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Hematology, plasma biochemistry, and trace element reference values for free-ranging adult Magellanic Penguins (*Spheniscus magellanicus*)

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Abstract

Blood parameters (hematology and biochemistry) are useful in assessing the physiological, nutritional, and overall health status of both captive and free-ranging wildlife. In this study, we established baseline values for blood parameters (hematology, plasma biochemistry, and trace elements) of free-ranging adult Magellanic Penguins (*Spheniscus magellanicus*) sampled at ten breeding colonies covering their full latitudinal distribution in Patagonia over two decades. Males had higher packed cell volume, heterophil and eosinophil relative counts, total solids, copper, and iron but lower magnesium than females. We also compared our results with those obtained in previous studies on both captive and free-ranging Magellanic Penguins and other *Spheniscus* species. Our study provides a baseline for ecological studies investigating the physiological responses of this species to natural and anthropogenic changes. Furthermore, our findings provide clinically relevant reference values for Magellanic Penguins under care at zoos, aquaria, and rehabilitation centers.

Keywords Argentina · Hematology · Magellanic Penguin · Patagonia · Plasma biochemistry · *Spheniscus magellanicus*

Introduction

Penguins are Southern Hemisphere seabirds, and two-thirds of penguin species are considered as threatened (Vulnerable, Endangered or Near Threatened) by IUCN (BirdLife International 2017). Magellanic Penguins (*Spheniscus magellanicus*) are the most numerous of the four *Spheniscus* species, with an estimated population of 1.3 million breeding pairs distributed along the coast of Argentina, Chile, and the Falklands/Malvinas Islands (Boersma et al. 2015). The species is currently classified as 'Near Threatened,' and its main conservation threats are fisheries bycatch and competition, marine pollution, disease, climate variability and change, and habitat degradation (Trathan et al. 2015; Birdlife International 2017).

Blood parameters (hematology and biochemistry) are useful in assessing the physiological, nutritional, and overall health status of wild species (Campbell 1994; Hochleithner 1994; Campbell and Ellis 2013). Moreover, these parameters are routinely used in the medical care of animals at rehabilitation centers and in zoos worldwide (Altman et al. 1997). The interpretation of blood parameters is reliant upon the availability of baseline values that can be used for comparison to detect deviations from expected or normal values.

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Although some studies have provided hematology and plasma biochemistry values from wild adult Magellanic Penguins (Ghebremeskel et al. 1989; Hawkey et al. 1989; D'Amico et al. 2014), most are based on small sample sizes from single breeding colonies. Moreover, these studies do not include many hematological and biochemical parameters that are routinely used for clinical purposes and physiological studies. Baseline data from permanently captive Magellanic Penguins are also available (Silva-Filho and Ruoppolo 2014; ZIMS 2018), but the extent to which some hematological and plasma biochemistry parameters may be altered due to the stress (or lack thereof), diet, and physiological challenges associated with captivity remains unknown (Dobado-Berrios et al. 1998; Alonso–Alvarez 2000; Moreno-Salas et al. 2014). As a result, when interpreting laboratory results of Magellanic Penguins, veterinarians and rehabilitators have often relied on reference values from other species of *Spheniscus* penguins (Wallace et al. 1995; Travis et al. 2006; Smith et al. 2008; Moreno-Salas et al. 2014; Parsons et al. 2015).

In this study, we established baseline values for blood parameters (hematology, plasma biochemistry, and trace elements) of wild, apparently healthy, adult Magellanic Penguins sampled across numerous colonies along the Argentinean Patagonian coast over several years. We then assessed the variability of these parameters between sexes. Finally, we discussed our results with those obtained in previous studies on captive and free-ranging Magellanic Penguins and other *Spheniscus* species.

Materials and methods

A total of 785 free-ranging Magellanic Penguins (Online Resource 1) were handled at ten colonies on the Argentinean Patagonian coast, from Península Valdés in the North of their range, to the Beagle Channel in Tierra del Fuego Island to the South: Punta Norte/San Lorenzo (42°04′31″S 63°47′19″W), Caleta Externa (42°16′02″S 63°38′05″W), Punta Tombo (44°03′08″S 65°13′20″W), Cabo Dos Bahías (44°53′59″S 65°34′43″W), Isla Vernacci Fondo (45°09′36″S 66°34′35″W), Isla de los Pájaros (47°45′11″S 65°58′04″W), Banco Cormorán (49°16′02″S 67°40′03″W), Monte León (50°21′50″S 68°54′35″W), Cabo Vírgenes (52°19′42″S 68°21′11″W), and Isla Martillo (54°54′26″S 67°23′06″W). Samples were obtained during seven expeditions conducted during the mid-to-late chick rearing (post-guard stage) over a 20-year period: (a) January 8–16, 1994, (b) January 2–24, 1995, (c) December 23, 1995 to January 10, 1996, (d) January 10–20, 1998, (e) January 18–20, 2001, (f) January 24–27, 2012, and (g) January 21–25, 2014. All penguins were adult-plumaged, not molting, appeared to be healthy (normal breathing and behavior, no visible or palpable lesions or abnormalities, and not emaciated), and were either resting in the colony or sitting on nests with chicks.

Penguins were caught using a flexible metal rod (approximately 1 m long) that was curved and padded at one end to form a hook to grasp the bird around its distal tibia or tarsus. Each bird was manually restrained and subjected to a rapid physical examination, measurements with a caliper (bill length, bill depth), weighing with a spring scale, and blood collection. Birds were temporarily marked (to avoid recapture) and released at their capture site. The entire capture, handling and sampling procedure lasted less than 5 min. In all expeditions and at all colonies, the captures were conducted during the morning (07:00–11:00 h) to avoid variations in blood parameters due to circadian rhythms and excessively hot temperatures. Furthermore, captures were not conducted in areas within 200 meters of tourist boardwalks or trails. Each day, the capture effort at a given colony was spread over multiple sites in order to minimize exposing birds to human presence before capture.

Blood ($<1\%$ of body mass) was drawn by venipuncture of the jugular vein, using 10 or 20 mL heparinized syringes and 0.7×22 mm needles. Samples were stored in plain and vacuum tubes (Benton-Dickinson, Rutherford, New Jersey, USA) and kept cool on ice. Blood samples were processed within four hours after collection. Thin blood smears were fixed with absolute methanol, and stained with modified Wright-Giemsa stain in the 1990s (Hematology Three-Step Stain, Accra Lab, Bridgeport, New Jersey, USA) and Wright-Rosenfeld stain in the 2010s (Rosenfeld 1947). Capillary tubes were centrifuged at 1000 G using a portable centrifuge (Mobilespin, Vulcan Technologies, Grandview, Missouri, USA), and packed cell volume (PCV) was visually estimated using a micro-hematocrit ruler. Total plasma solids (TS) were measured using a temperature-compensated refractometer (Schulco, Toledo, Ohio, USA) calibrated on site. Red blood cell (RBC) and white blood cell (WBC) counts were performed using a Neubauer hemocytometer and the Eosinophil Unopette 365851c and 5877 systems, respectively (Becton–Dickinson Vacutainer, Rutherford, New Jersey, USA) (Walberg 2001). Cell counts were conducted twice and results were only considered when the difference between counts was lower than 10%; biologically implausible values (as verified through the mean corpuscular volume value) were excluded. Differential leukocyte counts were performed for 200 cells. A coarse examination of blood smears for parasites was conducted in the 1990s (i.e., blood parasites were searched for concurrently with the leukocyte differential counts). A more thorough blood smear examination procedure and molecular testing for haemosporidian and piroplasmid parasites was conducted in the 2010s, and the results were presented elsewhere (Vanstreels et al. 2017).

The remaining blood was centrifuged at 1000 G for 20 min. Plasma was harvested and frozen in liquid

nitrogen. Samples were treated in a water bath at 56 °C for 2 h in accordance with US Department of Agriculture regulations before importation to the USA, and then were transferred to − 80 ºC freezer until blood chemistry analysis. Plasma chemistries and enzymes were processed using an automated analyzer (580 Auto Analyzer, Ciba Corning Diagnostics Corp., East Walpole, Massachusetts, USA) at a commercial laboratory (Vet Research, Farmingdale, New York, USA). The following parameters were measured: glucose, creatinine, uric acid, cholesterol, total protein, albumin, globulin, aspartate aminotransferase, creatine kinase, calcium, sodium, potassium, chloride, phosphorus, copper, iron, zinc, and magnesium. Trace elements boron (B), barium (Ba), cobalt (Co), manganese (Mn), and molybdenum (Mb) were analyzed by inductively coupled argon plasma emission spectroscopy as described by Stowe et al. (1985) at the Animal Health Diagnostic Lab (Michigan State University, East Lansing, Michigan, USA).

The discriminant function proposed by Bertellotti et al. (2002) was used to determine the sex of sampled birds based on the bill measurements. The following ratios were calculated: albumin to globulin (AG ratio), total protein to total solids (TPTS ratio), and heterophil to lymphocyte (HL ratio). The mean corpuscular volume was calculated as PCV divided by RBC and multiplied by 1000.

Sample sizes varied among different analyses due to field contingencies (clotting of blood samples, time or financial constraints, etc.) or because the results of a few samples were interpreted as laboratory errors (values that were clearly biologically implausible); other than this, however, no attempts were made to exclude outliers or to select data from "normal" individuals. Mean, standard deviation (SD), median, central 50% (first quartile–third quartile), central 95% (2.5nd percentile–97.5th percentile), range (minimum–maximum), median skewness (Sk), and kurtosis (Kt) were used to describe the distribution of the hematology or plasma biochemistry results. Data on the concentrations of some trace elements were heavily left-censored due to intrinsic quantification thresholds of the laboratory methods, and the 50th, 95th, and 99th percentiles (P_{50}, P_{95}, and P_{99}, respectively) and the maximum were used to represent these results.

The Anderson–Darling test was used to test the data for normality, and the following transformations were attempted: Box-Cox, log-normal, exponential, 2-parameter exponential, Weibull, smallest extreme value, largest extreme value, gamma, logistic, log–logistic, and Johnson. Hematological, plasma biochemistry, and trace element concentrations were compared between males and females using two-sample t tests (normal distribution) or Mann–Whitney tests (non–normal distribution). Significance level was 0.05 for all tests.

Results

No blood parasites were seen in the blood smears (see also Vanstreels et al. 2017). The results for body mass, hematology, plasma biochemistry, electrolytes, and trace elements are summarized in Table 1. Table 2 summarizes the results for the trace elements that had a left-censored concentration distribution. Males had a higher body mass, packed cell volume, heterophil and eosinophil relative counts, total solids, cholesterol, copper and iron concentration than females, whereas magnesium concentration was higher in females (Table 3). All other blood parameters analyzed did not differ between sexes (all tests $p > 0.05$).

Discussion

This is the first long-term study to compile baseline hematology and plasma chemistry parameters of adult Magellanic Penguins, including individuals from ten breeding colonies covering their full latitudinal distribution in the Argentinean Patagonia.

Sex differences in hematology and plasma biochemistry

Body mass differed significantly between sexes and mean values matched those previously reported for the species (Gandini et al. 1992; Yorio et al. 2001; Bertellotti et al. 2002; D'Amico et al. 2014). Our finding of higher packed cell volumes in males compared to females have also been recorded in previous studies on Magellanic, African (*Spheniscus demersus*) and Galapagos Penguins (*Spheniscus mendiculus*) (Moreno et al. 2002; Travis et al. 2006; Parsons et al. 2015), suggesting this may be a common pattern for this genus even though it was not observed in Humboldt Penguins (*Spheniscus humboldti*) (Smith et al. 2008). Considering that male Magellanic Penguins tend to be larger than females, and that maximum diving depth and duration are correlated to body size (Walker and Boersma 2003), perhaps the higher packed cell volume of males is related to the higher oxygen demands of longer dives. This might also help explain why in some circumstances male Magellanic Penguins tend to forage deeper (vertical distance), whereas females forage wider (horizontal distance) during years of food shortage (Raya-Rey et al. 2012). Overall, higher packed cell volume in males is not an uncommon pattern in birds (Fair et al. 2007).

Differences found in relative leukocyte counts suggest there may be differential exposure or response to factors such as stress or pathogens between males and females. During

Table 1 Descriptive statistics for body mass, hematology, plasma biochemistry, and trace elements of wild adult Magellanic Penguins (*Spheniscus magellanicus*) sampled during seven expeditions conducting during the mid-to-late chick rearing over a 21-year period (1994–2014) on the Argentinean coast

Parameter	Unit	Mean	SD	Median	Central 50%	Central 95%	Range	Sk	Kt	n
Body mass	kg	3.99	0.59	4.00	3.60–4.45	2.80–5.10	2.00–6.30	− 0.08	0.44	769
Packed cell volume	%	47.64	4.15	48.00	45.00–50.00	39.00–56.00	32.00–60.00	− 0.20	0.08	757
Mean corpuscular volume	fL	261.57	41.53	263.89	238.10–285.00	182.59–341.28	171.00–351.72	0.09	− 0.28	73
Red blood cells	10^{12} L^{-1}	1.88	0.35	1.82	1.64–2.08	1.36–2.71	1.24–2.90	0.78	0.49	74
White blood cells	10^{9} L^{-1}	25.12	12.60	22.70	17.10–28.94	10.64–55.64	7.70–122.20	2.61	12.12	393
Heterophils	%	46.91	12.57	47.00	38.00–54.86	21.85–72.00	13.00–88.00	0.08	− 0.03	715
Lymphocytes	%	39.56	12.23	39.00	31.00–46.00	18.85–70.15	9.00–82.00	0.58	0.63	715
Eosinophils	%	10.78	7.40	10.00	5.00–15.75	0.00–25.89	0.00–41.75	0.53	− 0.07	715
Monocytes	%	2.58	3.42	1.00	0.00–4.00	0.00–12.00	0.00–16.00	1.55	1.81	715
Basophils	%	0.17	0.51	0.00	0.00–0.00	0.00–2.00	0.00–4.00	3.94	18.23	715
HL ratio		1.42	0.91	1.20	0.86–1.71	0.34–3.65	0.02–9.78	2.56	13.08	715
Heterophils	10^{9} L^{-1}	11.31	5.14	10.75	7.64–13.75	4.64–23.35	2.69–43.99	1.89	7.22	393
Lymphocytes	10^{9} L^{-1}	10.96	8.92	8.45	5.78–12.64	2.31–33.48	1.17–72.10	2.81	11.40	393
Eosinophils	10^{9} L^{-1}	1.84	1.74	1.42	0.61–2.70	0.00–6.34	0.00–9.94	1.44	2.40	393
Monocytes	10^{9} L^{-1}	0.98	1.07	0.72	0.17–1.45	0.00–3.58	0.00–7.39	2.11	7.41	393
Basophils	10^{9} L^{-1}	0.03	0.11	0.00	0.00–0.00	0.00–0.41	0.00–0.82	4.06	18.80	393
Total solids	g L^{-1}	62.07	11.80	60.00	54.00–70.00	42.00–88.00	34.00–102.00	0.51	− 0.03	759
Total protein	g L^{-1}	53.63	9.07	52.00	48.00–59.00	40.00–72.23	28.00–120.00	1.32	6.85	392
TPTS ratio		0.90	0.10	0.90	0.84–0.96	0.68–1.10	0.52–1.32	− 0.09	1.71	375
Albumin	g L^{-1}	17.39	3.12	17.00	15.00–19.00	12.00–24.00	8.00–32.00	0.78	2.25	392
Globulin	g L^{-1}	36.24	7.46	35.00	31.00–40.00	24.00–51.90	20.00–88.00	1.31	5.69	392
AG ratio		0.49	0.12	0.50	0.40–0.50	0.30–0.72	0.30–1.50	2.66	16.64	392
Glucose	mmol L^{-1}	10.44	1.52	10.50	9.76–11.29	7.27–13.04	5.39–20.11	0.32	4.40	392
Creatinine	µmol L^{-1}	48.36	12.66	44.21	44.21–53.05	26.53–79.58	17.68–106.10	1.10	1.97	392
Uric acid	µmol L^{-1}	522.16	407.29	392.57	248.33–657.25	107.06–1696.07	77.32–2391.10	1.81	3.41	392
Cholesterol	mmol L^{-1}	5.38	1.40	5.28	4.43–6.14	3.08–8.18	2.59–15.95	1.38	7.71	392
Aspartate transaminase	U L^{-1}	158.00	89.78	149.50	100.00–198.25	41.78–329.90	10.00–974.00	2.84	19.58	392
Creatine kinase	U L^{-1}	16.03	22.38	10.00	4.00–19.00	1.00–88.45	0.00–235.00	4.54	30.12	392
Sodium	mmol L^{-1}	157.64	10.81	158.00	153.75–162.00	140.00–169.00	115.00–316.00	7.55	118.45	392
Chloride	mmol L^{-1}	112.17	9.24	112.00	108.75–116.00	95.55–123.23	79.00–238.00	6.05	88.29	392
Potassium	mmol L^{-1}	2.52	0.99	2.40	1.90–2.93	1.20–4.72	1.00–8.60	2.02	7.12	392
Calcium	mmol L^{-1}	9.73	1.01	9.80	9.20–10.23	8.08–11.42	5.40–19.20	1.92	20.21	392
Phosphorus	mmol L^{-1}	3.38	1.67	3.10	2.40–4.00	1.38–7.19	0.90–16.00	2.89	14.43	392
Magnesium	µg g^{-1}	28.73	9.29	25.90	23.20–30.20	20.00–56.99	15.40–77.50	2.38	6.39	372
Copper	µg g^{-1}	0.77	0.21	0.74	0.65–0.87	0.49–1.16	0.23–3.15	4.14	44.70	372
Iron	µg g^{-1}	1.47	1.21	1.08	0.79–1.71	0.32–4.95	0.12–8.27	2.65	8.70	372
Zinc	µg g^{-1}	2.18	0.56	2.14	1.75–2.51	1.29–3.45	1.01–4.80	0.71	0.95	372

SD Standard deviation, *Sk* Skewness, *Kt* Kurtosis, *n* Sample size

reproductive stage, males engage in violent fights over nests (Renison et al. 2006). The males' higher proportion of heterophils could therefore be related to the stress and immune responses associated with fights and resulting wounds. Concurrently, testosterone is known to induce increased eosinophil counts and decreased lymphocyte counts in other species of birds (Saino et al. 1995; Duffy et al. 2000). Several other factors may also influence the strength of the immune response in the two sexes in birds, including the amount of mating or reproductive effort expended and the timing of egg laying (Moreno et al. 1998, 2002; Klein 2000; Fargallo et al. 2001; Vleck and Vleck 2002; Fair et al. 2007; Palacios et al. 2018). There is evidence that female Magellanic Penguins experience higher mortality than males, which is thought to explain the apparently male-biased sex ratio of this species' adult population (Boersma 2008; Vanstreels et al. 2013).

Table 2 Descriptive statistics for plasma trace element concentrations in wild adult Magellanic Penguins (*Spheniscus magellanicus*) sampled during seven expeditions conducting during the mid-to-late chick rearing over a 20-year period (1994–2014) on the Argentinean coast

Parameter	Unit	P_{50}	P_{95}	P_{99}	Maximum	n
Boron	µg g^{-1}	1.000	1.250	1.500	1.990	372
Barium	µg g^{-1}	0.062	0.100	0.113	0.122	337
Cobalt	µg g^{-1}	0.100	0.125	0.143	0.167	338
Manganese	µg g^{-1}	0.050	0.062	0.074	0.083	338
Molybdenum	µg g^{-1}	0.200	0.250	0.286	0.333	338

P_{50} 50th percentile, P_{95} 95th percentile, P_{99} 99th percentile, *n* Sample size

Table 3 Descriptive statistics for parameters that were significantly different between male and female wild adult Magellanic Penguins (*Spheniscus magellanicus*) sampled during seven expeditions conducting during the mid-to-late chick rearing over a 21-year period (1994–2014) on the Argentinean coast

Parameter	Unit	Statistical test	Sex	Mean	SD	Median	Central 50%	Central 95%	Range	n
Body mass	kg	$W = 66{,}730$	Male	4.22	0.52	4.20	3.90–4.54	3.20–5.20	2.30–6.30	462
		$p < 0.001$	Female	3.63	0.52	3.60	3.30–3.95	2.61–4.55	2.00–5.50	285
Packed cell volume	%	$W = 91{,}385$	Male	48.03	4.18	48.00	45.00–51.00	39.45–56.00	32.00–60.00	459
		$p < 0.001$	Female	46.87	4.07	47.50	44.00–50.00	38.88–55.00	38.00–57.00	276
Heterophils	%	$t_{585} = -2.58$	Male	48.26	12.44	48.00	40.00–56.13	24.00–73.00	14.00–88.00	424
		$p = 0.010$	Female	45.81	12.02	45.50	38.00–52.50	23.40–70.30	13.00–80.00	269
Eosinophils	%	$W = 92{,}184.5$	Male	10.70	7.25	10.00	5.00–15.50	0.00–25.21	0.00–41.75	424
		$p < 0.001$	Female	10.64	7.77	10.00	4.00–15.28	0.00–26.55	0.00–36.00	269
Total solids	g L^{-1}	$W = 96{,}510$	Male	62.81	12.07	62.00	54.00–72.00	40.50–88.00	34.00–99.00	461
		$p = 0.042$	Female	61.28	11.39	60.00	52.00–68.00	44.00–88.00	41.00–102.00	276
Cholesterol	mmol L^{-1}	$t_{331} = -2.01$[a]	Male	5.50	1.22	5.40	4.68–6.37	3.29–8.04	2.85–8.81	196
		$p = 0.046$	Female	5.28	1.60	5.19	4.19–5.96	2.98–8.18	2.59–15.95	172
Magnesium	µg g^{-1}	$t_{348} = 2.31$[a]	Male	27.78	8.32	25.25	22.78–29.33	19.90–53.53	17.30–64.70	192
		$p = 0.022$	Female	29.57	9.82	26.45	23.90–30.98	20.00–55.81	15.40–69.90	166
Copper	µg g^{-1}	$W = 26{,}619.5$	Male	0.81	0.24	0.77	0.68–0.89	0.52–1.16	0.23–3.15	192
		$p = 0.001$	Female	0.74	0.16	0.72	0.64–0.84	0.43–1.07	0.23–1.25	166
Iron	µg g^{-1}	$t_{347} = -2.89$[a]	Male	1.61	1.25	1.13	0.86–1.79	0.52–5.15	0.31–8.27	192
		$p = 0.004$	Female	1.31	1.00	0.97	0.76–1.55	0.42–3.69	0.32–7.50	166

SD Standard deviation, *n* Sample size

[a]Test following Johnson transformation of data

Further investigations on sex differences in physiological and immunological responses to pathogen exposure of Magellanic Penguins would therefore be valuable to understand the ecology of this species.

Comparison with previous studies on Magellanic Penguins

Table 4 compares our results to those of previous publications on the hematology and plasma chemistry of adult Magellanic Penguins. We found higher packed cell volume, mean corpuscular volume, relative heterophil count and calcium and phosphorus concentrations, and lower relative lymphocyte count and creatinine level than those obtained by Hawkey et al. (1989) and Ghebremeskel et al. (1989) during pre and post-molt period. These differences are expected due to physiological variations induced by molting (Hawkey et al. 1989; Mazzaro et al. 2013). Furthermore, the values reported by Hawkey et al. (1989) and Ghebremeskel et al. (1989) were obtained from penguins sampled after a significant population mortality event, potentially introducing bias in their sample. When our hematological results are compared to those obtained by D'Amico et al. (2014), on the other hand, the main discrepancy is that we found a lower relative monocyte count, the significance of which is unclear.

Compared to our results, permanently captive Magellanic Penguins had lower white blood cell counts, higher

Table 4 Comparison of different studies on the hematological parameters ("Mean ± Standard deviation" or "Mean [Reference interval]") of adult Magellanic Penguins (*Spheniscus magellanicus*)

Parameter	Unit	This study	Ghebremeskel et al. (1989) and Hawkey et al. (1989)[a]		D'Amico et al. (2014)	ZIMS (2018)	Silva-Filho and Ruoppolo (2014)	Coraiola et al. (2014)
Context of sampling		Post-guard chick rearing	Pre-molt	Post-molt	Guard chick rearing	Permanent captivity	Permanent captivity	Rehabilitation
Sample preservation		Heparin	Heparin	Heparin	Not applicable	Heparin	Heparin	Heparin
Individuals studied		73–759	4–12	4–7	30	52–273	70	19
Packed cell volume	%	47.6 ± 4.1	42.0 ± 4.0	41.0 ± 3.0		46.3 [29.0–61.0]	40.8 ± 4.7	47.7 ± 0.7
Mean corpuscular volume	fL	261.6 ± 41.5	204.0 ± 38.0	231.0 ± 23.0		239 [123–500]	231.9 ± 36.3	264.1 ± 6.7
Red blood cells	10^{12} L^{-1}	1.90 ± 0.40	2.14 ± 0.49	1.78 ± 0.15		2.42 [1.46–3.45]	1.79 ± 0.31	1.83 ± 0.06
White blood cells	10^{9} L^{-1}	25.1 ± 12.6				15.5 [3.3–38.7]	12.2 ± 5.0	8.7 ± 0.8
Heterophils	%	46.9 ± 12.6	32.0 ± 14.0[b]		38.5 ± 1.8	52.9 [18.0–88.0]	68.7 ± 12.0	54.3 ± 2.0
Lymphocytes	%	39.6 ± 12.2	60.0 ± 17.0[b]		39.9 ± 1.8	41.7 [9.0–77.0]	24.8 ± 11.5	37.3 ± 2.1
Eosinophils	%	10.8 ± 7.4	6.4 ± 4.2[b]		13.6 ± 1.3	2.1 [0.0–10.0]	1.4 ± 2.6	3.4 ± 0.7
Monocytes	%	2.6 ± 3.4	1.2 ± 1.1[b]		7.5 ± 0.6	3.3 [0.0–12.0]	4.1 ± 2.6	2.8 ± 0.6
Basophils	%	0.2 ± 0.5	0.1 ± 0.3[b]		0.3 ± 0.1	1.4 [0.0–6.0]	1.1 ± 1.3	2.2 ± 0.4
HL ratio		1.4 ± 0.9	0.5[b]		1.1 ± 0.1	1.3	2.8	1.6 ± 0.2
Total solids	g L^{-1}	62.1 ± 11.8				62.0 [39–86]		68.1 ± 12.2
Total protein	g L^{-1}	53.6 ± 9.1	53.0 ± 8.4	38.0 ± 4.1		58.0 [41–82]		
TPTS ratio		0.9 ± 0.1				0.9		
Albumin	g L^{-1}	17.4 ± 3.1	19.5 ± 3.3	11.6 ± 1.5		19.0 [0–33]		
Globulin	g L^{-1}	36.2 ± 7.5	30.5 ± 5.2	26.4 ± 2.9		37.0 [0–62]		
AG ratio		0.5 ± 0.1	0.6	0.4		0.7 [0.3–1.4]		
Glucose	mmol L^{-1}	10.4 ± 1.5				12.0 [8.2–15.8]		
Creatinine	µmol L^{-1}	48.4 ± 12.7	75.8 ± 7.2	59.2 ± 9.5		27.0 [2–71]		
Uric acid	µmol L^{-1}	522 ± 407	1399 ± 278	326 ± 133		614 [131–1648]		
Cholesterol	mmol L^{-1}	5.4 ± 1.4				7.7 [4.7–11.3]		
Aspartate transaminase	U L^{-1}	158.0 ± 89.8				196.0 [65–455]		
Creatine kinase	U L^{-1}	16.0 ± 22.4				284.0 [60–868]		
Sodium	mmol L^{-1}	157.6 ± 10.8	144.0 ± 3.7	159.6 ± 4.6		152.0 [137–166]		
Chloride	mmol L^{-1}	112.2 ± 9.2	103.5 ± 2.6	109.2 ± 3.6		111.0 [96–122]		
Potassium	mmol L^{-1}	2.5 ± 1.0	16.8 ± 2.6	4.0 ± 3.0		4.4 [2.2–7.3]		
Calcium	mmol L^{-1}	9.7 ± 1.0	2.3 ± 0.3	2.2 ± 0.1		2.5 [2.2–3.0]		
Phosphorus	mmol L^{-1}	3.4 ± 1.7	2.3 ± 0.4	1.5 ± 0.2		1.1 [0.2–2.5]		
Iron	µg g^{-1}	1.5 ± 1.2	1.4 ± 0.5[c]	0.3 ± 0.1[c]				

[a]Ghebremeskel et al. (1989) and Hawkey et al. (1989) provide hematology and plasma chemistry results, respectively, based on samples from the same individuals. [b]Results from pre- and post-molt individuals were not statistically different and were therefore combined. [c]Values converted from µmol L^{-1} by assuming a plasma specific gravity of 1.0278 (Trudnowski and Rico 1974)

relative heterophil counts, and lower relative eosinophil counts (Silva-Filho and Ruoppolo 2014; ZIMS 2018). Furthermore, in comparison with our findings, permanently captive Magellanic Penguins had higher creatine kinase and uric acid levels, and lower creatinine, calcium, and phosphorus concentrations (ZIMS 2018). The higher

relative heterophil counts may be interpreted as a response to the stress experienced in captivity (Gross and Siegel 1983; Vleck et al. 2000), though stress is also frequently experienced by free-ranging wildlife. The lower total white blood cell and eosinophil counts are not unexpected considering the role played by these cells in the immune response to gastrointestinal helminths (Campbell and Ellis 2013), and the fact that captive penguins are regularly dewormed and subject to preventative treatments, thus reducing their exposure to parasites and pathogens (AZA 2014; Silva-Filho and Ruoppolo 2014). The red blood cell parameters, on the other hand, showed contradictory patterns depending on the study. Silva-Filho and Ruoppolo (2014) reported lower packed cell volume and mean corpuscular volume but similar red blood cell counts as those obtained in this study, whereas the ZIMS database (ZIMS 2018) shows similar packed cell volume but lower mean corpuscular volume and red blood cell counts. These disparities might be related to differences in the exercise routine among captive populations of penguins (e.g., exhibit design, access to pools, husbandry), which would affect their oxygen demands and modulate erythropoiesis (see Fair et al. 2007). Furthermore, comparisons with captive birds (ZIMS 2018) should be interpreted with caution given that difference in diets, food availability, and physical activity can affect plasma biochemistries (Dobado-Berrios et al. 1998; Alonso–Alvarez 2000, and references therein).

In contrast, penguins undergoing rehabilitation studied by Coraiola et al. (2014) had hematological parameters that were remarkably similar to those in this study. This suggests a closer resemblance to wild status, potentially related to a shorter time spent in captivity. The only notable difference was the lower relative eosinophil count observed in rehabilitation animals, which is possibly related to routine deworming treatments.

Iron levels in this study were comparable to those of pre-molt individuals studied by Ghebremeskel et al. (1989), whereas the post-molt individuals in that study had markedly lower iron levels, which suggests that plasma iron is affected by molt. The pre-molt individuals studied by Ghebremeskel et al. (1989) had lower sodium, chloride, calcium, and phosphorus concentrations but higher potassium concentrations than those observed in other studies on Magellanic Penguins, including ours. However, Mazzaro et al. (2013) did not observe significant changes in these parameters in African Penguins (*Spheniscus demersus*) undergoing molt in captivity. This suggests that the electrolyte concentration changes observed by Ghebremeskel et al. (1989) were related to indirect factors associated with the pre-molt period (e.g., dietary changes which would not have occurred in captive individuals) rather than inherent physiological mechanisms associated with molt.

Comparison with other species of Spheniscus penguins

The hematology and plasma biochemistry results of this study are generally comparable to those obtained by similar studies on African, Galapagos, and Humboldt Penguins (Table 5). Discrepancies of note are the moderately higher relative eosinophil counts and the apparently lower glucose and creatine kinase of Magellanic Penguins. Considering the prominent role that eosinophils play in the response to helminth infection (Campbell and Ellis 2013), the higher eosinophil counts of Magellanic Penguins might be related to the fact that this species stands out as having one of the highest known helminth diversities of all penguins (Brandão et al. 2014). Parasitological surveys of adult wild Magellanic Penguins on the Patagonian coast have found that 100% of individuals are infected by nematodes, with a mean intensity of 600 to 1000 helminths per host (Diaz et al. 2010; Garbin et al. 2013; D'Amico et al. 2014).

With regard to glucose and creatine kinase, both parameters can rise as a result of physical stress and exercise (Hochleithner 1994). It is possible that the lower values we obtained reflect venipuncture of the jugular vein, which allows for rapid blood collection resulting in less physical struggle than might occur during restraint for blood collection from the ulnar or metatarsal veins. Alternatively, Magellanic Penguins could have a greater tolerance to capture stress-induced increases in glucose and creatine kinase than the other species, perhaps as a serendipitous consequence of high physiological demands related to this species' generalist foraging habits and long-distance winter migration (see Boersma et al. 2015).

Our results for some plasma electrolytes and trace elements were overall similar to those reported in previous studies for other *Spheniscus* penguins (Table 5), with the following exceptions: (a) plasma iron levels were higher in the Humboldt Penguins studied by Smith et al. (2008) than in this study; and (b) calcium and phosphorus concentrations were markedly higher in this study and in the Humboldt Penguins studied by Smith et al. (2008) than in other studies. Considering that penguins can occasionally ingest seashells to supplement calcium (Boersma et al. 2004), and that fish and squid differ substantially in their iron, calcium, and phosphorus contents (Sidwell et al. 1977), it seems plausible that the differences observed among these studies could be related to dietary differences.

Concluding remarks

The hematology, plasma biochemistry, and trace element results obtained in this study provide a baseline for ecological studies investigating the physiological responses of

Table 5 Comparison of hematological parameters (Mean ± Standard deviation) in adult *Spheniscus* penguins

Parameter	Unit	This study	Wallace et al. (1995)	Smith et al. (2008)	Moreno-Salas et al. (2014)	Travis et al. (2006)	Parsons et al. (2015)
Species		*S. magellanicus*	*S. humboldti*	*S. humboldti*	*S. humboldti*	*S. mendiculus*	*S. demersus*
Context of sampling		Post-guard chick rearing	Pre-breeding	Post-guard chick rearing	Post-guard chick rearing	Asynchronous breeding	Post-guard chick rearing
Sample preservation		Heparin	Heparin	Heparin	Heparin	Heparin	No anticoagulant
Individuals studied		73–759	45–51	78–87	21	53–83	103–107
Packed cell volume	%	47.6 ± 4.1	49.4 ± 5.0	47.2 ± 3.6	41.0 ± 9.1	44.1 ± 6.8	46.0 ± 5.7
Mean corpuscular volume	fL	261.6 ± 41.5					251.0 ± 35.6
Red blood cells	10^{12} L^{-1}	1.9 ± 0.4					1.8 ± 0.3
White blood cells	10^{9} L^{-1}	25.1 ± 12.6	23.3 ± 7.1	12.9 ± 11.5	12.5 ± 4.2		17.7 ± 8.4
Heterophils	%	46.9 ± 12.6	52.5	45.6 ± 10.6	53.6	57.6 ± 16.0	
Lymphocytes	%	39.6 ± 12.2	34.7	47.3 ± 10.0	37.6	31.5 ± 15.5	
Eosinophils	%	10.8 ± 7.4	1.8	3.6 ± 4.0	2.2	8.0 ± 7.5	
Monocytes	%	2.6 ± 3.4	3.3	3.2 ± 2.4	5.4	2.4 ± 2.1	
Basophils	%	0.2 ± 0.5	1.7	0.0	< 0.1	0.4 ± 0.7	
H/L ratio		1.4 ± 0.9	1.5	1.0	1.4	1.8	
Total solids	g L^{-1}	62.1 ± 11.8		56.4 ± 6.7	58.0 ± 10.0		
Total protein	g L^{-1}	53.6 ± 9.1	55.9 ± 7.9	52.2 ± 8.9		56.0 ± 7.7	59.0 ± 9.6
TP/TS ratio		0.9 ± 0.1		0.9			
Albumin	g L^{-1}	17.4 ± 3.1	13.8 ± 4.4	18.7 ± 2.7		12.2 ± 1.5	19.3 ± 4.0
Globulin	g L^{-1}	36.2 ± 7.5	42.1 ± 7.2	33.5 ± 7.1		43.8	39.8 ± 6.3
A/G ratio		0.5 ± 0.1	0.3	0.6		0.3	0.5 ± 0.1
Glucose	mmol L^{-1}	10.4 ± 1.5	13.6 ± 1.4	14.4 ± 2.6		12.1 ± 1.9	11.8 ± 2.2
Creatinine	µmol L^{-1}	48.4 ± 12.7	57.2 ± 14.4	53.1 ± 11.5			24.1 ± 11.9
Uric acid	µmol L^{-1}	522.2 ± 407.3	377.0 ± 184.0	808.9 ± 612.6	440.0 ± 210.0	1100.0 ± 400.0	394.0 ± 221.0
Cholesterol	mmol L^{-1}	5.4 ± 1.4	5.5 ± 1.4	5.0 ± 0.9			5.4 ± 1.4
Aspartate transaminase	U L^{-1}	158.0 ± 89.8	206.8 ± 88.2	208.1 ± 118.8	145.0 ± 76.0	282.5 ± 89.9	218.0 ± 90.0
Creatine kinase	U L^{-1}	16.0 ± 22.4	222.4 ± 148.0	361.5 ± 172.9	59.0 ± 79.0	174.4 ± 144.5	419.0 ± 272.0
Sodium	mmol L^{-1}	157.6 ± 10.8	154.0 ± 3.5	149.6 ± 13.1		156.3 ± 6.1	154.0 ± 6.0
Chloride	mmol L^{-1}	112.2 ± 9.2		108.0 ± 10.4		118.5 ± 4.6	121.0 ± 6.1
Potassium	mmol L^{-1}	2.5 ± 1.0	3.5 ± 0.7	2.8 ± 0.7		3.9 ± 1.3	5.1 ± 2.5
Calcium	mmol L^{-1}	9.7 ± 1.0	3.5 ± 1.3	10.0 ± 1.4		2.5 ± 0.2	2.8 ± 0.8
Phosphorus	mmol L^{-1}	3.4 ± 1.7	1.4 ± 0.7	3.7 ± 1.3		1.6 ± 0.8	1.5 ± 0.6
Magnesium	µg g^{-1}	28.7 ± 9.3		26.9 ± 4.6[a]			
Copper	µg g^{-1}	0.8 ± 0.2		0.6 ± 0.1[a]			
Iron	µg g^{-1}	1.5 ± 1.2		2.5 ± 2.3[a]			
Zinc	µg g^{-1}	2.2 ± 0.6		2.3 ± 0.56[a]			

[a]Values converted from µg mL^{-1} by assuming a plasma specific gravity of 1.0278 (Trudnowski and Rico 1974)

populations of this species to natural and anthropogenic changes. Furthermore, our findings can be used as reference values for the clinicopathological assessment of Magellanic Penguins under care at zoos, aquaria, and rehabilitation centers.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed, and research was conducted under permits approved by Dirección de Conservación y Áreas Protegidas and Dirección de Fauna y Flora Silvestre of Chubut Province, Consejo Agrario of Santa Cruz Province, and Dirección de Fauna y Flora de Tierra del Fuego.

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