Austral J Vet Sci **49, 27-33 (2017)**

SHORT COMMUNICATION

Haematological and serum biochemical reference values for urban-working equines in Chile

Karina Aros[a], Jorge Carrasco[b], Rodrigo Briones[bc], Tamara A. Tadich[a*]

ABSTRACT. Blood variables are an important tool when assessing the health and welfare of working horses. Commonly, reference intervals established abroad for Thoroughbreds are used, which not necessarily apply for local conditions. Thus, the aim of this study was to establish haematological and biochemical reference intervals for apparently healthy working horses under Chilean local husbandry conditions, and compare them with those proposed in the literature. A group of 320 working horses were sampled, and reference intervals were calculated for 11 haematological and 15 serum biochemical variables according to IFCC (International Federation of Clinical Chemistry) standards. The reference intervals calculated were then compared with those established in the literature for sport and working horses. The percentage of horses below and above the reference intervals from the literature was also calculated. Reference intervals for red blood cell count, haemoglobin and haematocrit were lower for 92.6%, 34.7% and 62.4% of Chilean working horses respectively when compared to reference intervals for UK horses. On the other hand, enzyme reference intervals were higher for CK, LDH and AST, and were higher for 100%, 94.9% and 70.6% of Chilean working horses when compared to reference intervals for UK horses. In conclusion, results show that reference intervals established overlap with those from the literature. The main differences were found when comparing the reference values with those established for sport horses such as Thoroughbreds, whereas values adequate better to those established for working horses in Pakistan.

Key words: working horses, haematology, serum biochemistry, reference intervals.

RESUMEN. Las variables sanguíneas son una herramienta importante para evaluar salud y bienestar en equinos de trabajo. Comúnmente se utilizan intervalos de referencia (IR) establecidos fuera de Chile para equinos Fina Sangre de Carrera, los que no necesariamente aplican bajo condiciones locales. El objetivo del estudio fue establecer IR para variables sanguíneas en equinos de trabajo aparentemente sanos en Chile y compararlos con los de la literatura. Se muestrearon 320 equinos de trabajo, calculándose los IR para 11 variables hematológicas y 15 de bioquímica sanguínea de acuerdo con los estándares de la IFCC (International Federation of Clinical Chemistry). Los IR calculados fueron comparados con aquellos de la literatura para equinos de deporte y trabajo. El porcentaje de equinos por sobre y bajo los IR fueron calculados. La mayoría de los IR calculados se superponen con los de la literatura. Los IR para recuento de eritrocitos, hemoglobina y hematocrito fueron más bajos en 92,6%, 34,7% y 62,4% de los equinos de trabajo en Chile, respectivamente, en comparación a los IR para equinos del Reino Unido. Por el contrario, los IR calculados para enzimas fueron más altos en 100% de los equinos de trabajo para CK, en 94,9% para LDH y en 70,6% para AST, en comparación con los IR establecidos para equinos del Reino Unido. En conclusión, las diferencias más relevantes se encontraron al comparar los intervalos de referencia calculados con aquellos establecidos para equinos de deporte, adecuándose mejor a los intervalos calculados para equinos de trabajo en Pakistán.

Palabras clave: equinos de trabajo, hematología, bioquímica sanguínea, intervalos de referencia.

INTRODUCTION

In the assessment of animal welfare the use of physiological indicators is common. Within these, blood reference values have been an important tool for practitioners in order to monitor health and welfare at individual or group level, or the immediate response of an animal towards a determined stressor (Waran 2007, Geffré *et al* 2009, Cozzi *et al* 2011). For example, changes in plasma concentration of glucose, urea and proteins have been associated to significant metabolic cost by the animal, while immunosuppression can indicate a potential health risk, or a reduction in growth rate (Barnett and Hemsworth 1990).

To identify those individuals that have health alterations, values from the blood analysis are usually compared with the population means or ranges of standard values (Herdt 2000), which in the case of horses are usually based on Thoroughbreds, sport or pleasure horses living under good husbandry conditions (Pritchard *et al* 2009). The use of inappropriate reference values increases the risk of erroneous conclusions by the clinician or the researcher, and may lead to further unnecessary or inappropriate analysis (Tsang *et al* 1998).

Haemato-biochemichal values obtained abroad may not be fully applicable under local conditions due to genetic factors, differences in environmental and husbandry practices and animal's function (Gul *et al* 2007, Pritchard *et al* 2009). Gul *et al* (2007) reported haemato-biochemical values for apparently healthy equids in Pakistan, finding increased values for biochemichal variables in horses when compared to the literature. Pritchard *et al* (2009) established haematological and biochemical reference intervals for working horses in Lahore (Pakistan), finding differences with reference limits from the United Kingdom (UK), for the variables studied.

Accepted: 04.08.2016.

[a]Departamento de Fomento de la Producción Animal, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile.

[b]Escuela de Medicina Veterinaria, Universidad Mayor, Temuco, Chile.

[c]Centro Clínico y de Investigación Equino, Padre Las Casas, Temuco, Chile.

*Corresponding author: TA Tadich; Santa Rosa 11735, La Pintana, Santiago, Chile; tamaratadich@u.uchile.cl

In Chile working horses are still the main source of income for many families (Tadich and Stuardo 2014). When the welfare of these horses is evaluated blood variables are used for assessing their health state and international reference intervals are usually applied (Tadich *et al* 2011).

The aim of this study was to establish haematological and biochemical reference intervals for apparently healthy working horses under Chilean local husbandry conditions, and compare them with those proposed in the literature.

MATERIAL AND METHODS

ANIMALS

The determination of reference intervals was done *de novo* from measurements made in reference individuals from a urban working horses reference population according to definitions provided by Geffré *et al* (2009). The recruitment of horses was done through a free clinic programme for working horses provided by the Veterinary Faculty of the Universidad de Chile. The selection criteria for inclusion of horses developed by Pritchard *et al* (2009) was applied, under the understanding that a healthy horse was one actively working and with no clinical signs of disease in order to be included in the study. A total of 320 horses were sampled, including mares, geldings and stallions, all with body condition scores between 2 and 4 (scale of 1 to 5), and between 2 and 14 years of age. All horses were urban draught horses living in urban and peri-urban areas of the cities of Santiago, Viña del Mar, Talca, Linares, Temuco, and Valdivia. The main products they transport are construction material, wood, sand and market products such as vegetables. Horses do not work every day and work between 4-8 hours daily on working days. Water is provided by owners once they return from work and feedstuff usually consists of alphalpha hay and a small proportion of owners also provides some type of grain (Tadich *et al* 2008).

HAEMATOLOGICAL AND BIOCHEMICAL VARIABLES

Horses were sampled by jugular puncture during resting days. Blood samples were divided into 4 tubes (4 mL with EDTA, 4 mL with no additive, 2 mL with citrate and 4 mL with heparin). For haematology, haemoglobin (Cyanomethemoglobin method using Hitachi®, Photometer 4020 (Boehringer Mannhein)), haematocrit, red blood cells (RBC) count, mean corpuscle volume (MCV), white blood cell (WBC) count, neutrophils (N), lymphocytes (L), neutrophils-lymphocytes ratio (N:L), monocytes, eosinophil's and platelets were determined (Abacus Junior Vet®). Differential leucocyte counts and erythrocyte morphology were performed on blood-stained smears using a Romanowski stain (Corzap 1, Hemogram®) at 1,000× (Olympus CX31®). Plasma activities of aspartate aminotransferase (AST; EC, 2.6.1.1), gamma glutamyl transferase (GGT; EC, 2.3.2.2), alkaline phosphatase (AST; EC, 3.1.3.1), lactate dehydrogenase (LDH; EC 1.1.1.27) and creatine kinase (CK; EC, 2.7.3.2) were analysed by Human kits (Human ®); and glutathione peroxidase (GPx; EC, 1.11.1.9) by Ransel (Randox®). All biochemical analysis was quantified using an autoanalyzer (Metrolab 2300®, Wiener Lab). Plasma concentration of total protein, albumin, globulin, urea, and creatinine, was analysed using Human kits (Human®) and lactate using Sentinel kit (Sentinel CH®). Fibrinogen (Biofuge haemo Heraeus), calcium (Ca) (Atomic absorption spectrometry, Thermo Electron Corporation ® S Serie), phosphate (P) (photometric determination with molybdate, Ultra Violet Auto analyser Wiener lab ®, Metrolab 2300), and Ca:P ratio were also determined.

STATISTICAL ANALYSIS

The reference intervals (RIs) were established according to the norms of the American Society of Veterinary Clinical Pathology (Friedrichs *et al* 2012). First aberrant values were eliminated from each variable, then the normality of the data was assessed through the Shapiro-Wilk test ($P>0.05$) (Statistix 8.0 ®). Non normal data was normalised with the logarithm method when possible. Posteriorly possible outliers were identified through the Dixon method and eliminated (Dixon 1953). For parametric variables the means methods was used to calculate the reference interval ($RI = m \pm 2$ SD) according to the International Federation of Clinical Chemistry (IFCC) (Wittwer 2012). For non-parametric variables the RI was calculated as established by Solberg (1987) where the lower reference limit (LRL) and upper reference limit (URL) were calculated with a nonparametric test based in the 2.5 and 97.5 percentiles. Descriptive statistics (mean, median, standard deviation and minimum and maximum values) were also calculated for each variable. The percentage of horses, with values below and above each of the reference intervals, from the literature was calculated. For calculation of RIs and descriptive statistics the Microsoft Excel ® programme was used.

The RIs calculated were then compared to those proposed in the literature for Chilean Creole horses, as a means of comparing with horses under the same geographical and climate conditions (Wittwer 2012); for Thoroughbreds (Knottenbelt 2006), and an international reference for working horses in Pakistan, for comparing with horses performing similar work (Pritchard *et al* 2009).

RESULTS AND DISCUSSION

The RIs for common haemato-biochemical variables were calculated for apparently healthy urban-working horses in Chile (table 1) and compared with those in the literature (table 2) for horses with different functions and geographical locations.

Table 1. Haematological and biochemical reference intervals for urban working horses in Chile, number of horses, descriptive statistics, number of outliers and confidence interval of 90% for lower and upper limits of reference and method applied.

Variable	N	Mean	SD	Median	Min	Max	RI (LRL-URL)	90% CI LRL	90% CI URL	n outliers	Method
Haematology											
RBC count $x10^{12}$/L	246	6.87	1.11	6.72	4.22	12.44	4.64-9.09	4.65-5.25	9.02-9.92	0	means
Haemoglobin g/L	245	120	16.2	115	79	172	83.2-148.1	82-90	149-163	0	means
Haematocrit (PCV) %	245	33.6	5.1	33	22.1	51	25-46	22.8-26.1	43-50	1	percentiles
Mean corpuscle volume fL	245	49.2	4.1	49.4	33.7	63	40-56.9	37-41.2	55-63	1	percentiles
WBC count $x10^9$/L	246	8.60	2.14	8.40	4.09	15.40	4.31-12.9	4.40-5.40	12.80-15.20	0	means
Neutrophils $x10^9$/L	246	4.69	1.69	4.52	0.93	12.32	1.31-8.07	1.08-2.23	7.71-11.12	0	means
Lymphocytes $x10^9$/L	246	3.31	1.46	2.99	0.38	9.00	1.23-7.19	0.77-1.40	6.32-8.35	0	percentiles
N:L	245	1.67	0.98	1.46	0.39	5.75	0.45-4.74	0.41-0.58	3.98-5.7	1	percentiles
Monocytes $x10^9$/L	190	0.23	0.19	0.14	0.03	1.11	0.06-0.78	0.06-0.06	0.61-1.02	0	percentiles
Eosinophils $x10^9$/L	188	0.37	0.29	0.27	0.06	1.41	0.07-1.19	0.07-0.08	0.99-1.34	0	percentiles
Platelets $x10^9$/L	225	173.17	93	159	20	930	71-422	46-97	310-664	0	percentiles
Biochemestry											
Urea mmol/L	316	7.31	1.8	7.19	2.3	13.8	3.73-10.9	2.7-4.3	10.6-12	0	means
Globulin g/L	256	37	9.3	37	11	59	17.8-55.3	13-21	51-57	0	means
Fibrinogen g/L	165	1.01	1.45	2	1	6	1.2-6.00	1-1.4	4.0-6.0	1	percentiles
Proteins g/L	316	72	11.2	72	32	95	43-91	36-49	89-92	0	percentiles
Albumin g/L	316	36	6.5	37	14	54	21-50	17-23	47-52	0	percentiles
Calcium mmol/L	315	4.5	3.4	2.9	1.6	14.1	2-12.4	1.8-2.2	11.9-13.7	0	percentiles
Phosphate mmol/L	316	1.62	1.3	1.1	0.3	7.2	0.5-5.3	0.5-0.6	4.8-6	0	percentiles
Ca:Pi	191	2.95	1.36	2.6	0.9	9.6	1.3-6.7	1-1.6	6.1-8.1	0	percentiles
Creatinine umol/L	267	90	26.9	86.7	44	334	53.9-134.3	53-58	132.6-176.8	1	percentiles
AST IU/L	313	315	176	296	10	1705	100-732	49-137	663-1180	1	percentiles
GGT IU/L	316	25.8	26.4	18	2	218	7-112	6.7-8	69-168	0	percentiles
CK IU/L	308	323	179	286	53	1093	107-821	83-113	752-888	8	percentiles
ALP IU/L	252	296	121	277	24	779	108-565	91-139	525-673	1	percentiles
LDH IU/L	315	807	515	714	196	5101	353-1746	295-386	1411-3858	0	percentiles
GPx IU/g Hb	287	112	82	98	8	394	11-278	10.0-13.0	259-321	0	percentiles

*SD=standard deviation; Min= minimum value; Max= maximum value; RI= reference interval; LRL=lower reference limit; URL= upper reference limit.

A total of eleven haematological and fifteen biochemical variables were assessed, from a population of 320 sampled working horses. Due to damage of samples during handling, aberrant results provided by the laboratory and the establishment of outliers, not all variables have the same sample size, being 165 horses the smallest sample (fibrinogen) and 316 individuals the largest one (urea, proteins, albumin, phosphate and GGT) for calculating the reference interval (table 1). This allowed excluding atypical values that could have been the product of inadequate analysis (Wagemann *et al* 2014) or from horses with an underlying pathology that was not detected at the moment of clinical examination. Despite elimination of aberrant data and outliers all variables included over 120 individuals, in accordance to the minimum sample size recommended by the IFCC (Solberg 1987, Friedrichs 2012).

The final sample size, descriptive statistics, the RIs established, the method applied, and number of outliers are presented in table 1. Differences in the RIs established could be product of differences in laboratory analysis, but could also reflect the diversity in genetic characteristics, husbandry practices, geoclimatic conditions and adaptation to the work performed by the individuals that were evaluated (Satué *et al* 2012, Padalino *et al* 2014).

Table 2 shows the comparison between RIs for haematological and biochemical variables established for Chilean urban-working horses and those established for Thoroughbred horses in Europe (Knottenbelt 2006), Chilean

Creole Horses in Chile (Wittwer 2012) and working horses in Pakistan (Pritchard *et al* 2009). The RIs calculated were similar to those reported for working horses in Pakistan (Pritchard *et al* 2009), and the Chilean Creole horses studied by Wittwer (2012); and differed more from those proposed by Knottenbelt (2006) for Thoroughbred horses, especially for RBC, Haemoglobin and enzymes (table 2).

Red blood cell count and haemoglobin RIs calculated are similar to those proposed by Pritchard *et al* (2009) and lower than those provided by Knottenbelt (2006) and Wittwer (2012). The later two intervals were developed for horses dedicated mainly to sports, this could explain the difference since these horses are usually under better feeding practices; at the same time training could also influence the concentration of erythrocytes and haemoglobin. In relation to this higher RBC counts Satué *et al* (2012) reported that "hot-blooded breeds" have higher RBC count, Hb and PCV than draught horses, ponies or the "cold-blooded breeds", for which PCV as low as 24% can be found in healthy animals. This cold-blooded breeds category includes Chilean creole horses and the crossbred horses used for draught work in Chile. On the same line, training can affect basal levels of RBC count, Hb and PCV. For example endurance trained horses have lower resting values of these variables (Satué *et al* 2012), type of exercise comparable to the draught work performed by urban working horses. On the other hand, a decrease in RBC and haemoglobin due to anaemia has

Table 2. Reference intervals (RI) established for Chilean working horses and those reported in the literature for sport horses in the UK, Chilean Creole Horse in Chile and working horses in Pakistan.

Variable	Reference Intervals			
	RI UK Horses[1]	RI Chilean Creole Horse[2]	RI Pakistan Working Horse[3]	RI Chilean Working Horse
Hematology				
RBC count $x10^{12}$ L	8.5-12.5	5.9-9.4	4.97-8.18	4.64-9.09
Hemoglobin g/L	110-180	107-167	89-139	83.2-148.1
Hematocrit (PCV)%	35-46	30-47	25-40.3	25-46
Mean Corpuscle Volume fL	41-49	40-61	43.9-55.6	40-56.9
WBC count $x10^9$/uL	6.0-12.0	5-11	5.72-13.7	4.31-12.9
Neutrophils $x10^9$ /uL	2.7-6.7	2.2-6.1	2.22-7.25	1.31-8.07
Lymphocytes $x10^9$/uL	1.5-5.5	1.5-6.5	1.44-6.78	1.23-7.19
N:L	-	-	-	0.45-4.74
Monocytes $x10^9$L	0.0-0.2	0.0-0.6	0.0-0.62	0.060-0.779
Eosinophils $x10^9$ L	0.1-0.6	0.1-0.8	0.0-1.13	0.070-1.187
Platelets $x10^9$uL	240-550	90-210	85-276	71-422
Biochemestry				
Urea mmol/L	3.5-8	3.6-8.8	3.6-11.4	3.73-10.9
Globulin g/L	17-40	25-41	34-53	17.8-55.3
Fibrinogen g/L	1.5-3.0	1.0-5.0	0.67-2.08	1.2-6.00
Proteins g/L	62.5-70	68-84	57-76	43-91
Albumin g/L	30-36	26-38	18-28	21-50
Calcium mmol/L	2.5-4.0	2.49-3.21	2.68-3.14	2-12.4
Phosphate mmol/L	1.0-2.0	0.90-1.50	0.39-1.64	0.5-5.3
Ca:Pi	–	–	–	1.3-6.7
Creatinine umol/L	90-200	85-115	61.3-133	53.9-134.3
AST IU/L	80-250	<480	189-456	100-732
GGT IU/L	<40	<62	11-32	7-112
CK IU/L	<50	<140*	123-358	107-821
ALP IU/L	<250	<530	59-319	108-565
LDH IU/L	76-400	<700	–	353-1746
GSH-Px IU/g Hb	–	>130	–	11-278

[1] Reference intervals from Knottenbelt (2006).
[2] Reference intervals from Wittwer (2012).
[3] Reference intervals from Pritchard *et al* (2009).
*< 500 post exercise.

been considered as a cause of low values, but Chile is free of equine infectious anaemia and *Trypanosoma* spp.[1]; and iron deficiency in horses has been poorly reported (Reed *et al* 2004), but considering the sometimes inappropriate feeding practices that working horses receive this possibility cannot be ruled out. Most working horses in Chile have been reported to have access to pasture, either in public green areas in the city or near rubbish dumps (Tadich *et al* 2008), being likely that they could be obtaining iron from the soil (Humphries *et al* 1983, Brommer and Sloet van Oldruitenborgh-Oosterbaan 2001).

The N:L ratio has been reported as a more reliable indicator of stress than the use of cortisol alone (Stull and Rodiek 2000), with a normal fluctuation between 1.5 and 2.5 (Rossdale *et al* 1982). The N:L ratio could be of value when studying working horses, and increases could be expected to occur in an overworked horse. No reference intervals for this ratio were provided by the literature used for comparison (Knottenbelt 2006, Pritchard *et al* 2009, Wittwer 2012) (table 2).

The percentage of horses that presented variables above or below the RI provided in the literature (table 3) is higher when comparing to the RIs for Thoroughbreds (Knottenbelt 2006), and horses tend to adjust better to the RI for working horses in Pakistan (Pritchard *et al* 2009) and Chilean creole horses (Wittwer 2012). These results support Pritchard *et al* (2009) conclusions that the reference intervals established by them are more appropriate when assessing a working horse population. The major differences were found for enzymes, where the median and mean of LDH and GSH-Px were out of the reference limits established by Knottenbelt (2006) and Wittwer (2012) respectively, no reference limit was provided by Pritchard *et al* (2009) for these enzymes. GSH-Px participates in reduction of oxidative processes and contains the major percentage of blood selenium (López Alonso *et al* 1997, Oh *et al* 1974). The assessment of GsHPx in chilean horses

[1] SAG, Servicio Agrícola y Ganadero. 2011. Situación sanitaria equina en Chile: Resultados vigilancia epidemiológica, primer semestre del 2011. Available at www.sag.cl/sites/default/files/situacion_sanitaria_equinos_I_semestre_2011.pdf

Table 3. Percentage of Chilean working horses below and above the reference intervals established in the literature for Thoroughbreds in the United Kingdom (UK) (Knottenbelt 2006), Chilean horses in Chile (Wittwer 2012) and working horses in Pakistan (Pritchard *et al* 2009).

Variable	UK		Chile		Pakistan	
	% below RI	% above RI	% below RI	% above RI	% below RI	% above RI
RBC count $x10^{12}$ L	92.6	0.0	17.9	2.4	1.2	2.8
Haemoglobina g/L	34.7	0.0	30.6	0.4	1.2	8.6
Haematocrit %	62.4	1.6	23.7	1.2	2.0	9.8
Mean corpuscle volume fL	3.3	48.9	0.4	6.5	7.3	3.7
WBC count $x10^9$/uL	8.1	6.5	2.0	14.2	6.5	1.6
Neutrophils $x10^9$ /uL	8.9	11.8	3.3	18.3	3.3	6.9
Lymphocytes $x10^9$/uL	5.3	9.8	5.3	3.7	4.9	2.8
Monocytes $x10^9$L	0.0	37.4	0.0	5.8	0.0	4.2
Eosinophils $x10^9$ L	11.2	17.0	11.2	9.6	0.0	3.2
Platelets $x10^9$uL	90.7	1.3	3.6	14.2	3.1	6.7
Urea mmol/L	1.6	31.3	1.9	19.0	1.9	1.9
Globulin g/L	1.6	38.7	10.9	32.8	39.5	2.0
Fibrinogen g/L	6.7	8.5	0.0	4.2	0.0	8.5
Proteins g/L	14.9	58.5	29.7	8.5	8.5	38.6
Albumin g/L	13.6	51.3	6.3	35.4	1.3	56.6
Calcium mmol/L	14.9	21.3	14.9	24.1	22.9	26.3
Phosphate mmol/L	37.3	19.9	28.5	28.2	0.3	24.4
Creatinine umol/L	58.4	0.7	46.1	10.1	6.0	3.7
AST IU/L	1.9	70.6	0.0	7.3	11.5	8.0
GGT IU/L	0.0	13.3	0.0	5.7	0.0	16.8
CK IU/L	0.0	100.0	0.0	90.3	6.2	31.8
ALP IU/L	0.0	57.1	0.0	3.6	0.4	38.9
LDH IU/L	0.0	94.9	0.0	53.0	0.0	0.0
GsHPx IU/g Hb	0.0	0.0	61.7	0.0	0.0	0.0

is important since clinical cases of miodegeneration and steatosis due to selenium deficiency have been reported (Araya *et al* 2004), together with insufficient concentrations of selenium in Chilean grasses in order to satisfy equine requirements established by the National Research Council (NRC 2005, Ceballos *et al* 1999). Over 60% of horses in the present study presented selenium deficiency identified by a decrease in serum GSH-Px according to the reference value provided by Wittwer (2012) (table 3), but none presented clinical signs of selenium deficiency.

In relation to CK and AST, the upper reference limits established were higher than those reported in the literature for both enzymes (Knottenbelt 2006) (table 2), but similar to other reports in working horses (Gul *et al* 2007, Pritchard *et al* 2009, Tadich *et al* 1997). High serum concentrations of these enzymes could reflect a lack of adaptation to work resulting in a low-grade chronic muscular damage (Tadich *et al* 1997). This differs with the results of Vergara and Tadich (2015) for tourism working horses in Chile, where no significant increases of these enzymes were found after work.

The upper reference limit for calcium was higher in the Chilean working horses than in the other three reference intervals. In the horse, the intestine is not a regulatory point for calcium homeostasis and intestinal calcium absorption is always turned on, thus feeding high dietary calcium increases the amount of calcium that enters the blood (NRC 2005). A more detailed study on feeding practices in working horses is required to better understand these changes.

In conclusion, the reference intervals calculated differ from those found in the literature for other equines, mainly those calculated for sport horses. Working horses seem to share some similarities around different countries, which could be the result of adaptation to work and precarious husbandry conditions, especially in what refers to feeding practices that are reflected in their haemato-biochemical values.

ACKNOWLEDGEMENTS

Funding source: FONDECYT Iniciación en la Investigación Nº11121467.

REFERENCES

Araya O, R Urzúa R, Bustamante H. 2004. The effect of injectable barium selenite on the Glutathione peroxidase activity of horses on pastures. *Arch Med Vet* 36, 31-37.

Barnett JL, Hemsworth PH. 1990. The validity of physiological and behavioural measures of animal welfare. *Appl Anim Behav Sci* 25, 177-187.

Brommer H, Sloet van Oldruitenborgh-Oosterbaan MM. 2001. Iron deficiency in stabled dutch warmblood foals. *J Vet Intern Med* 15, 482-485.

Ceballos A, Wittwer F, Contreras P, Quiroz A, Böhmwald H. 1999. Blood activity of glutathione peroxidase and its correlation with blood selenium concentration in grazing dairy cattle. *Pesq Agropec Bras* 34, 2331-2338.

Cozzi G, Ravarotto L, Gottardo F, Stefani AL, Contiero B, *et al*. 2011. Reference values for blood parameters in Holstein dairy cows: effects of parity, stage of lactation, and season of production. *J Dairy Sci* 94, 3895-3901.

Dixon WJ. 1953. Processing data for outliers. *Biometrics* 9, 74-89.

Friedrichs K, Harr KE, Freeman K, Szladovits B, Walton R, *et al*. 2012. ASVCP reference interval guidelines: determination of *de novo* reference intervals in veterinary species and other related topics. *Vet Clin Pathol* 41, 441-453.

Geffré A, Friedrichs K, Harr K, Concordet D, Trumel C, *et al*. 2009. Reference values: a review. *Vet Clin Pathol* 38, 288-298.

Gul ST, Ahmad M, Khan A, Hussain I. 2007. Haemato-biochemical observations in apparently healthy equine species. *Pakistan Vet J* 27, 155-158.

Herdt TH. 2000. Variability characteristics and test selection in herd-level nutritional and metabolic profile testing. *Vet Clin North Am Food Anim Pract* 16, 387-403.

Humphries WR, Philippo M, Young BW, Bremmer I. 1983. The influence of dietary iron and molybdenum on copper metabolism in calves. *Br J Nutr* 49, 77-86.

Knottenbelt D. 2006. Vital signs, normal values. In: Knottenbelt D (ed). *Saunders Equine Formulary*. W.B. Saunders Co., Philadelphia, USA, Pp 7.

López Alonso M, Miranda M, Hernández J, Castillo C, Benedito JL. 1997. Glutathione peroxidase (GSH-Px) in ruminants associated to selenium deficiency pathologies. *Arch Med Vet* 29, 171-180

NRC, National Research Council. 2005. *Mineral Tolerance of animals*. 2nd revised edition. The National Academies Press, Washington DC, USA, Pp 97-108.

Oh S, Ganther HE, Hoekstra WG. 1974. Selenium as a component of glutathione peroxidase isolated from ovine erythrocytes. *Biochemistry* 13, 1825-1829.

Padalino B, Rubino G, Lacinio Dbio R, Petazzi F. 2014. Observations on the hematology of standardbred horses in training and racing in southern Italy. *J Equine Vet Sci* 34, 398-402.

Pritchard JC, Burn CC, Barr ARS, Whay HR. 2009. Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. *Res Vet Sci* 87, 389-395.

Reed SM, Bayly WM, Sellon DC. 2004. *Equine Internal Medicine*. WB Saunders, St Louis, MO, USA.

Rossdale PD, Burguez PN, Cash RSG. 1982. Changes in blood neutrophil/lymphocyte ratio related to adrenocortical function in the horse. *Equine Vet J* 14, 293-298.

Satué K, Hernández A, Muñoz A. 2012. Physiological factors in the interpretation of equine haematological profile. In: Lawrie C (ed). *Hematology, science and practice*. Intech, Rijeka, Croatia.

Solberg H. 1987. Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. *Clin Chim Acta* 179, s12-s32.

Stull CL, Rodiek AV. 2000. Physiological responses of horses to 24 hours of transportation using a commercial van during summer conditions. *J Anim Sci* 78, 1458-1466.

Tadich N, Méndez G, Wittwer F, Meyer K. 1997. Blood biochemical values of load cart draught horses in the city of Valdivia (Chile). *Arch Med Vet* 29, 45-61.

Tadich T, Escobar A, Pearson RA. 2008. Husbandry and welfare aspects of urban draught horses in the south of Chile. *Arch Med Vet* 40, 267-273.

Tadich TA, Elgueta A, Galecio JS, Menarim B. 2011. Welfare assessment of urban draught horses in the south of Chile: preliminary results. *Rev Colom Cienc Pecu* 24, 369-370.

Tadich TA, Stuardo Escobar L. 2014. Strategies for improving the welfare of working equids in the Americas: a Chilean example. *Rev Sci Tech Off Int Epiz* 33, 203-211.

Tsang CW, Lazarus R, Smith W, Mitchell P, Koutts J, *et al*. 1998. Hematological indices in an older population simple: derivation of healthy reference values. *Clin Chem* 44, 96-101.

Vergara F, Tadich TA. 2015. Effect of the work performed by tourism carriage horses on physiological and blood parameters. *J Equine Vet Sci* 35, 213-218.

Wagemann C, Wittwer F, Chihuailaf R, Noro M. 2014. Reference intervals for blood markers of mineral balance for groups of dairy cows in the South of Chile. *Arch Med Vet* 46, 121-125.

Waran N. 2007. *The welfare of horses*. The Netherlands' Kluwer Academic Publisher, Dordrecht, The Netherlands.

Wittwer F. 2012. *Manual de patología clínica veterinaria*. 2da ed. Imprenta América, Valdivia, Chile.