BLOOD PARAMETERS OF EMPEROR PENGUIN *APTENODYTES FORSTERI* CHICKS AT THEIR NORTHERNMOST ANTARCTIC COLONY

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Received 22 March 2016, accepted 13 May 2016

Among penguin species, the Emperor Penguin *Aptenodytes forsteri* is one of the most vulnerable to the effects of global warming (Jenouvrier *et al*. 2014), since, along with the Adelie Penguin *Pygoscelis adelie*, it occurs only in Antarctica, the region most affected by global climate change worldwide (Convey *et al*. 2009). Breeding colonies are circumpolar, distributed between 64° and 77°S, and the population is estimated at about 238 000 pairs in total (Fretwell *et al.* 2012, Libertelli & Coria 2014). Emperor Penguins breed on fast ice, which, in order to allow penguins to complete a successful breading season, should remain frozen from April to December (Williams 1995). Given that the thickness and the extent of sea ice are expected to change, it is predicted that the population of Emperor Penguins will decrease in the northern portion of their range (Ainley *et al*. 2010). Population dynamics are critical for the species' conservation, especially because of the potential population decline caused by global warming. Consequently, Emperor Penguins are now considered "Near Threatened" (IUCN 2015).

In order to improve our understanding of Emperor Penguin populations, it is important to monitor the physical condition of individuals (SCAR 2010). Blood parameters, including blood cell counts and biochemical profiles, are promising indicators of population health; their use in the context of conservation biology has grown considerably in the last decade (Deem *et al*. 2001, Cooke *et al*. 2013). In this article, we report blood parameters related to the health and immune status of Emperor Penguin chicks at their northernmost colony: Cerro Nevado/Snow Hill Island, Antarctica.

During the winter, i.e. August–September 2014, we established camp approximately 300 m from the Emperor Penguin's breeding site at Cerro Nevado/Snow Hill Island (64.52°S, 57.44°W). Although weather conditions were harsh, we were able to capture and sample 21 chicks, 6–8 weeks of age, based on their body weight (mean 2.2 ±SD 9.1 kg) (Williams 1995); chicks were in recently established crèches. We hand-captured, sampled and released chicks that were in groups of at least three, distributed randomly in the crèche. Each bird was weighed with a spring scale (Pesola) with 20 g accuracy. Blood samples (~0.3 mL) were extracted from the foot vein using 27 gauge needles, and blood smears were prepared with a drop of fresh blood on individual slides. The smears were air-dried, fixed with ethanol for 3 min and stained with Tinción 15 (Biopur, Argentina).

We measured blood carbohydrates and lipids to assess current nutritional status (Brown 1996). Due to the small blood volumes obtained, blood glucose and lipid values (cholesterol and triglycerides) were obtained *in situ* using Accutrend Plus (Roche) with the specific Accutrend test strip. To validate the method, we determined the values of glucose (mg dL^{-1}), cholesterol (mg dL^{-1}) and triglycerides (mg dL^{-1}) obtained with the Accutrend test strips by comparing with those from analyses using a spectrophotometer (Metrolab 1600 Plus, Argentina), previously carried out for Magellanic Penguin *Spheniscus magellanicus* chicks. Results showed non-significant differences (t test = -0.24, -1.31 and 1.15, respectively, all $P > 0.05$) (V. D'Amico, unpubl. data). Blood smears were analyzed with a light microscope, scanning monolayer fields with similar densities of erythrocytes to estimate the total leukocyte count and the proportion of the five leukocyte types (heterophils, eosinophils, basophils, lymphocytes and monocytes). These analyses were done to provide information on the cellular components of the immune system (Campbell 1995). The total leukocyte count per 10 000 erythrocytes was estimated by counting the number of erythrocytes in one microscopic visual field and multiplying it by the number of microscopic visual fields scanned, until 100 leukocytes were counted (Lobato *et al*. 2005). The proportion of each leukocyte type was obtained from a sample of 100 leukocytes viewed under 1 000× magnification (oil immersion) (Campbell 1995). Total counts for each leukocyte type were obtained by multiplying the total leukocyte count by the respective percentage. The heterophil/lymphocyte ratio (H/L), considered a reliable measure of stress in birds (Davis *et al*. 2008), was calculated based on the corresponding leukocyte count.

Most of the values found for Emperor Penguin chicks (Table 1) were within the blood parameter range reported for Adelie Penguin chicks of about the same age sampled at Stranger Point, South Shetland Islands (D'Amico *et al*. 2016). Although Emperor and Adélie penguins breed in different seasons (austral winter and summer, for Emperor and Adélie penguins, respectively), we made this comparison because both populations are at the northern extent of their respective ranges, and, therefore, are subject to the reduction of the sea ice caused by global warming (Carlini *et al*. 2009; Lynch *et al*. 2012). However, no such reduction has yet been observed for Emperor Penguins in the study region.

As described above, blood glucose and cholesterol levels were within the range reported for Adélie chicks. Also, all triglycerides values were lower than 75 mg dL^{-1}, which is considerably lower when compared to those reported for Adelie as well as Gentoo Penguin *Pygoscelis papua* chicks sampled in northern Antarctic sites, i.e. 126.6 mg dL^{-1} and 131.5 mg dL^{-1}, respectively (D'Amico *et al*. 2016). This could be related to the phenology of Emperor chicks. From about seven weeks after hatching until independence, chicks are in nurseries, also referred to as crèches (Williams 1995), which require the metabolism of triglycerides and proteins, involved in thermogenesis (Tomas & George 1975, Masoro 1995).

Concerning leukocyte counts, lymphocytes and heterophils were the most abundant cells, yielding an H/L ratio <1.0, which could be indicative of low physiological stress (Davis *et al*. 2008). Total counts and percentages of basophils, eosinophils and monocytes remained low, which is normal in healthy birds (Campbell 1995) (Table 1). It is difficult to make statements about the values shown by Emperor chicks for two main reasons: 1) The composition of leukocytes in chicks may reflect the ontogeny of the immune system rather than stress (Masello *et al*. 2009, Dehnhard *et al*. 2011). Thus, leukocytes are more frequently studied in adults than in chicks (Jakubas *et al*. 2015); 2) This is the first time leukocyte data were collected for this species. Leukocytes constitute not only the primary line of defense against pathogens (Roitt *et al*. 2001), but they are also influenced by physiological stress in animal populations (Davis *et al*. 2008), depending on a number of factors, including the birds' habitat. Therefore, different pathogens and environmental factors found at different locations can lead to understanding specific patterns of leukocyte production and activation (Campbell 1995).

We are aware that one shortcoming of this work is the modest sample size. However, due to the extreme weather conditions at the site during the winter season, it was impossible to gather more samples. Such conditions are likely to be experienced in any study of this species. As previously indicated, the values presented here constitute a first report for Emperor Penguin chicks at their northernmost colony. The blood parameters collected can become the basis for systematic monitoring of this population and for comparison with other Emperor Penguin populations.

ACKNOWLEDGEMENTS

We are grateful for the invaluable assistance provided by the staff of Base Marambio (Argentina), especially by the Instituto Antártico Argentino, for the use of their logistic facilities. We thank Jorge Lusky and Daniel Pedreira for technical assistance in the camp. We thank M. G. Palacios for her comments and corrections of the manuscript; M. V. Gonzalez Eusevi, from Inglés Científico (www.inglescientifico.com), who corrected the English version of the manuscript. Permission to work in the study area was given by the Instituto Antártico Argentino (Dirección Nacional del Antártico). We appreciate the comments of V. Ruoppolo and D. Ainley, which helped to improve the paper.

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TABLE 1
Blood parameters obtained for Emperor Penguin chicks at Cerro Nevado/Snow Hill, Antarctica

Parameter	Mean ± SD	Range
Mass (g)	2 219 ± 421.2	1 500–3 000
Glucose (mg dL^{-1})	162.9 ± 43.1	74–240
Cholesterol (mg dL^{-1})	246.1 ± 57.7	152–300
Triglycerides (mg dL^{-1})	64.8 ± 3.4	60–70
Total counts		
Leukocytes	68.2 ± 33.7	37–158
Basophils	11.4 ± 23.8	0–68
Eosinophils	334.1 ± 344.5	54–1 431
Heterophils	2 473.5 ± 1 543.7	958–7 402
Lymphocytes	3 729.4 ± 1 856	1 783–8 456
Monocytes	262.9 ± 175.1	0–712
Percentages		
Basophils	0.2 ± 0.5	0–2
Eosinophils	4.9 ± 3.3	1–15
Heterophils	36.1 ± 9.3	17–51
Lymphocytes	54.9 ± 8.1	45–74
Monocytes	4.1 ± 2.5	0–10
Heterophil/lymphocyte ratio	0.7 ± 0.3	0–1

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