

Clinical, hematological, total plasma protein and fibrinogen parameters of magellanic penguins (*Spheniscus magellanicus*) pre- and post-rehabilitation¹

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ABSTRACT.- Coraiola A.M., Kolesnikovas C.K.M., Krul R., Mangini P.R. & Locatelli-Dittrich R. 2014. **Clinical, hematological, total plasma protein and fibrinogen parameters of magellanic penguins (*Spheniscus magellanicus*) pre- and post-rehabilitation.** *Pesquisa Veterinária Brasileira* 34(Supl.1):43-48. Laboratório de Patologia Clínica Veterinária, Departamento de Medicina Veterinária, Universidade Federal do Paraná, Rua dos Funcionários 1540, Curitiba, PR 80035-050, Brazil. E-mail: angela.coraiola@gmail.com

Magellanic penguins (*Spheniscus magellanicus*) usually arrive in poor body conditions at Brazilian beaches during the winter. Hematology provides valuable information about clinical and immunity status of the animals. The aims of this study were to determine the hematologic, total plasma protein (TPP) and fibrinogen profiles of young and adult magellanic penguins in PROAMAR and CETAS-SC, relating these results with the state of health and survival possibility of the animals. In Paraná 14 animals were evaluated in pre and eight in post-rehabilitation and 29 animals were evaluated in Santa Catarina after rehabilitation. Before rehabilitation, all animals showed weakness. In hematological exams of these animals, we found that anemia was present in 83% of the penguins that died and 50% of those which survived. The heterophils/lymphocytes (H/L) ratio was 3.87 ± 0.57 in animals that died, significantly higher than the average of 2.20 ± 0.30 for animals that survived. These two parameters are useful to assess the survival possibility of animals to rehabilitation. The body condition score was positively correlated with hematocrit and TPP, and negatively correlated with H/L ratio. After rehabilitation, the values were similar to other animals of the family Spheniscidae, with averages ranging from 1.64 to 1.90×10^6 erythrocytes/ μL ; 43.38 to 48.80% of hematocrit; 12.45 to 13.52g/dL of hemoglobin; 8,684 to 14,011 leukocytes/ μL ; 4,767 to 8,041 heterophils/ μL ; 3,215 to 4,951 lymphocytes/ μL ; 95 to 655 eosinophils/ μL ; 179.8 to 277.9 monocytes/ μL ; 141 to 184.9 basophils/ μL ; and 1.26 to 1.74 of H/L ratio. These parameters can therefore be used as reference values and release parameters for young and adult Magellanic penguins in captivity on the rehabilitation centers.

INDEX TERMS: *Spheniscus magellanicus*, penguin, seabirds, hemograma, anemia, H/L ratio.

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RESUMO.- [Parâmetros clínicos, hematológicos, de proteína plasmática total e fibrinogênio em pinguins-de-magalhães (*Spheniscus magellanicus*) pré e pós-reabilitação.] Pinguins-de-magalhães (*Spheniscus magellanicus*) geralmente chegam debilitados às praias do Brasil durante o inverno. A hematologia fornece informações valiosas sobre estado clínico e imunidade dos animais. Os objetivos do presente estudo foram determinar o perfil hematológico, de proteína plasmática total (PPT) e fibrinogênio de pinguins-de-magalhães jovens e adultos no PROAMAR e no CETAS-SC, relacionando esses resultados com o estado de saúde e possibilidade de sobrevivência dos animais. Foram avalia-

dos no Paraná 14 animais na pré e oito na pós-reabilitação e 29 animais em Santa Catarina após a reabilitação. Antes da reabilitação, todos os animais apresentavam debilidade. Nos exames hematológicos desses animais, observou-se que a anemia estava presente em 83% dos pinguins que foram a óbito e em 50% dos que sobreviveram. A relação heterófilos/linfócitos (H/L) foi de $3,87 \pm 0,57$ nos animais que foram a óbito, significativamente maior que a média de $2,20 \pm 0,30$ dos animais que sobreviveram. Esses dois parâmetros são úteis na avaliação da possibilidade de sobrevivência dos animais à reabilitação. O escore corporal apresentou correlação positiva com hematócrito e PPT, e correlação negativa com relação H/L. Após a reabilitação os valores foram semelhantes aos de outros animais da família Spheniscidae, com médias variando de 1,64 a $1,90 \times 10^6$ eritrócitos/ μL ; 43,38 a 48,80% de hematócrito; 12,45 a 13,52g/dL de hemoglobina; 8.684 a 14.011 leucócitos/ μL ; 4.767 a 8.041 heterófilos/ μL ; 3.215 a 4.951 linfócitos/ μL ; 95 a 655 eosinófilos/ μL ; 179,8 a 277,9 monócitos/ μL ; 141 a 184,9 basófilos/ μL ; e 1,26 a 1,74 de relação H/L. Esses parâmetros, portanto, podem ser utilizados como valores de referência e parâmetros para soltura para pinguins-de-magalhães jovens e adultos em cativeiro nos centros de reabilitação.

TERMOS DE INDEXAÇÃO: *Spheniscus magellanicus*, pinguim, aves marinhas, hemograma, anemia, relação H/L.

INTRODUCTION

Magellanic penguins (*Spheniscus magellanicus* Forster, 1781) breed during the summer on the Patagonian mainland and the Falkland Islands, and in winter they migrate north, reaching southern Brazil and Uruguay (Gandini et al. 1998, Pütz et al. 2007). The animals that arrive on beaches in Brazil are generally weak due to the long journey, difficulty in finding food, oil contamination and diseases (Silva-Filho & Ruoppolo 2006, Rodrigues et al. 2010).

Hematology is useful for assessing and diagnosing diseases in birds (Coles 1986, Campbell 2007). Nevertheless, few studies have been conducted to determine hematological parameters of penguins in Central and South America. There are reference haematological values for Rock-hopper Penguins (*Eudyptes crestatus*), gentoo penguins (*Pygoscelis papua*) and the magellanic penguins on the Patagonian coast in the wild (Hawkey et al. 1989), Humboldt penguins (*Spheniscus humboldti*) in captivity and free-living in the coastal regions of Chile (Wallace et al. 1995, Villouta et al. 1997), free-living blue penguins (*Eudyptula minor*) (Sergeant et al., 2004) and free-living galapagos penguins (*Spheniscus mendiculus*) (Travis et al. 2006). No study assessed the animals during rehabilitation or established hematological parameters to determine release.

In a study conducted by Rodrigues et al. (2010) with magellanic penguins arriving along the coast of Rio Grande do Sul, contaminated by oil or not, the authors found that hematocrit and body weight of young animals are helpful indicators for the assessment of the health status and survival chances during rehabilitation.

Currently, there are standardized protocols for treatment and rehabilitation of magellanic penguins in Brazil;

however, the clinical and hematological values used for determining release criteria of these animals are from research on rehabilitation of other species (Ruoppolo et al. 2004).

This study was aimed at determining hematologic, total plasma protein (TPP) and fibrinogen profiles of young and adult magellanic penguins, pre and post rehabilitation, and relating the parameters to animals' health and survival.

MATERIALS AND METHODS

The study was conducted at Pontal do Parana, in the state of Parana, with 14 magellanic penguins received in PROAMAR (Project for Studies and Recovery of Birds, Mammals and Reptiles), from the Center of Sea Research, Federal University of Paraná (UFPR) in the years 2010 and 2011; and at Florianopolis, in the State of Santa Catarina, with 29 magellanic penguins received in CETAS (Screening Center of Wild Animals), from the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) in 2011. The project was approved by the Ethics Committee on Animal Use - CEUA/UFPR under number 046/2010 and by (ICMBio) SISBIO # 25013-2.

The rehabilitation period in both sites was from one to two months. In PROAMAR the animals were received in the period from June to October, and blood collections were carried out in the beginning of the rehabilitation process and in the end of the process from August to October. In CETAS-SC, the penguins were received from June to September, and the blood collections were carried out from September to November, in the end of the rehabilitation process.

Animal handling during rehabilitation was different in the two locations. In PROAMAR, in the first days the animals were fed approximately 100g of fish per day, and this diet was increased gradually to about 500g of fish per day. The Penguins were fed discarded trash fish from shrimp fisheries or frozen sardines. Nutritional supplements were provided with thiamine (vitamin B1) (75mg five times per week), and vitamins A (0.25ml twice a week) and E (0.5ml once a week). The most debilitated animals were fed liquid diets with the same supplementation.

In CETAS-SC, each animal was fed 500g of frozen sardines per day, and they were also provided nutritional supplement with thiamine (75mg doses three times per week). When debilitated, the animals were fed up to 10 sardines per day, plus oral rehydration. When they showed to be extremely weak, they received Pot-nay Gold B12® (1ml) and two doses of Iron Injectable Ferrodex®, with one week interval.

Deworming was performed at both sites after stabilizing the animals. Drontal Plus® tablets were administered twice (1/4 of 660mg tablet) at an interval of 15 days.

Sanitary handling of the animals was performed similarly in the two sites, with daily cleaning of the rooms and swimming pool with water and sodium hypochlorite.

The release criteria after rehabilitation were different. In Proamar, the general condition of the animals was assessed, including body weight measurements and inspection of the feathers (feather loss or damage), posture and good performance in the pool. In CETAS-SC, a complete clinical examination (with emphasis on auscultation) was performed using IFAW (International Found for Animal Welfare) criteria, which consider the general condition of the animals, waterproofing properties of feathers, hematocrit not less than 38%, retention of leukocytes in capillaries maximum of 2% and total protein level greater than or equal to 3g/dl (Silva-Filho & Ruoppolo 2006).

In Parana, the animals were monitored during rehabilitation period with physical examinations, body weight measurements and blood sample collections in the beginning (up to two days af-

ter animal's arrival) and in the end of the rehabilitation period. The animals received in Santa Catarina were assessed only in the end of the rehabilitation period using physical examinations, body weight measurements and blood sample collections. The monitoring protocols were different due to the recovery criteria used by the rehabilitation centers.

Physical examination was performed in physical restrained animals, assessing buccal and eye mucosa, inspecting their bodies for injuries or oil stains and palpating the pectoral muscles to determine the body condition allocating a body score, ranging from 1 (extremely thin, when the keel is very prominent and observed without palpation), 2 (thin, when the keel is not very prominent but can be felt) or 3 (normal, when the keel is not prominent or easily felt).

Blood samples were collected after an 8 hour fast (PROAMAR) and 24 hour fast (CETAS-SC) by puncturing the median metatarsal vein. The fasting protocols were different due to different feeding methods used in the sites. Blood samples of approximately 3ml were collected from each animal into tubes containing the anticoagulant heparin. Blood smears were made immediately after sample collection on samples without anticoagulant.

The samples were transported under refrigeration to the Laboratory of Veterinary Clinical Pathology, UFPR, for analysis.

The hemogram included the erythrogram (erythrocytes count, hematocrit and hemoglobin concentration and RBC indices), the leucocyte count (Total leukocyte and differential leukocyte count) and thrombocytes count. Furthermore, the plasma concentrations of TPP were determined and the plasma fibrinogen levels were estimated.

Erythrocytes and leukocytes counts were performed using a hemocytometer (Improved Neubauer chamber®) with blood samples diluted 1:20 in cresyl blue. The thrombocyte count estimate was performed by slide estimation method, determining the average number of thrombocytes per field. The hematocrit (Hct) was measured immediately using micro-haematocrit method and blood centrifugation in capillary tubes at 9000 rpm for 5 minutes (Coles 1986). After completion of the hematocrit, plasma was used to determine TPP concentrations and estimate the concentration of Fibrinogen in plasma by refractometry (Millar et al. 1971). Hemoglobin concentration was measured using cyanomethaemoglobin method with centrifugation before reading the absorbance by spectrophotometer (Coles 1986).

The MCV (mean corpuscular volume) and MCHC (mean corpuscular hemoglobin concentration) were measured according to Wintrobe (1933).

Blood extensions were stained with Wright stain and the slides were examined with an optical microscope. The erythrocytes were examined for their color, size, shape, immaturity and for the presence of inclusion bodies or parasites. A hundred erythrocytes per slide were examined for determining erythrocytic nuclear abnormalities (ENA) (micronuclei and budding) to calculate the percentage of ENA/100 erythrocytes. Differential leukocyte counts were performed on 100 leukocytes (heterophils, basophils, eosinophils, lymphocytes and monocytes) and they were assessed for the presence of toxic changes, immature or degenerated cells and the presence of inclusion bodies or parasites. Obtained cell counts were used for calculation of the relative proportion of heterophils to lymphocytes. (H/L ratio).

The parameters were analyzed statistically with the software BioEstat 5.0 using the Student's t-test and linear regression analysis.

RESULTS

Fourteen penguins were monitored, 13 young and one adult, at PROAMAR. Physical examination before rehabi-

litation revealed that all animals had green diarrhea. Six animals that were severely debilitated died during the first week. The remaining eight animals were slightly to moderately debilitated and recovered within two months. Two penguins had skin lesions suggestive of trauma and four showed signs of oil contamination.

Twenty-nine magellanic penguins were monitored at CETAS in the State of Santa Catarina, in 2011, 19 of them were adults and 10 were young and all of them were clinically healthy. The rehabilitation period of these animals was from one to two months.

The results of haematological examinations, TPP, Fibrinogen, body condition score and body weight of the animals, pre and post-rehabilitation, in PROAMAR and CETAS-SC are shown in Table 1. The blood smear-estimated thrombocyte count was not performed in animals from CETAS-SC, since all blood smears showed platelets aggregation.

In pre-rehabilitation, comparing the parameters of the animals that survived and those who came to death, there was a significant difference by Student's t test ($p < 0.05$) in the mean relative values of heterophils and lymphocytes and H/L ratio (Table 2).

Comparing pre and post-rehabilitation data only from the animals that survived in Parana, there were significant differences ($p < 0.05$) between means of body weight, absolute and relative values of eosinophils and TPP (Table 3).

DISCUSSION

No significant difference ($p > 0.05$) was identified in animal's body weight pre-rehabilitation between animals who survived and those who died, which differs from the study conducted by Rodrigues et al. (2010), who found that the magellanic penguins received at the rehabilitation center in Rio Grande do Sul had significantly lower body weight on arrival, and that the animals that died during rehabilitation had lower body weight on arrival than those who survived.

After rehabilitation, the body weight of the animals in Parana was significantly lower than those in Santa Catarina. This difference may have been due to different feeding methods used in the sites. The animals in Parana were fed discarded trash fish from shrimp fisheries. On the other hand, the animals in Santa Catarina were fed only sardines.

The main fish species discarded at Pontal do Parana are: *Cathorops spixii*, *Selene setapinnis*, *Cynoscion* spp., *Larimus breviceps*, *Paralichthys brasiliensis* and *Stellifer* spp. (Cattani et al. 2011). The sardines have higher levels of lipids, proteins and calories than *Cynoscion* spp. and *P. brasiliensis* (Andrade et al. 2009, Martins & Oetterer 2010, Minozzo 2010). This fact may have been responsible for the higher body weight observed in animals after rehabilitation in Santa Catarina.

In the study conducted by Rodrigues et al. (2010), the magellanic penguins that survived the rehabilitation in Rio Grande do Sul showed body weight similar to the animals after rehabilitation in CETAS-SC. Even so, the animals at PROAMAR gained weight during the recovery period, because the body weight was significantly higher ($p < 0.05$) after rehabilitation.

Table 1. Means and standard deviations of haematological parameters, total plasma protein, fibrinogen and body weight of young and adult magellanic penguins (*Spheniscus magellanicus*) received at PROAMAR and CETAS-SC, pre- and post-rehabilitation

Parameters*	Parana		Santa Catarina	
	Young and adult pre-rehabilitation(n=14)	Young and adult post-rehabilitation(n=8)	Young post-rehabilitation(n=10)	Adult post-rehabilitation(n=19)
Body weight (kg)	2,26±0,1 ^a	2,81±0,15 ^b	3,39±0,1 ^c	3,5±0,08 ^c
Erythrocytes (x10 ⁶ /μL)	1,53±0,11 ^a	1,64±0,07 ^{ab}	1,90±0,06 ^b	1,83±0,06 ^b
Hematocrit (%)	37,5±2,15 ^a	43,38±1,15 ^{ab}	48,8±1,04 ^b	47,74±0,71 ^b
Hemoglobin (g/dL)	10,99±0,93 ^a	12,45±0,52 ^{ab}	12,84±0,42 ^{ab}	13,52±0,24 ^b
MCV ¹ (fL)	252,4±14,45 ^a	266,5±8,95 ^a	257,9±6,9 ^a	264,1±6,69 ^a
MCHC ² (%)	29,09±1,54 ^a	28,72±0,97 ^a	26,3±0,57 ^a	28,32±0,24 ^a
Total leukocyte (/μL)	16.122±2.368 ^a	14.011±1.100 ^{ab}	10.100±948,1 ^{ab}	8.684±868,7 ^b
Heterophil (%)	69,21±2,84 ^a	56,75±3,3 ^b	50,8±3,29 ^b	54,26±1,96 ^b
Heterophils (/μL)	11.407±1.902 ^a	8.041±959 ^{ab}	5.115±573,2 ^b	4.767±516,1 ^b
Lymphocytes (%)	27,14±2,31 ^a	36,25±3,51 ^{ab}	44,9±3,52 ^b	37,26±2,14 ^b
Lymphocytes (/μL)	4.204±637,7 ^a	4.951±496,2 ^a	4.568±531,3 ^a	3.215±365,2 ^a
Eosinophils (%)	1,64±0,62 ^{ab}	4,5±1,1 ^a	1,0±0,49 ^b	3,42±0,69 ^{ab}
Eosinophils (/μL)	243,5±116 ^a	655±175,7 ^b	95±48,65 ^a	265,3±51,84 ^a
Monocytes (%)	1,5±0,51 ^a	1,12±0,48 ^a	1,8±0,84 ^a	2,84±0,57 ^a
Monocytes (/μL)	196,2±88,28 ^a	179,8±87,33 ^a	181±90,94 ^a	277,9±78,06 ^a
Basophils (%)	0,5±0,23 ^a	1,37±0,5 ^{ab}	1,5±0,4 ^{ab}	2,21±0,44 ^b
Basophils (/μL)	70,86±46,81 ^a	184,9±59,6 ^a	141±38,28 ^a	159,5±26,53 ^a
Thrombocytes/field	3,92±0,41 ^a	4,75±0,59 ^a	-	-
Policromatophils/field	3,46±0,59 ^a	3,59±0,6 ^a	1,8±0,33 ^b	1,45±0,16 ^b
H/L ³	2,91±0,37 ^a	1,74±0,26 ^b	1,26±0,17 ^b	1,62±0,16 ^b
ENA/100 ⁴	0,93±0,3 ^a	0,5±0,19 ^a	0,7±0,3 ^a	0,89±0,28 ^a
TPP ⁵ (g/dL)	4,99±0,29 ^a	6,15±0,28 ^b	6,96±0,21 ^b	6,81±0,12 ^b
Fibrinogen (g/dL)	0,32±0,04 ^a	0,22±0,04 ^{ab}	0,28±0,07 ^{ab}	0,14±0,04 ^b

* Values are expressed as mean ± standard deviation. ^{abc} Different letters in each row indicate statistically significant differences (p<0,05). ¹ MCV = Mean corpuscular volume, ² MCHC = Mean corpuscular hemoglobin concentration, ³ H/L = heterophil/lymphocyte ratio, ⁴ ENA/100 = erythrocytic nuclear abnormalities in 100 erythrocytes, ⁵ TPP = Total plasma protein.

In the erythrogram of penguins assessed after rehabilitation, there was significant variation (p<0.05) in the parameters according to age (young or adult) and site (or Parana Santa Catarina). The samples were collected at the two sites almost at the same time, so probably there was no variation in parameters influenced by the season. However, the feeding methods were different in the sites, which may have been the cause of the differences observed.

The values of erythrocytes count, hemoglobin count and hematocrit of these animals were similar to those obtained from other Family Spheniscidae penguins, captive and free living (Hawkey et al. 1989, Villouta et al. 1997, Sergent et al. 2004), including magellanic penguins (Hawkey et al. 1989).

Despite not having anemia, the penguins showed higher MCV values and the MCHC values were lower than those found in the family Spheniscidae's literature (Hawkey et al. 1989, Villouta et al. 1997, Sergent et al. 2004), including free-living magellanic penguins, which showed MCV values of 215±34 fL and MCHC values of 33,1±2,8% (Hawkey et al. 1989).

The TPP of the animals in Parana was similar to healthy Humboldt penguins in captivity (Villouta et al. 1997) and healthy free-living blue penguins (Sergent et al. 2004). The animals in Santa Catarina showed TPP values significantly higher (p<0.05), possibly due to the sardine diet.

The levels of erythrocytes count, hemoglobin count and hematocrit in the penguins before rehabilitation in Parana indicate the presence of anemia, as they were significantly lower (p<0.05) than in the penguins assessed after rehabilitation in Santa Catarina.

Table 2. Means and standard deviations of haematological parameters, total plasma protein, fibrinogen and body weight pre-rehabilitation of magellanic penguins (*Spheniscus magellanicus*) received at PROAMAR

Parameters*	Death (n=6)	Survival (n=8)
Body weight (kg)	2,13±0,14 ^a	2,35±0,13 ^a
Erythrocytes (x10 ⁶ /μL)	1,52±0,2 ^a	1,54±0,13 ^a
Hematocrit (%)	33,5±3,27 ^a	40,5±2,5 ^a
Hemoglobin (g/dL)	9,93±1,48 ^a	11,79±1,19 ^a
MCV ¹ (fL)	228,1±17,7 ^a	270,7±19,99 ^a
MCHC ² (%)	29,18±2,08 ^a	29,02±2,32 ^a
Total leukocyte (/μL)	19.988±3.486 ^a	13.223±2.986 ^a
Heterophil (%)	75,83±3,81 ^a	64,25±3,19 ^b
Heterophils (/μL)	15.114±2.815 ^a	8.627±2.223 ^a
Lymphocytes (%)	21,17±2,06 ^a	31,63±2,91 ^b
Lymphocytes (/μL)	4.335±860,8 ^a	4.107±960 ^a
Eosinophils (%)	1,67±1,48 ^a	1,62±0,26 ^a
Eosinophils (/μL)	310,2±279,4 ^a	193,5±32,33 ^a
Monocytes (%)	1,17±0,98 ^a	1,75±0,56 ^a
Monocytes (/μL)	207,2±186,2 ^a	188±82,01 ^a
Basophils (%)	0,17±0,17 ^a	0,75±0,37 ^a
Basophils (/μL)	22,17±22,17 ^a	107,4±80,09 ^a
Thrombocytes/field	3,85±0,79 ^a	3,97±0,47 ^a
Policromatophils/field	3,43±0,97 ^a	3,49±0,8 ^a
H/L ³	3,87±0,57 ^a	2,2±0,3 ^b
ENA/100 ⁴	1,5±0,56 ^a	0,5±0,27 ^a
TPP ⁵ (g/dL)	4,73±0,6 ^a	5,17±0,26 ^a
Fibrinogen (g/dL)	0,27±0,04 ^a	0,36±0,06 ^a

* Values are expressed as mean ± standard deviation. ^{ab} Different letters in each row indicate statistically significant differences (p<0,05). ¹ MCV = Mean corpuscular volume, ² MCHC = Mean corpuscular hemoglobin concentration, ³ H/L = heterophil/lymphocyte ratio; ⁴ ENA/100 = erythrocytic nuclear abnormalities in 100 erythrocytes; ⁵ TPP = Total plasma protein.

Table 3. Means and standard deviations of haematological parameters, total plasma protein, fibrinogen and body weight pre and post-rehabilitation of magellanic penguins (*Spheniscus magellanicus*) that survived at PROAMAR

Parameter*	Pre-rehabilitation (n=8)	Post-rehabilitation (n=8)
Body weight (kg)	2,35±0,13 ^a	2,81±0,15 ^b
Erythrocytes (x10 ⁶ /μL)	1,54±0,13 ^a	1,64±0,07 ^a
Hematocrit (%)	40,5±2,5 ^a	43,38±1,15 ^a
Hemoglobin (g/dL)	11,79±1,19 ^a	12,45±0,52 ^a
MCV ¹ (fL)	270,7±19,99 ^a	266,5±8,95 ^a
MCHC ² (%)	29,02±2,32 ^a	28,72±0,97 ^a
Total leukocyte (/μL)	13.223±2.986 ^a	14.011±1.100 ^a
Heterophil (%)	64,25±3,19 ^a	56,75±3,3 ^a
Heterophils (/μL)	8.627±2.223 ^a	8.041±959 ^a
Lymphocytes (%)	31,63±2,91 ^a	36,25±3,51 ^a
Lymphocytes (/μL)	4.107±960 ^a	4.951±496,2 ^a
Eosinophils (%)	1,62±0,26 ^a	4,5±1,1 ^b
Eosinophils (/μL)	193,5±32,33 ^a	655±175,7 ^b
Monocytes (%)	1,75±0,56 ^a	1,12±0,48 ^a
Monocytes (/μL)	188±82,01 ^a	179,8±87,33 ^a
Basophils (%)	0,75±0,37 ^a	1,37±0,5 ^a
Basophils (/μL)	107,4±80,09 ^a	184,9±59,6 ^a
Thrombocytes/field	3,97±0,47 ^a	4,75±0,59
Policromatophils/field	3,49±0,80 ^a	3,59±0,6 ^a
H/L ³	2,2±0,3 ^a	1,74±0,26 ^a
ENA/100 ⁴	0,5±0,27 ^a	0,5±0,19 ^a
TPP ⁵ (g/dL)	5,17±0,26 ^a	6,15±0,28 ^b
Fibrinogen (g/dL)	0,36±0,06 ^a	0,22±0,04 ^a

* Values are expressed as mean ± standard deviation. ab Different letters in each row indicate statistically significant differences (p<0,05).

¹ MCV = Mean corpuscular volume, ² MCHC = Mean corpuscular hemoglobin concentration, ³ H/L = heterophils/lymphocytes ratio; ⁴ ENA/100 - erythrocytic nuclear abnormalities in 100 erythrocytes; ⁵ TPP - Total plasma protein.

In the pre-rehabilitation, the combination of anemia and hypoproteinemia was more frequent in those that died (83%) than in those that survived (50%). Likewise, Rodrigues et al. (2010), who monitored magellanic penguins during rehabilitation in Rio Grande do Sul, found significantly lower hematocrit in the animals that died than in those who survived.

Erythrocytic nuclear abnormalities (ENA) were taken as biomarkers to assess exposure to petroleum hydrocarbons and their derivatives (Netto et al. 2000). Yet, in the present study there was no correlation between the presence of ENA and oil contamination.

After rehabilitation, the total leukocyte count in animals in Parana was significantly higher (p<0.05) than the young and adult animals in Santa Catarina. Villouta et al. (1997) found that the total leukocyte count varied according to the time of captivity in Humboldt penguins, and there was no evidence of disease in the animals. This study revealed that newly captured penguins had a mean total leukocyte count of 11,700±5,600 leukocytes/μL. After three weeks of captivity this value increased to 15,900±4600 leukocytes/μL and after seven weeks of captivity it was 9,800±2,400 leukocytes/μL. The variation in the number of total leukocyte observed in the animals of this study can therefore be considered normal.

In post-rehabilitation the animals showed a higher number of heterophils than lymphocytes, which was also

observed in healthy Humboldt penguins free-living (Wallace et al. 1995) and in captivity (Villouta et al., 1997), free-living blue penguins (Sergent et al. 2004) and free-living galapagos penguins (Travis et al. 2006).

Before rehabilitation, leukocytosis was more frequent in penguins that died (66%) than in those that survived (25%). All the animals that died showed relative and absolute heterophilia and relative lymphopenia. According to Hawkey et al. (1989), the absolute number of leukocyte is relatively stable in healthy adult birds, while the heterophils count is much more susceptible to variations under the influence of stress or microbial infection. The penguins with leukocytosis that survived showed heterophilia and absolute lymphocytosis.

The H/L ratio in animals post-rehabilitation was significantly lower (p<0.05) than in pre-rehabilitation. In these animals, the mean H/L ratio of penguins that died was 3.87±0.57, significantly different (p<0.05) from the mean 2.20±0.30 of those that survived.

Leukocyte profiles can be directly related to stress and the levels of circulating corticosteroids (Davis et al. 2008). Corticosteroid-induced leukocytosis reveals mature heterophilia (slight to moderate) and lymphopenia (Campbell 2007). Therefore, the heterophils/lymphocytes (H/L) ratio has been proposed as a sensitive measure of chronic stress in birds, although it also changes in case of diseases (Davis et al. 2008).

Hawkey et al. (1985) found that the H/L ratio in healthy gentoo penguins was 2.3, whereas in animals with pododermatitis it was 4.4, demonstrating the correlation between this parameter and the disease severity. These data demonstrate that interpreting changes in leukocyte profiles can be problematic, especially if infection status is not known. The two factors are closely related: the stress is known for increasing vulnerability to disease and diseases for increasing stress (Davis et al. 2008).

Eosinophils numbers varied significantly (p<0.05) in post-rehabilitation among young (95±48.65/μL) and adult animals (265.3±51.84/μL) received in Santa Catarina and (655±175.72/μL) in Parana. This aspect has been observed by other authors in family Spheniscidae penguins. Villouta et al. (1997) found wide variation in this type of cells in healthy Humboldt penguins, and animals in captivity for three days had an average of 400±600 eosinophils/μL, while animals in captivity for 15 weeks had 1,100±900 eosinophils/μL. Similarly, comparing pre and post-rehabilitation of animals that survived in Parana, there were significant differences (p<0.05) in relative and absolute values of eosinophils.

In mammals, eosinophils are responsible for immediate hypersensitivity reactions and for fighting parasitic agents. In birds, however, there is little correlation between eosinophilia and parasitic infections, and these cells seem not to contribute to acute hypersensitivity reactions. In this case, eosinophilia is often associated with inflammatory response (Mitchell & Johns 2008). In animals from this present study there was no apparent correlation between inflammatory response and eosinophilia.

The monocyte counts found in animals after rehabilitation were slightly smaller, and the basophil values were similar to those previously reported for the family Sphe-

niscidae penguins (Hawkey et al. 1989, Wallace et al. 1995, Villouta et al. 1997, Sergeant et al. 2004, Travis et al. 2006).

The function of Fibrinogen in birds, as in mammals, is activating the blood coagulation system and promoting systemic inflammatory response with nonspecific criteria (Harr, 2010). However, no correlations between increased levels of Fibrinogen and inflammatory response were observed in the animals assessed.

CONCLUSIONS

The body condition score was positively correlated with hematocrit and TPP, and negatively correlated with the H/L ratio.

The presence of anemia in magellanic penguins in their pre-rehabilitation indicates higher risk of death. Penguins with high H/L ratios (3.87 ± 0.57) before rehabilitation are possibly at greater risk of death than those with lower H/L ratios (2.20 ± 0.30). Feeding the penguins a diet of supplemented sardines during rehabilitation seems to be more effective for full recovery and subsequent release.

The data suggest that the hematologic criteria for animals release should be hematocrit above 45%, TPP greater than or equal to 6.0g/dL, total leukocyte count less than $10,000/\mu\text{L}$ and (H/L) ratio less than 1.5.

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