukocyte values and the neutrophil/lymphocyte ratio at 5 hours post-application.

The effects observed in the hematological parameters of the vicuñas could be associated with dehydration events due to intense muscular exercise, as well as deficiencies by some management communities in the application of the capture and handling methods carried out during herding, capture, and shearing. These effects could also be associated with acute stress events, but in the present study no biochemical tests were performed to corroborate this physiological change in the vicuñas.

Recently, manuals of animal welfare best practices and sanitary measures for the sustainable use of vicuña fiber were implemented (MMAyA *et al.* 2021), to apply these practices in the wild vicuña harvesting activities in Bolivia. The results obtained are in line with the parameters obtained in most communities; however, it is necessary to continue strengthening in other communities and regions for adequate application. Likewise, similar studies should continue to monitor this situation, and understand other factors such as habitat destruction, carrying capacity for animals on the *altiplano*, and the presence of sarcoptic mange, and thereby further improve vicuña conservation efforts.

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