Hematology and Blood Chemistry Reference Values of Captive Adult Black-Faced Ibis (Theristicus melanopis melanopis)

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Simple Summary: Wildlife and human interactions are increasing all over the world, with many injured wild animals needing to be treated or rehabilitated. Reference values are of great help when treating injured animals. The black-faced ibis is a bird common in South America and is one of the few ibises in this region. The human population has increased the risk of interaction with these birds, and rehabilitation centers are receiving injured birds. The aim of this work was to establish the normal or reference hematology and blood chemistry values in this species. The result may be used to elucidate the health and welfare status of injured or recovering black-faced ibis in rehabilitation centers and to act with the required therapeutic measures. This in turn may increase rates of survival and may indicate the correct moment of returning of the birds back to the wild while contributing to the conservation of this species.

Abstract: Hematology and blood chemistry reference values in wildlife animals are considered a key element to evaluate their health and welfare status. The incidence of birds rescued is increasing, thus, rehabilitation centers worldwide need valid reference values to improve medical care for wild individuals. The objective of this study was to obtain the reference values of the adult black-faced ibis (Theristicus melanopis). Blood was taken from adult rehabilitated birds and analyzed to obtain red and white line values such as hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), heterophils, lymphocytes, monocytes, eosinophils and basophils. Values for blood glucose, proteins, albumin, globulin, calcium, phosphorus, blood urea nitrogen (BUN), creatinine, alkaline phosphatase, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and albumin:globulin (A/G) ratio were also obtained. The results were similar to others reported for species of the Threskiornithidae family (bald, glossy and Puna ibises), but showed higher values for white blood cells (WBC), heterophils, monocytes and heterophil-lymphocyte (H/L) ratio, but lower values for basophils and eosinophils. Moreover, higher values in albumin, BUN and CPK were observed. This is the first report of the hematology references values for the black-faced ibis showing differences to other closely related species. The results may be of use in rescue and rehabilitation centers for animal welfare and health assessments of the black-faced ibis.

Keywords: conservation health; rehabilitation; ecosystems; wild bird health; wild bird welfare
1. Introduction

The black-faced ibis (Theristicus melanopis) is a native bird of Chile and is also found in some areas of Peru and Argentina [1,2]. There are only a few studies of this bird, mostly on ecology [3,4] and reproduction biology [5] due to the limited captive population and the complicated capture methods in the wild. It is categorized as a least concern species in the International Union for Conservation of Nature Red List [2]. However, the current legislation in Chile [6] classified T. melanopis as beneficial to agriculture and considered it as an endangered species in the north, vulnerable in the central and southern regions and least concerned in the austral region.

The black-faced ibis can inhabit multiple landscapes such as dry grasslands, swampy valleys, river banks or agricultural fields [7], and its diets consist of insects, annelids, and amphibians [8]. However, much of their habitat has been reduced because urbanization has increased [9]. This phenomenon may have been the cause of the increment in the number of black-faced ibises received in wildlife rehabilitation centers, mainly due to traumatic injuries, becoming the most common species received for rehabilitation during 2017 in Chile (personal communication with the Wildlife Rehabilitation Center “Metrenco” and Rescue Centre for Wildlife “CEREFAS” of Universidad Austral, Chile).

In addition, available information has shown limited success in releases back into the wild from rehabilitation centers worldwide [10,11].

Hematological and blood chemistry variables for wild birds are scarce, and those published have proved to be good indicators of the health and welfare status of wild birds in rehabilitation centers [12]. For example, in a rehabilitation center, a low packed cell volume (<50%) was related to high mortality risk in raptors [13], with similar findings for adult African penguins (Spheniscus demersus) [14]. These studies showed the importance of having reference values in rehabilitation centers for birds at the national or regional level, thus ensuring a correct determination of health and welfare.

There are no studies considering the range for hematology and blood chemistry variables in the black-faced ibis. This lack of information may deter veterinarians from performing proper evaluations of health and welfare status of this specie. Furthermore, due to the wide geographical range of this species, it may be of importance to have normal range values specifically for the black-faced ibis populations in Chile. This is sustained from the evidence reported in other species of wild bird such as raptors, psitaccids and other ibises, showing that hematological variables can differ from geographically different regions attributed mainly to diet [15–17].

This study was carried out to establish the normal hematological and blood chemistry values in the black-faced ibis, and differences related to sex and sampling location were also assessed. This information could be used as a complementary tool for veterinary medicine in rehabilitation centers.

2. Materials and Methods

The samples were collected from clinically normal adult black-faced ibises that were born and held in captivity. The study was conducted in accordance with national legislation on the use of animals for research and ARRIVE guidelines [18]. A total of 22 birds were sampled across different rehabilitation centers in Chile (La Serena, Santiago, Rancagua and Linares cities) from 29°54′16.31′′ S to 35°50′48.01′′ S. This territorial coverage represents the north, central and south-central zones of Chile, where the majority of the human population exists and bird interactions can occur. All birds were clinically normal with no sign of illness. The sex of birds was determined and recorded by observation of cloacal region during sampling. Birds were manually immobilized, and blood samples were taken from the brachial vein and placed in ethylenediaminetetraacetic acid (EDTA) tubes. Samples were immediately stored between 2 to 8 °C and submitted to the certified diagnostic laboratory (Laboratorio Veterinario Especializado, VetLab, Santiago, Chile) within 24 h of sampling.

Samples were analyzed by the private veterinary laboratory using an automatic hematological analyzer (BC-3600, Mindray®). Hematology profile was obtained for the 22 birds, and serum biochemistry profiles were obtained from 11 bird samples. The reduced sample size for blood biochemistry was due
Animals 2020, 10, 2227 to small final volume obtained in the 11 samples. Hematology variables were red blood count (RBC), hematocrit, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cells (WBC), heterophils, eosinophils, lymphocytes, monocytes and the heterophil/lymphocyte ratio (H/L ratio). Automatic blood chemistry was carried out using an analyzer (BS-480, Mindra®). Glucose, total proteins, albumin, globulin, albumin:globulin ratio (A/G), calcium, phosphorus, blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) were determined.

Normality tests were carried out using the Kolmogorov–Smirnov test. Means, standard deviations, minimum and maximum values and quartiles were calculated according to standard and suggested methods to obtain references values in animals [19]. Geographical differences in RBC, hemoglobin, MCH, MCHC, MCV, WBC, heterophils, eosinophils, basophils, lymphocytes and monocytes were detected using analysis of variance and for hematocrit, and for the H/L ratio, the Kruskal–Wallis test was used. To detect any correlation between continuous variables, a Pearson test was carried out. All statistical analyses were carried out using R software [20].

3. Results

The mean ± standard deviation, minimum and maximum values and quartiles for each hematological variable can be observed in Table 1.

Table 1. Mean ± standard deviation, minimum and maximum values and quartiles and of hematological variables of captive black-faced ibis (Theristicus melanopis) (N = 22).

| Variable                  | Mean +/- SD | Min  | Max  | 2.5% | 50%  | 97.5% |
|---------------------------|-------------|------|------|------|------|-------|
| RBC (10^6 cells/µL)      | 3.03 +/- 0.46 | 1.81 | 3.94 | 2.08 | 3.04 | 3.75 |
| Hematocrit (%)            | 49.9 +/- 4.4  | 40.0 | 56.0 | 40.0 | 50.5 | 56.0 |
| Hemoglobin (g/dL)         | 16.7 +/- 2.2  | 9.8  | 19.9 | 11.3 | 17.0 | 19.6 |
| MCH (pg/cell)             | 57.2 +/- 4.0  | 50.6 | 67.4 | 51.3 | 56.5 | 65.1 |
| MCHC (g/dL)               | 33.2 +/- 2.4  | 25.0 | 36.0 | 27.6 | 34.0 | 35.7 |
| MCV (fL)                  | 173.4 +/- 17.6 | 142.0 | 220.0 | 147.2 | 172.0 | 211.6 |
| WBC (cell/µL)             | 9704 +/- 4249 | 3800 | 19,800 | 4325 | 9550 | 19,590 |
| Heterophils (cell/µL)     | 5830 +/- 3206 | 1634 | 16,632 | 1828 | 5688 | 12,992 |
| Eosinophils (cell/µL)     | 307 +/- 466    | 0    | 2166 | 8    | 159 | 1436 |
| Basophils (cell/µL)       | 19 +/- 43      | 0    | 128  | 0    | 120  |
| Lymphocytes (cell/µL)     | 3087 +/- 1563  | 930  | 7372 | 946  | 3192 | 6337 |
| Monocytes (cell/µL)       | 446 +/- 375    | 52   | 1552 | 100  | 345  | 1465 |
| H/L ratio                 | 2.4 +/- 2.1    | 0.6  | 10.5 | 0.7  | 1.8  | 7.7  |

Note: RBC = red blood count, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, MCV = mean corpuscular volume, WBC = white blood count, H/L = heterophil-lymphocyte ratio, SD = standard deviation, Min = minimum value, Max = maximum value.

Likewise, blood chemistry variables are shown in Table 2. All variables adjusted to normality except basophils, eosinophils, monocytes, H/L ratio, BUN and A/G ratio (p < 0.05).

Table 2. Mean ± standard deviation, minimum and maximum values and quartiles of of blood chemistry variables of captive black-faced ibis (Theristicus melanopis) (N = 11).

| Variable           | Mean +/- SD | Min  | Max  | 2.5% | 50%  | 97.5% |
|--------------------|-------------|------|------|------|------|-------|
| Glucose (mg/dL)    | 209 +/- 84   | 79   | 302  | 81   | 254  | 298   |
| Total proteins (g/dL) | 4.4 +/- 1.8 | 0.6  | 6.9  | 1.2  | 4.1  | 6.9   |
| Albumin (g/dL)     | 2.1 +/- 0.8  | 1.2  | 3.5  | 1.2  | 1.7  | 3.4   |
| Globulin (d/dL)    | 3.1 +/- 1.3  | 1.7  | 6.1  | 1.8  | 2.7  | 5.7   |
| Calcium (mg/dL)    | 8.8 +/- 1.5  | 6.3  | 10.6 | 6.4  | 9.1  | 10.6  |
| Phosphorus (mg/dL) | 9.9 +/- 5.3  | 3.2  | 20.9 | 3.8  | 8.9  | 20.2  |
| BUN (mg/dL)        | 7.59 +/- 7.9 | 1.9  | 27.8 | 2.1  | 4.7  | 25    |
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| Variable       | Mean +/- SD | Min | Max | 2.5% | 50% | 97.5% |
|----------------|-------------|-----|-----|------|-----|-------|
| Creatinine (mg/dL) | 0.5 +/- 0.3 | 0.0 | 0.8 | 0.05 | 0.5 | 0.8   |
| ALP (IU/L)      | 402.8 +/- 179.4 | 195.8 | 715.5 | 199.1 | 353.6 | 708.4 |
| AST (IU/L)      | 241 +/- 75 | 122 | 410 | 134 | 227 | 381   |
| GGT (IU/L)      | 5.9 +/- 4.2 | 1.8 | 13.9 | 1.9 | 4.2 | 13.5  |
| LDH (IU/L)      | 1485 +/- 819 | 612 | 3600 | 639 | 1477 | 3183  |
| CPK (IU/L)      | 995 +/- 342 | 403 | 1423 | 453 | 1040 | 1418  |
| A/G ratio       | 0.68 +/- 0.1 | 0.51 | 1.16 | 0.52 | 0.57 | 1.08  |

Note: BUN = blood urea nitrogen, ALP = alkaline phosphatase, AST = aspartate aminotransferase, GGT = gamma-glutamyl transeptidase, LDH = lactate dehydrogenase, CPK = creatine phosphokinase, A/G = albumin-globulin ratio, SD = standard deviation, Min = minimum value, Max = maximum value.

There were no statistically significant differences in hematological (Table 3, p > 0.05) or biochemistry variables (Table 4, p > 0.05) in regard to sampling location.

### Table 2. Cont.

| Variable | Mean +/- SD | Min | Max | 2.5% | 50% | 97.5% |
|----------|-------------|-----|-----|------|-----|-------|
| Creatinine (mg/dL) | 0.5 +/- 0.3 | 0.0 | 0.8 | 0.05 | 0.5 | 0.8   |
| ALP (IU/L)      | 402.8 +/- 179.4 | 195.8 | 715.5 | 199.1 | 353.6 | 708.4 |
| AST (IU/L)      | 241 +/- 75 | 122 | 410 | 134 | 227 | 381   |
| GGT (IU/L)      | 5.9 +/- 4.2 | 1.8 | 13.9 | 1.9 | 4.2 | 13.5  |
| LDH (IU/L)      | 1485 +/- 819 | 612 | 3600 | 639 | 1477 | 3183  |
| CPK (IU/L)      | 995 +/- 342 | 403 | 1423 | 453 | 1040 | 1418  |
| A/G ratio       | 0.68 +/- 0.1 | 0.51 | 1.16 | 0.52 | 0.57 | 1.08  |

### Table 3. Mean ± standard deviation of hematological variables of captive black-faced ibis (Theristicus melanopis) according to sampling location (N = 22).

| Variable        | La Serena (n = 3) | Santiago (n = 11) | Rancagua (n = 6) | Linares (n = 2) | p Value |
|-----------------|-------------------|-------------------|------------------|----------------|---------|
| RBC (10^6 cells/µL) | 2.97 +/- 0.04 | 3.07 +/- 0.49 | 3.08 +/- 0.54 | 2.68 +/- 0.50 | 0.73    |
| Hematocrit (%)  | 50.3 +/- 1.5 | 49.1 +/- 4.7 | 52.3 +/- 2.8 | 46.5 +/- 9.1 | 0.49    |
| Hemoglobin (g/dL) | 17.3 +/- 0.6 | 16.5 +/- 2.7 | 17.4 +/- 1.6 | 15.5 +/- 3.7 | 0.71    |
| MCH (pg/cell)    | 52.8 +/- 2.2 | 57.2 +/- 4.0 | 59.4 +/- 4.0 | 57.3 +/- 3.0 | 0.08    |
| MCHC (g/dL)      | 34.3 +/- 0.2 | 32.9 +/- 3.3 | 33.2 +/- 1.3 | 33.0 +/- 1.4 | 0.69    |
| MCV (fL)         | 169.3 +/- 3.0 | 175.0 +/- 19.5 | 172.6 +/- 22.8 | 173.0 +/- 14. | 0.88    |
| WBC (cell/µL)    | 11,400 +/- 7660 | 9283 +/- 3022 | 7733 +/- 2787 | 15,500 +/- 5515 | 0.12    |
| Heterophils (cell/µL) | 8568 +/- 7289 | 5249 +/- 2054 | 4615 +/- 1720 | 8562 +/- 1609 | 0.18    |
| Eosinophils (cell/µL) | 114 +/- 76 | 425 +/- 601 | 62 +/- 42 | 678 +/- 138 | 0.25    |
| Basophils (cell/µL) | 32.0 +/- 55 | 30.1 +/- 52 | 0.0 +/- 0.0 | 0.0 +/- 0.0 | 0.48    |
| Lymphocytes (cell/µL) | 2016 +/- 410 | 3150 +/- 1282 | 2767 +/- 1554 | 5310 +/- 2916 | 0.11    |
| Monocytes (cell/µL) | 670 +/- 642 | 379 +/- 221 | 288 +/- 105 | 950 +/- 851 | 0.09    |
| H/L ratio        | 4.8 +/- 4.9 | 1.9 +/- 1.2 | 2.0 +/- 1.1 | 1.8 +/- 0.6 | 0.79    |

Note: RBC = red blood count, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, MCV = mean corpuscular volume, WBC = white blood count, H/L = heterophil-lymphocyte ratio.

### Table 4. Mean ± standard deviation of blood biochemistry variables of captive black-faced ibis (Theristicus melanopis) according to sampling location (N = 11).

| Variable          | Santiago (n = 3) | Rancagua (n = 6) | Linares (n = 2) | p Value |
|-------------------|------------------|------------------|-----------------|---------|
| Glucose (mg/dL)   | 159 +/- 105 | 208 +/- 75 | 283 +/- 26 | 0.29    |
| Total proteins (g/dL) | 4.6 +/- 1.9 | 3.9 +/- 1.9 | 5.3 +/- 2.3 | 0.68    |
| Albumin (g/dL)    | 1.6 +/- 0.6 | 2.2 +/- 0.9 | 2.4 +/- 0.9 | 0.69    |
| Globulin (dL/dL)  | 2.9 +/- 1.4 | 3.3 +/- 1.5 | 2.9 +/- 1.3 | 0.92    |
| Calcium (mg/dL)   | 8.9 +/- 2.3 | 9.0 +/- 1.4 | 7.9 +/- 0.4 | 0.69    |
| Phosphorus (mg/dL) | 10.0 +/- 7.5 | 10.0 +/- 5.5 | 9.6 +/- 4.3 | 0.99    |
| BUN (mg/dL)       | 2.7 +/- 0.6 | 6.6 +/- 5.2 | 17.8 +/- 14.1 | 0.08   |
| Creatinine (mg/dL)| 0.4 +/- 0.3 | 0.5 +/- 0.3 | 0.4 +/- 0.0 | 0.76    |
| ALP (IU/L)        | 413.1 +/- 270.0 | 404.4 +/- 151.3 | 382.4 +/- 245.4 | 0.98   |
| AST (IU/L)        | 206 +/- 75 | 257 +/- 87 | 243 +/- 34 | 0.66    |
| GGT (IU/L)        | 5.0 +/- 1.5 | 6.9 +/- 5.6 | 4.5 +/- 0.5 | 0.74    |
| LDH (IU/L)        | 1952 +/- 1484 | 1178 +/- 421 | 1705 +/- 323 | 0.41    |
| CPK (IU/L)        | 777 +/- 231 | 977 +/- 353 | 1379 +/- 36 | 0.15    |
| A/G ratio         | 0.59 +/- 0.10 | 0.68 +/- 0.24 | 0.83 +/- 0.02 | 0.16    |

Note: BUN = blood urea nitrogen, ALP = alkaline phosphatase, AST = aspartate aminotransferase, GGT = gamma-glutamyl transeptidase, LDH = lactate dehydrogenase, CPK = creatine phosphokinase, A/G = albumin-globulin ratio.
Additionally, no statistical differences were found between the sex of birds for hematological variables (Table 5, $p > 0.05$) or blood biochemistry variables (Table 6, $p > 0.05$).

**Table 5.** Mean $\pm$ standard deviation of hematological variables of captive black-faced ibis (*Theristicus melanopis*) according to sex (N = 22).

| Variable          | Male ($n$ = 12) | Female ($n$ = 10) | $p$ Value |
|-------------------|-----------------|-------------------|-----------|
| RBC (10$^6$ cells/µL) | 3.16 +/- 0.53 | 2.86 +/- 0.31 | 0.11      |
| Hematocrit (%)    | 49.5 +/- 4.9   | 50.3 +/- 4.3     | 0.68      |
| Hemoglobin (g/dL) | 17.3 +/- 2.7   | 16.1 +/- 1.5     | 0.22      |
| MCH (pg/cell)     | 56.8 +/- 4.4   | 57.9 +/- 3.9     | 0.55      |
| MCHC (g/dL)       | 33.9 +/- 2.9   | 32.5 +/- 1.7     | 0.19      |
| MCV (µL)          | 171.3 +/- 20.4 | 176.0 +/- 14.6   | 0.35      |
| WBC (cell/µL)     | 9742 +/- 3867  | 9660 +/- 4884    | 0.97      |
| Heterophils (cell/µL) | 5913 +/- 3715 | 5730 +/- 2662    | 0.89      |
| Eosinophils (cell/µL) | 354 +/- 595   | 259 +/- 250      | 0.61      |
| Basophils (cell/µL) | 25.0 +/- 45.5  | 12.8 +/- 40.5    | 0.52      |
| Lymphocytes (cell/µL) | 3025 +/- 1108 | 3161 +/- 2047    | 0.85      |
| Monocytes (cell/µL) | 412 +/- 320   | 486 +/- 447      | 0.66      |
| H/L ratio         | 2.4 +/- 2.6    | 2.3 +/- 1.3      | 0.86      |

Note: RBC = red blood count, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, MCV = mean corpuscular volume, WBC = white blood count, H/L = heterophil-lymphocyte ratio.

**Table 6.** Mean $\pm$ standard deviation of blood biochemistry variables of captive black-faced ibis (*Theristicus melanopis*) according to sex (N = 11).

| Variable         | Male ($n$ = 3) | Female ($n$ = 8) | $p$-Value |
|------------------|---------------|-----------------|-----------|
| Glucose (mg/dL)  | 273 +/- 17    | 184 +/- 87      | 0.12      |
| Total proteins (g/dL) | 3.6 +/- 2.8 | 4.6 +/- 1.5     | 0.43      |
| Albumin (g/dL)   | 2.4 +/- 1.1   | 1.9 +/- 0.7     | 0.46      |
| Globulin (d/dL)  | 4.2 +/- 1.7   | 2.7 +/- 0.9     | 0.08      |
| Calcium (mg/dL)  | 9.5 +/- 1.1   | 8.5 +/- 1.6     | 0.38      |
| Phosphorus (mg/dL) | 11.0 +/- 9.0 | 9.5 +/- 4.1     | 0.71      |
| BUN (mg/dL)      | 9.3 +/- 6.8   | 6.9 +/- 8.6     | 0.68      |
| Creatinine (mg/dL) | 0.7 +/- 0.1 | 0.4 +/- 0.3     | 0.13      |
| ALP (IU/L)       | 549.2 +/- 263.7 | 347.9 +/- 116.1 | 0.09      |
| AST (IU/L)       | 228 +/- 29    | 246 +/- 87      | 0.76      |
| GGT (IU/L)       | 3.6 +/- 2.8   | 6.8 +/- 4.4     | 0.28      |
| LDH (IU/L)       | 695 +/- 374   | 1781 +/- 868    | 0.10      |
| CPK (IU/L)       | 1117 +/- 393  | 672 +/- 360     | 0.12      |
| A/G ratio        | 0.55 +/- 0.02 | 0.74 +/- 0.21   | 0.17      |

Note: BUN = blood urea nitrogen, ALP = alkaline phosphatase, AST = aspartate aminotransferase, GGT = gamma-glutamyl transeptidase, LDH = lactate dehydrogenase, CPK = creatine phosphokinase, A/G = albumin-globulin ratio.

### 4. Discussion

The results presented in this communication are the first attempt to establish reference values for the black-faced ibis in Chile. The sample size in our study is small, but it is common for wild birds and is in accordance with guidelines for determination of reference values in wild animals [21]. However, the values for hematology were relatively similar to other stork species, such as the northern bald ibises (*Geronticus eremita*) [22], Puna ibis (*Plegadis ridgewayi*) [23] and white and black storks (*Ciconia ciconia* and *Ciconia nigra*) [24–26]. For example, our results showed higher values for WBC, heterophils, monocytes and H/L ratio, but lower values for basophils and eosinophils than those reported for bald ibises [15,27]. In contrast, no differences in hematology reference values of glossy ibises (*Plegadis falcinellus*) [28] or Puna ibises [23] were observed. A plausible explanation may be related to the habitats and dietary differences of the species, where bald ibises inhabit coastal areas of Africa [29], while the glossy and Puna ibises inhabit inland marshes and flatlands of Europe, North and
also South America, similar to black-faced ibises [30,31]. Furthermore, different types of diet may
impose dietary deficiencies, such as iron, which could have an effect on some blood components such
as hematocrit or hemoglobin [32,33]. Moreover, dietary composition may have an impact on exposure
to pathogens (virus, bacteria, protozoa, and coccidias) which may increase numbers of immune system
cells such as lymphocytes and monocytes [34] and trigger metabolic changes that could be observed in
blood chemistry variables, such as those related to liver function [35].

There are only a few studies exploring blood chemistry in the Threskiornithidae family, and the
results from this study showed differences in some of these variables. The blood chemistry values
were similar to the values reported for adult bald ibises [27], but we obtained higher values for
phosphorus and LDH and lower values for calcium in the black-faced ibises analyzed in this study.
In contrast, the sampled birds exhibited higher values in albumin, BUN and CPK when compared to
Puna ibises [23]. There is no clear explanation for these differences, and they could be species-specific
rather than from stress. Future studies should be conducted to elucidate plausible explanations for the
observed differences in some blood chemistry parameters in ibises.

Stress, more specifically chronic stress, has been biological well characterized in many human an
non-human animal species and may trigger a state of continuous catabolism affecting different systems
that can be reflected in the modification of blood chemistry variables [36] or hematology, such as the
H/L ratio [37].

Furthermore, no differences were observed for the sex of birds or sampling location, suggesting
that both hematology and blood chemistry variables are not affected by these factors. More studies
should be conducted to ensure a large sample size and confirm these preliminary results.

Finally, it is important to point out that, although the number of total animals sample
are in accordance with or even higher than many other studies in wild birds [15,16,25,28] and
recommendations for obtaining reference values in wild animals [19,21], it is recommended to increase
the number of individuals to increase the statistical power and analysis. This will depend on the
number of birds entering rehabilitation centers, and the sampling should be carried out before returning
them to the wild.

5. Conclusions

This study provided the first report for hematological parameters and blood chemistry reference
values in adult black-faced ibises in Chile. Most of the results obtained were similar to others previously
published for other ibises’ species, but differences both in hematology and blood chemistry were
observed. These differences may be specie-specific or a result of the different diet of the birds at a
regional or national level, as there were no differences among various sampling locations of Chile.
These preliminary results should be further explored in terms of number of sampled birds, age and
seasons. However, these results may be of use in rescue and rehabilitation centers as a tool to make
informed and scientifically based decisions for animal welfare and health in the black-faced ibis living
in the southern American hemisphere.

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References

1. Vuilleumier, F. Forest birds of patagonia: Ecological geography, speciation, endemism, and faunal history. Ornithol. Monogr. 1985, 255–304. [CrossRef]

2. BirdLife International. Theristicus melanopis. In IUCN Red List of Threatened Species; Amended Version of 2016 Assessment; International Union for Conservation of Nature and Natural Resources: Cambridge, UK, 2017. [CrossRef]

3. Azpíroz, A.B.; Isacch, J.P.; Dias, R.A.; Di Giacomo, A.S.; Fontana, C.S.; Palarea, C.M. Ecology and conservation of grassland birds in southeastern South America: A review. J. Field Ornithol. 2012, 83, 217–246. [CrossRef]

4. Raimilla, V.; Rau, J.R.; Niklitschek, E.J. Use of exotic conifers as nesting sites by black-faced ibis (Theristicus melanopis melanopis) in an urban area of southern Chile. Stud. Neotrop. Fauna Environ. 2015, 1–7. [CrossRef]

5. Gantz, A.; Yañez, M. Breeding biology of the black-faced ibis (Theristicus melanopis) in Southern Chile. Waterbirds 2016, 39, 346–355. [CrossRef]

6. SAG. Servicio Agrícola y Ganadero Ley de Caza; SAG: Santiago, Chile, 2015; 112p.

7. Del Hoyo, J.; Elliott, A.; Sargatal, J. Family Threskiornithidae (ibises and spoonbills). In Handbook of the Birds of the World, Vol. 1, Ostriches to Ducks; del Hoyo, J., Elliott, A., Sargatal, J., Eds.; Lynx Edicions: Barcelona, Spain, 1992; pp. 472–506.

8. Gantz, A.; Schlatter, R. La dieta de la bandurria (Theristicus caudatus melanopis Gmelin, 1789) en praderas agrícolas del sur de Chile. Medio Ambiente 1995, 12, 35–38.

9. Cornelius, C.; Cofre, H.; Marquet, P.A. Effects of habitat fragmentation on bird species in a relict temperate forest in semiarid Chile. Conserv. Biol. 2000, 14, 534–543. [CrossRef]

10. Hernandez, C.L.; Oster, S.C.; Newbrey, J.L. Retrospective study of raptors treated at the southeastern raptor center in Auburn, Alabama. J. Raptor Res. 2018, 52, 379–388. [CrossRef]

11. Fix, A.S.; Barrows, S.Z. Raptors rehabilitated in Iowa during 1986 and 1987: A retrospective study. J. Wildl. Dis. 1990, 26, 18–21. [CrossRef]

12. Doussang, D.; Palma, C.; Moreno, L.; Zambrano, B.; Pavéz, E.; Cerda, F.; González-Acuña, D. Hematological and Biochemical Parameters of Captive Andean Condors. J. Raptor Res. 2018, 52, 72–81. [CrossRef]

13. Molina-López, R.A.; Casal, J.; Darwich, L. Prognostic indicators associated with early mortality of wild raptors admitted to a wildlife rehabilitation centre in Spain. Vet. Q. 2015, 35, 9–15. [CrossRef]

14. Parsons, N.J.; Vanstreels, R.E.T.; Schaefer, A.M. Prognostic indicators of rehabilitation outcomes for adult african penguins (Spheniscus demersus). J. Wildl. Dis. 2018, 54, 54–65. [CrossRef] [PubMed]

15. Stanclova, G.; Schwendenwein, I.; Merkel, O.; Kenner, L.; Dittami, J.; Fritz, J.; Scope, A. The effects of migratory flight on hematologic parameters in northern bald ibises (Geronticus eremita). J. Zoo Wildl. Med. 2017, 48, 1154–1164. [CrossRef] [PubMed]

16. Artacho, P.; Soto-Gamboa, M.; Verdugo, C.; Nespolo, R.F. Using haematological parameters to infer the health and nutritional status of an endangered black-necked swan population. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 2007, 147, 1060–1066. [CrossRef] [PubMed]

17. Minias, P. The use of haemoglobin concentrations to assess physiological condition in birds: A review. Conserv. Physiol. 2015, 3, cov007. [CrossRef]

18. Kilkenny, C.; Browne, W.J.; Cuthill, I.C.; Emerson, M.; Altman, D.G. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. Plos Biol. 2010, 8, 1–5. [CrossRef]

19. Horowitz, G. Establishment and use of reference values. In Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics; Burtis, C., Bruns, D., Eds.; Saunders: Boston, MA, USA, 2015; pp. 60–71.

20. R Development Core Team. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Viena, Asutria, 2014; Available online: https://www.R-project.org (accessed on 1 June 2020).

21. Friedchirs, K.R.; Harr, K.E.; Freeman, K.P.; Szladovits, B.; Walton, R.M.; Barnhart, K.F.; Blanco-Chavez, J. American Society for Veterinary Clinical Pathology ASVCP reference interval guidelines: Determination of de novo reference intervals in veterinary species and other related topics. Vet. Clin. Pathol. 2012, 41, 441–453. [CrossRef]

22. Dutton, C.J.; Allchurch, A.F.; Cooper, J.E. Comparison of hematologic and biochemical reference ranges between captive populations of northern bald ibises (Geronticus eremita). J. Wildl. Dis. 2002, 38, 583–588. [CrossRef]
23. Coke, R.L.; West, G.D.; Hoover, J.P. Hematology and plasma biochemistry of captive Puna ibis (*Plegadis ridgwayi*). *J. Wildl. Dis.* 2004, 40, 141–144. [CrossRef]

24. Puerta, M.L.; Pulido, R.M.; Huecas, V.; Abelenda, M. Hematology and blood chemistry of chicks of white and black storks (*Ciconia ciconia* and *Ciconia nigra*). *Comp. Biochem. Physiol. Part A Physiol.* 1989, 94, 201–204. [CrossRef]

25. Alonso, J.C.; Huecas, V.; Alonso, J.A.; Abelenda, M.; Muñoz-Pulido, R.; Puerta, M.L. Hematology and blood chemistry of adult white storks (*Ciconia ciconia*). *Comp. Biochem. Physiol. Part A Physiol.* 1991, 98, 395–397. [CrossRef]

26. Han, J.; Jang, H.; Na, K. Hematologic and serum biochemical reference intervals of the oriental white stork (*Ciconia boyciana*) and the application of an automatic hematologic analyzer. *J. Vet. Sci.* 2016, 17, 399–405. [CrossRef] [PubMed]

27. Villegas, A.; Guzmán, J.M.S.; Corbacho, C.; Corbacho, P.; Vargas, J.M. Blood values of bald ibis (*Geronticus eremita*) in captivity: Comparative ranges and variability with age, sex and physical condition. *J. Ornithol.* 2004, 145, 98–104. [CrossRef]

28. Celldrán, J.; Polo, F.J.; Peinado, V.I.; Viscor, G.; Palomeque, J. Haematology of captive herons, egrets, spoonbill, ibis and gallinule. *Comp. Biochem. Physiol. Part A Physiol.* 1994, 107, 337–341. [CrossRef]

29. BirdLife International. *Geronticus eremita*. In *IUCN Red List of Threatened Species*; International Union for Conservation of Nature and Natural Resources: Cambridge, UK, 2016. [CrossRef]

30. BirdLife International. *Plegadis falcinellus*. In *IUCN Red List of Threatened Species*; International Union for Conservation of Nature and Natural Resources: Cambridge, UK, 2016. [CrossRef]

31. BirdLife International. *Plegadis ridgwayi*. In *IUCN Red List of Threatened Species*; International Union for Conservation of Nature and Natural Resources: Cambridge, UK, 2016. [CrossRef]

32. Norambuena, M.C.; Bozinovic, F. Effect of malnutrition on iron homeostasis in black-necked swans (*Cygnus melanocoryphus*). *J. Zoo Wildl. Med.* 2009, 40, 624–631. [CrossRef] [PubMed]

33. Mete, A.; Hendriks, H.G.; Klaren, P.H.M.; Dorrestein, G.M.; van Dijk, J.E.; Marx, J.J.M. Iron metabolism in mynah birds (*Gracula religiosa*) resembles human hereditary haemochromatosis. *Avian Pathol.* 2003, 32, 625–632. [CrossRef]

34. Fassbinder-Orth, C.A.; Karasov, W.H. Effects of feed restriction and realimentation on digestive and immune function in the Leghorn chick. *Poult. Sci.* 2006, 85, 1449–1456. [CrossRef]

35. Graczyk, T.K.; Cranfield, M.R.; Bicknese, E.J. Evaluation of serum chemistry values associated with avian malaria infections in African black-footed penguins (*Spheniscus demersus*). *Parasitol. Res.* 1995, 81, 316–319. [CrossRef]

36. Moberg, G.P.; Mench, J.A. *The Biology of Animal Stress: Basic Principles and Implication for Animal Welfare*, 1st ed.; CABI: Wallingford, UK, 2000; 384p.

37. Vleck, C.M.; Vertalino, N.; Vleck, D.; Bucher, T.L. Stress, corticosterone, and heterophil to lymphocyte ratios in free-living Adélie penguins. *Condor* 2000, 102, 392–400. [CrossRef]

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