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ORIGINAL PAPER

# Blood chemistry values in nestlings of Rockhopper Penguins (*Eudyptes chrysocome)*: the effect of sex and body condition

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## Abstract

Hematological studies concerned with the determination of normal values of blood parameters in animals have been increasing. However, studies on normal concentration of blood constituents of free-living birds still are not very common, and less than 5% of the species of birds have been analyzed, mostly in captivity. Avian hematology has been used in ornithological studies, because it provides biological data about these animals, their biology, and can be very important in the understanding of ecological and behavioral issues. The main purpose of the study was to investigate the concentrations of certain plasma biochemical parameters in nestlings of Rockhopper Penguins (*Eudyptes chrysocome)* at the crèche phase and the potential influence of some factors such as sex. We captured 95 nestling Rockhopper during the period 24–31 January 2017. All nestlings were randomly selected from colonies in Saunders Island (Falkland Islands). All the sampled birds were between 25 and 45 days of age, with mean weight of 1.778 ± 0.314 kg and mean bill length of 36.0 ± 2.8 mm. No differences in blood parameters or body condition between sexes were found. No parameters but total protein and urea were related to body index. Body index showed a negative significant relationship with urea levels in blood, with penguins in worse condition (those relatively lighter) showing higher levels of urea in blood than those that were relatively heavier. Same trend was observed for total proteins. Urea concentration in blood would be used as a tool in future studies, particularly in young Rockhoppers when they are in crèche phase, a period of high level of mortality mainly by predation. Plasma urea was the single variable that reflects the best body index and also has a rationale background explaining this relationship.

**Keywords** Nestling Rockhopper · Crèche · Blood chemistry · Nutritional condition · Urea · Penguins · Falkland

## Introduction

Hematological studies carried out on several aspects of the biochemistry and physiology of birds have increased, in particular those concerned with the determination of normal values of blood chemistry parameters (Nirmalan and Robinson 1971; Carpenter 1975; Gee et al. 1981). However, studies on normal concentration of blood constituents of free-living birds are not very common, and less than 5% of the species of birds have been analyzed, mostly in captivity (Ferrer 1993 and references therein). Avian blood chemistry has been used in ornithological studies, because it provides biological data about these animals, their biology, and the detection of possible pathological states. Determination of nutritional and physiological conditions can be very important in the understanding of ecological and behavioral issues.

Hematological values, including chemical components, are known to be influenced by many factors: physiological state, age, sex, nutritional condition, circadian rhythm, seasonal changes, captivity, pollutants, and plasma storing methods (Okumura and Tasaki 1969; Twiest and Smith 1970; Chilgren and DeGraw 1977; DeGraw et al. 1979; Gee et al. 1981; Rehder et al. 1982; Rehder and Bird 1983; Chaplin et al. 1984; Groscolas 1986; Ferrer et al. 1987; Garcia-Rodriguez et al. 1987a, b; Cherel and Le Maho 1988a, b; Viñuela et al. 1991; Jenni-Eiermann and Jenni 1992). So when we try to study the influence of one of these factors, we must be sure that the others are controlled (Ferrer 1990).

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An adequate knowledge of blood chemistry is greatly recommended for those projects involving research and management of populations as far as they can be valuable for the assessment of the nutritional levels and health status of species (Ferrer 1993; Ferrer and Dobado-Berrios 1998; Ferrer et al. 2017). Additionally, many hypotheses in ecology rely in differences among individuals in nutritional levels (Ferrer 1993; Angelier et al. 2011). These kinds of studies performed in long-lived birds, like seabirds, are important providing basic data that are interesting in veterinary, taxonomy, and ecology (Balasch et al. 1976; Polo et al. 1992; Work 1996; Uhart et al. 2003; Groscolas et al. 2008; Bourgeon et al. 2010; Crossin et al. 2012; Ferrer et al. 2017). Populations of many penguin species have declined substantially in the past two decades (Trathan et al. 2015) due to several reasons, including increasing pollution of the oceans. Consequently, developing new monitoring tools, for example blood analyses to detect abnormal effects in blood constituents, would be necessary. To do that, we need to know which the reference values are in the species and which factors are modulating them.

There are several species of penguins whose biochemical parameters in blood have been described (*Aptenodytes forsteri*, Groscolas 1982; *A. patagonicus,* Cherel et al. 1988a, b, c; *Pygoscelis adeliae, P. antarctica*, and *P. papua*, Aguilera et al. 1993; *Spheniscus humboldti*, Wallace et al. 1995; *Spheniscus mendiculus*, Travis et al. 2006). As far as we know, there are some published data on hematology of Rockhopper Penguins (*Eudyptes chrysocome,* Dehnhard et al. 2011) and also biochemical parameters (Ghebremeskel et al. 1989), but they focused on change in values before and after molt of adult birds. There are no reference values for nestlings of this species at the end of crèche phase. Those reference values could prove to be of interest for the conservation of this species as far as nutritional conditions at the beginning of juvenile dispersal seem to affect dispersal distances in other species (Ferrer and Morandini 2017) and because during the end of crèche phase there is a high predation rates that would be related to physiological conditions (Morandini and Ferrer pers. observ.).

The main purpose of the study was to investigate the concentrations of plasma biochemical parameters mainly related to fat and protein metabolism (urea, uric acid, triglycerides, cholesterol), in nestlings of Rockhopper Penguins at the crèche phase, to provide with reference values for this species and to analyze the potential influence of some factors such as sex. Even when sexual differences in blood chemistry are not very common in birds, being more frequent among hematocytological parameters has never been studied in this penguin species. In addition, we try to determine potential correlations between biochemical blood parameters and nutritional status, using for that body index. We investigate if concentration of some intermediate metabolism substances would be used as indicator of nutritional status, particularly, fat and protein metabolism products.

## Materials and methods

### Study area and species

We conducted this study in Saunders Island (51.37°S 60.09°W), located at the north coast of Falkland Islands, with 12,500 ha (Fig. 1). The Falkland Islands are located in the southwest region of the South Atlantic Ocean and it is home to penguins (*Aptenodytes patagonicus, Pygoscelis papua, Eudyptes chrysocome*, and *Spheniscus magellanicus*), shags (*Phalacrocorax magellanicus* and *P. atriceps*), and Black-browed Albatrosses (*Thalassarche melanophrys*).

The Rockhopper is the smallest yellow-crested penguin with a weight of 2–3.5 kg. The Southern Rockhopper Penguin (*Eudyptes chrysocome chrysocome*) has a global population of around 1 million. The Falkland Islands currently supports one of the largest populations (Baylis et al. 2013). The Southern Rockhopper Penguin status has been classified as vulnerable by the IUCN due to a declined by about one-third in the last 30 years. Nevertheless, recent studies showed a significant recovery of the population (Baylis et al. 2013). They breed in large colonies located from sea level to cliff-tops.

### Body measurements

We captured 95 nestling Rockhoppers during the period 24–31 January 2017. All nestlings were randomly selected from colonies in Saunders Island. According to flipper length (after Dehnhard et al. 2011), all the sampled birds were between 25 and 45 days of age, in the crèche phase (Poisbleau et al. 2010). We weighed and measured all the nestlings using calipers and a rule. We measured bill length (expose culmen, see Aguilera et al. 1993) and bill depth (to the nearest 0.1 mm with digital calipers), and flipper length (to the nearest mm) and with a spring balance recorded body mass (to the nearest 10 g). To reduce variability, most of the measurements (> 90%) were made by the same observer. To estimate nutritional conditions in nestlings independently of blood parameters, we conducted a regression between cube root of mass and bill length keeping residuals of this regression (body index) as proxy of body condition. We select bill length because it is the structural measure with the best linear relationship with the age of the chicks (Poisbleau et al. 2010). Caution must be taken with this approach due to high variability in body mass in penguins' chicks, depending on the time since the last meal was provided by their parents. We did not measured chicks that have been fed during our stay at the colony but we cannot discard this possibility.

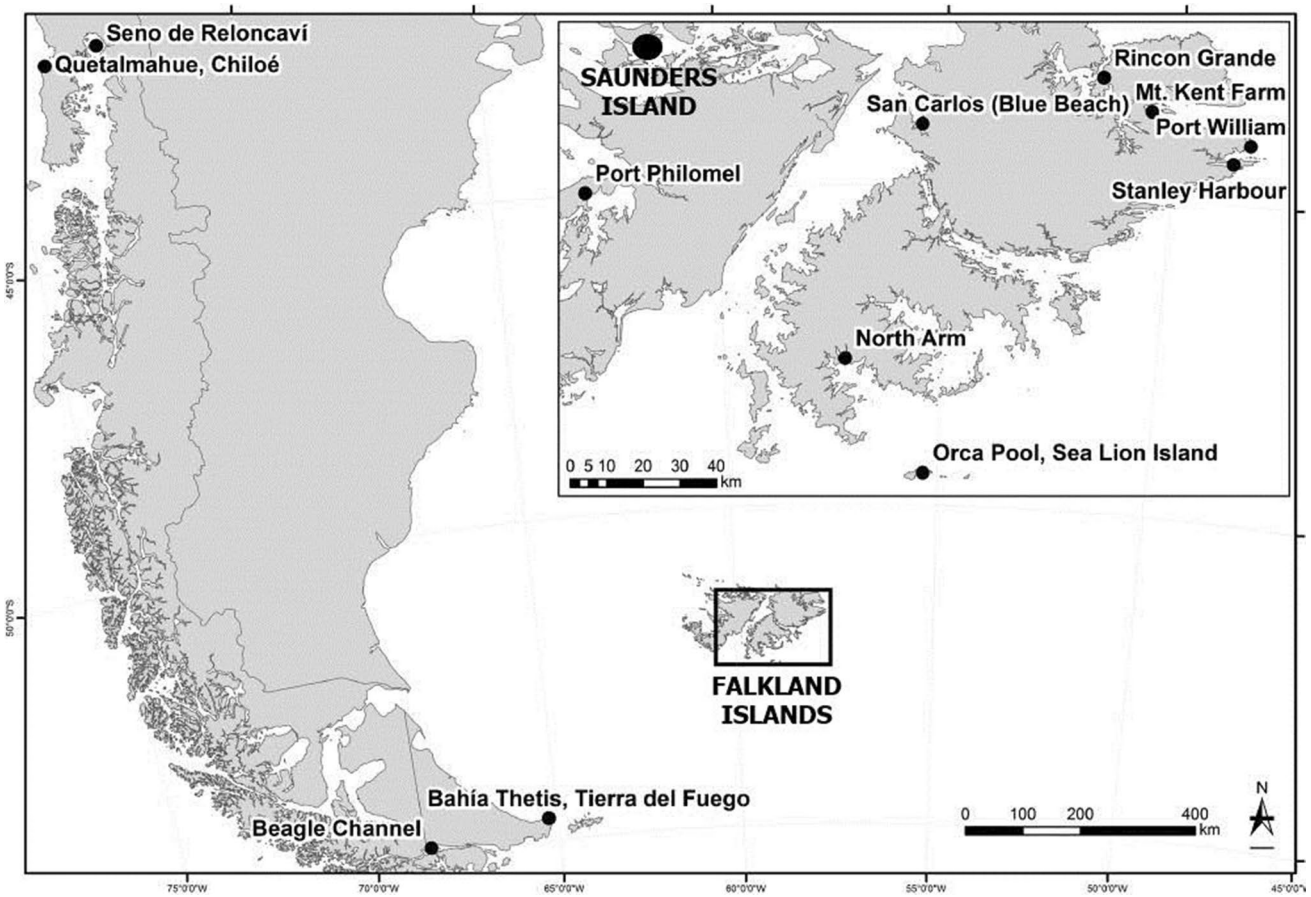


**Fig. 1** Study area, Saunders Island (51.37°S 60.09°W) in northwest of Falkland Islands

## Blood collecting procedures

Blood samples were collected between 11 a.m. and 3 p.m. avoiding variations in blood parameters due to circadian rhythms (Garcia-Rodriguez et al. 1987b; Ferrer 1990; Ferrer et al. 1994). Samples, up to 2 ml, were extracted using a 23-gauge needle and a 5-ml heparinized syringe. Blood samples were stored on portable refrigerator around 6 °C. Blood samples were carried to the laboratory within 8 h after blood withdrawal and were centrifuged at 3000 rpm for 10 min. We kept plasma stored frozen at − 24°C until analysis. Handling time including bleeding time of birds caught was below 3 min. All the birds were released at the capture point.

## Tested parameters and sex determination

We performed biochemical analyses on a computer process-controlled multichannel autoanalyzer (Cobas Integra 400, Roche Diagnostic). We made 11 determinations in nestling birds (Table 1). Sample reproductability was > 97.3% for all parameters and was calculated measuring a sample 20 times for each parameter. Protein metabolism or catabolism affect levels of urea and uric acid, being cholesterol, triglycerides, and

**Table 1** The 11 determinations conducted in blood from Rockhopper Penguin (*Eudyptes crestatus*) nestlings

| Parameter | Method |
|---|---|
| Glucose | GOD-PAP Method |
| Urea | Urease Method |
| Uric Acid | Uricase Method |
| Cholesterol | CHOH-PAP Method |
| Triglycerides | Enzymatic Method |
| Creatine Kinase | Ck, Enzymatic Method |
| Alkaline Phosphatase | Alp, Cresolphthalein Phosphate Hydrolysis |
| Total Protein | Biuret Reaction |
| Calcium | Cresolphthalein Complexone Reaction |
| Lactate dehydrogenase | LDH-UV |
| β-hydroxybutyrate | Kinetic Method |

The name of the parameter and methods used in determinations are shown

β-hydroxybutyrate a reflection of fat metabolism (Garcia-Rodriguez et al. 1987a; Cherel et al. 1988a, 1993; Alonso-Alvarez et al. 2003). Alkaline phosphatase and calcium levels vary according to ossification process (Viñuela et al. 1991; Dobado-Berrios and Ferrer 1997) and creatine kinase is related to muscular activity (Alonso-Alvarez et al. 2003). LDH is useful in diagnosing tissue damage. The analyses of plasma parameters were carried out in the Laboratory of Ecophysiology of the Doñana Biological Station (CSIC), Seville, Spain.

Sex was determined by means of PCR amplification of sections from CHD1-Z and CHD1-W genes that are located on the avian sex chromosomes (Griffiths et al. 1998). Using this technique, we identified 22 females and 31 males. The analyses were carried out in the laboratory of ecophysiology of the Doñana Biological Station (CSIC), Seville, Spain. Due to small samples, not all the analyses were conducted in all the birds.

### Statistical analyses

All data are expressed as mean ± standard deviations (SD). Normality in distribution of variables was tested. We used a MANOVA analyses to look for the effect of sex on blood biochemistry parameters. Regression analyses were used to study relationship between body condition index and nutritional indicators (urea levels and total proteins). Statistica 8.0 software statistical package was used to perform statistical procedures, and we used an alpha value of 0.05 to assess significance of results.

## Results

Mean weight of penguins was 1.778 ± 0.314 kg (range 1.000–2.600 kg) and mean bill length was 36.0 ± 2.8 mm (range 29.6–42.0). We identified 52 males and 41 females in our sample (2 were not determined due to small sample). Body measurements by sexes are presented in Table 2. As expected in penguins, females are smaller and lighter than males. However, no differences in blood parameters or body condition between sexes were found (Table 2). Mean, range, and SD of blood parameters and body condition index are presented in Table 3.

There was a significant positive relationship between cube root of body weight and bill length ($r = 0.433$, $n = 95$, $p < 0.0001$). The residuals of this regression are what we used as a proxy of body index. A multiple regression analysis trying to find significant relationship between body index and blood parameters was conducted (Table 4). No parameters but total protein and urea were related to body index. Body index showed a negative significant relationship with urea levels in blood ($r = -0.309$, $n = 83$, $p = 0.004$; Fig. 2) and total proteins ($r = -0.244$, $n = 83$, $p = 0.030$; Fig. 3), with penguins in worse condition (those relatively lighter) showing higher levels of urea and total proteins in blood than those that were relatively heavier.

Cholesterol, triglycerides, and β-hydroxybutyrate showed a non-significant negative trend, decreasing when body index increases. Uric acid concentration, contrarily, seems to show a positive relationship but again non-significant. We did not find any significant correlation among blood parameters.

**Table 2** Mean, SD, and ANOVA analyses of morphometric measurements of Rockhopper Penguins (*Eudyptes crestatus*) during the crèche phase

| Measurements | Males ($n = 52$) | Females ($n = 41$) | $F$ | $p$ |
|---|---|---|---|---|
| Body mass (Kg) | 1.860 ± 0.344 | 1.680 ± 0.310 | 6.806 | 0.010 |
| Bill depth (mm) | 19.1 ± 1.9 | 18.2 ± 1.3 | 6.390 | 0.008 |
| Bill width (mm) | 15.3 ± 1.3 | 14.7 ± 1.2 | 4.763 | 0.031 |
| Bill length (mm) | 37.6 ± 6.3 | 33.9 ± 2.0 | 67.565 | < 0.0001 |
| Flipper length (mm) | 164 ± 6 | 160 ± 7 | 10.632 | 0.001 |
| Diagonal tarsus (mm) | 28.1 ± 2.4 | 26.9 ± 1.9 | 7.133 | 0.011 |

**Table 3** MANOVA. Test of significance (with sigma-restricted parameterization) of the effect of sex in blood parameters and body condition of young *Eudyptes crestatus*

| | Test | Value | $F$ | Effect–df | Error–df | $p$ |
|---|---|---|---|---|---|---|
| Intercept | Wilks | 0.0098 | 509.680 | 12 | 61 | 0.0000 |
| SEX | Wilks | 0.8405 | 0.964 | 12 | 61 | 0.4917 |

Results demonstrate no effect of sex

**Table 4** Means and SD of biochemical blood parameters in nestlings' Rockhopper Penguins (*Eudyptes crestatus*) at the crèche phase

| Parameter | *n* | Mean | Minimum | Maximum | SD |
|---|---|---|---|---|---|
| Body index | 93 | 0.000 | −0.538 | 0.469 | 0.173 |
| ALP (U $L^{-1}$) | 81 | 439.842 | 128.360 | 1066.800 | 181.143 |
| β-hydroxybutyrate (μmol $L^{-1}$) | 76 | 1808.200 | 307.471 | 6167.210 | 1152.527 |
| Calcium (μmol $L^{-1}$) | 85 | 0.593 | 0.388 | 1.006 | 0.112 |
| Cholesterol (μmol $L^{-1}$) | 85 | 1.498 | 5.448 | 34.444 | 5.872 |
| CK (U $L^{-1}$) | 85 | 2058.671 | 508.000 | 5683.000 | 1115.006 |
| Glucose (μmol $L^{-1}$) | 84 | 10.801 | 0.038 | 14.073 | 3.254 |
| LDH (U $L^{-1}$) | 83 | 590.277 | 282.000 | 1503.000 | 246.296 |
| Total proteins (g $dL^{-1}$) | 85 | 3.711 | 1.190 | 5.530 | 0.756 |
| Triglycerides (μmol $L^{-1}$) | 85 | 11.591 | 0.530 | 40.925 | 6.445 |
| Uric acid (μmol $L^{-1}$) | 85 | 0.485 | 0.002 | 1.217 | 0.298 |
| Urea (μmol $L^{-1}$) | 85 | 0.979 | 0.218 | 5.267 | 0.871 |

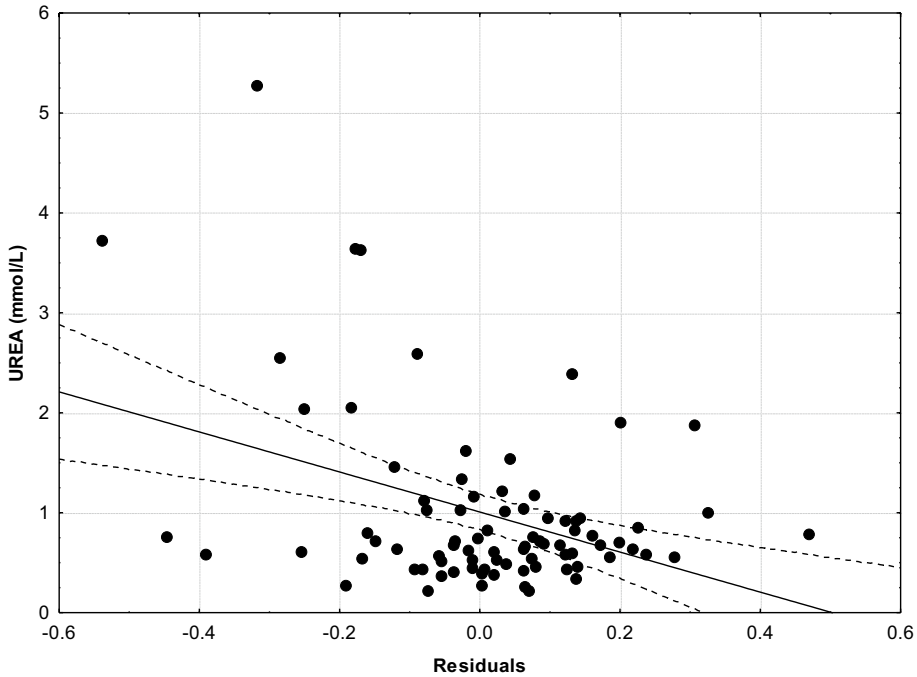


**Fig. 2** Significant relationship between body index and urea levels in blood ($r = -0.309$, $n = 83$, $p = 0.004$). Young *Eudyptes crestatus* penguins relatively lighter (body index below cero) showing higher levels of urea in blood than those that were relatively heavier

## Discussion

The main objective of this study was to establish reference values at the population level for several blood chemistry parameters in Rockhopper nestlings, and to determine their means and variability using free-living birds. Anyway, caution must be taken with these results. It is known that reference values in wild populations can vary among nestlings inhabiting different geographical areas (Ferrer and Dobado-Berrios 1998). Those dissimilarities are probably accounting for quantitative and qualitative differences in their respective diets. More information from other colonies of Rockhoppers in different areas is needed to stablish definitive reference values for this species. In addition to reference values, we analyzed the potential effect of a factor such as sex in these values. We used relatively high sample sizes of wild bird species to good purpose. No differences between sexes were found for any of the studied constituents. Both our present results and other previous results support that sex-related differences are exhibited mainly in hematocytological parameters (red cell number, hematocrit, hemoglobin, etc.) rather than in plasma variables (Mulley 1979; Puerta et al. 1989; Tell and Citino 1992; Ferrer and Dobado-Berrios 1998; Ferrer et al. 2017). Nevertheless, in some species of raptors, sexual differences in plasma chemistry parameters have been found, related probably with large sexual dimorphism in size (Balbontín and Ferrer 2005).

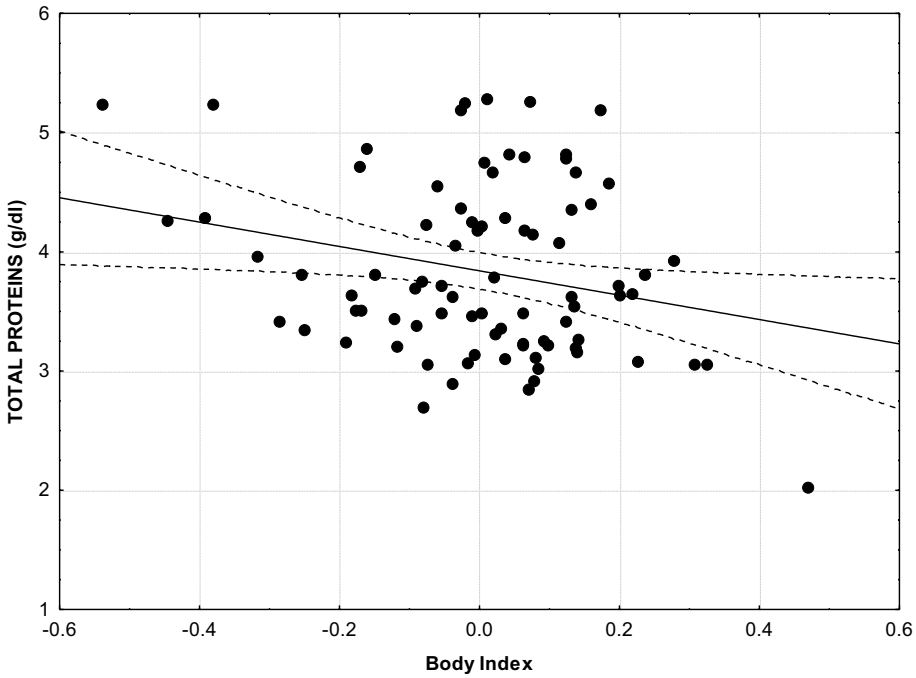


**Fig. 3** Significant relationship between body index and total proteins in blood ($r = -0.244$, $n = 83$, $p = 0.030$). Young *Eudyptes crestatus* penguins relatively lighter (body index below cero) showing higher levels of total protein in blood than those that were relatively heavier

Blood values in young Rockhopper Penguins were similar to those described for other penguin species (Aguilera et al. 1993) or adults of the same species (Ghebremeskel et al. 1989, see Tables 5, 6), with the expected differences between adults and juveniles in some parameters as has been described in several species (Casado et al. 2002; Ferrer et al. 2017 and references therein). Metabolic status is different between adult and nestlings. In our case, young penguins exhibited lower values of uric acid and higher values for urea, cholesterol, or triglycerides than adults (Ghebremeskel et al. 1989) or adults of other penguin species (Aguilera et al. 1993). Contrary, levels of ALP are higher in our study because plasma ALP activity is higher in immature birds than in adults (Puerta et al. 1989; Viñuela et al. 1991; Dobado-Berrios and Ferrer 1997; Ferrer et al. 2017). Those differences are due to the different rate of osteoblastic activity and bone growth in young and adults birds (Viñuela et al. 1991; Dobado-Berrios and Ferrer 1997). The mean concentration of urea in the blood of the young Rockhoppers was greater than those for uric acid, even though they are considered to be uricotelic (Gee et al. 1981). This is interesting, because adult *Pygoscelis* penguins usually exhibit blood urea concentration lower than those for uric acid (Aguilera et al. 1993). Mean levels of urea and uric acid and also creatinine decrease with age in other species (Rosskopf et al. 1982; Casado et al. 2002) suggesting that adults are in better

**Table 5** Results of a multiple regression analysis between body index and blood parameters in young *Eudyptes crestatus*

| | Beta | Beta S.E. | B | B S.E. | *t* (59) | *p* |
|---|---|---|---|---|---|---|
| Intercept | | | −0.160012 | 0.209518 | −0.763 | 0.448 |
| ALP | 0.040 | 0.128 | 0.000033 | 0.000104 | 0.317 | 0.751 |
| β-hydroxybut. | −0.104 | 0.135 | −0.000013 | 0.000017 | −0.765 | 0.445 |
| Calcium | 0.266 | 0.155 | 0.021167 | 0.012380 | 1.709 | 0.092 |
| Cholesterol | −0.020 | 0.151 | −0.000029 | 0.000215 | −0.136 | 0.891 |
| CK | 0.159 | 0.123 | 0.000022 | 0.000017 | 1.292 | 0.201 |
| Glucose | −0.142 | 0.157 | −0.000429 | 0.000476 | −0.899 | 0.371 |
| LDH | 0.000 | 0.165 | 0.000001 | 0.000100 | 0.005 | 0.995 |
| Total proteins | −0.332 | 0.149 | −0.065117 | 0.029349 | −2.218 | **0.030** |
| Triglycerides | −0.034 | 0.136 | −0.000045 | 0.000180 | −0.249 | 0.080 |
| Uric acid | 0.277 | 0.163 | 0.007806 | 0.004592 | 1.699 | 0.094 |
| Urea | −0.651 | 0.250 | −0.006952 | 0.002676 | −2.597 | **0.004** |

Only urea and total proteins showed significant relationship with body index

Bold values are statistical significant

**Table 6** Comparisons of blood chemistry parameters among our study with young *Eudyptes crestatus* and adults of the same species and other species of penguins

| Parameters | Present study | *Eudyptes crestatus* | *Pygoscelis Adeliae* | *Pygoscelis antarctica* | *Pygoscelis papua* | *Spheniscus mendiculus* | *Spheniscus magellanicus* |
|---|---|---|---|---|---|---|---|
| ALP (U $L^{-1}$) | 439.84 | 64.9 | 86.1 | 356.6 | 128 | | 89.8 |
| Calcium (µmol $L^{-1}$) | 0.59 | 2.96 | 2.56 | 2.55 | 2.57 | 2.5 | 2.25 |
| Cholesterol (µmol $L^{-1}$) | 1.49 | | 13.5 | 7.18 | 13.1 | | |
| Glucose (µmol $L^{-1}$) | 10.80 | | 15.91 | 15.13 | 13.14 | 12.1 | |
| LDH (U $L^{-1}$) | 590.2 | | 201.0 | 374.2 | 514.7 | | |
| Total proteins (g $dL^{-1}$) | 3.7 | 5.7 | 4.1 | 4.1 | 4.7 | 5.6 | 5.3 |
| Triglycerides (µmol $L^{-1}$) | 11.59 | | 81.4 | 75.9 | 102.3 | | |
| Uric acid (µmol $L^{-1}$) | 0.48 | | 0.71 | 0.47 | 0.67 | 1.1 | |
| Urea(µmol $L^{-1}$) | 0.97 | 1 | 0.57 | 1.84 | 0.43 | | 2.7 |

condition than young. In other seabird species, however, these seem to be the opposite, with young birds in better nutritional status than adults (Ferrer et al. 2017).

An additional aim of this study was to find a proxy of body condition using intermediate metabolite in blood. This parameter would be used as a tool in future studies, particularly in young Rockhoppers when they are in crèche phase, a period of high level of mortality mainly by predation. Plasma urea was the single variable that reflects the best body index and also has a rationale background explaining this relationship. The increase in plasma urea level could be explained by the use of own proteins as energy source during fasting period (Garcia-Rodriguez et al. 1987a; Castellini and Rea 1992). Urea levels in blood have been used as index of body condition in raptors (e.g., Ferrer 1992, 1993, 1994). Urea levels increase as a response to starvation and decrease after refeeding because proteins are actively mobilized as energy source, increasing the nitrogenous excretion components released into the blood (Garcia-Rodriguez et al. 1987a; Alonso-Alvarez et al. 2002, 2003). Urea is not sensitive to recent ingest (in contrast to glucose concentration for example), and increase and decrease in blood concentration are slow (Garcia-Rodriguez et al. 1987a; Ferrer 1990). Consequently, this could be considered a good indicator not only of acute fasting, but also of mid-term nutritional condition. Thanks to these characteristics, urea level in blood has been used as indicators of nutritional condition in several species in different ecological contexts (Ferrer et al. 1987; Ferrer 1992, 1994; Alonso-Alvarez and Ferrer 2001; Casado et al. 2002; Balbontín and Ferrer 2005).

It is well known that uric acid concentration increases during fasting periods (Garcia-Rodriguez et al. 1987a; Cherel et al. 1988a, b; Robin et al. 1988; Alonso-Alvarez et al. 2003) because it is the major compound for nitrogen excretion in birds. In contrast, changes in urea concentrations are usually thought to be of minor importance. However, the increase of plasma urea concentration found in several species of large size penguins (Groscolas 1982; Cherel et al. 1988a; Cherel and Le Maho 1988a, b; Cherel et al. 1993) as well as smaller ones (Cherel et al. 1993; Alonso-Alvarez et al. 2003) is in line with evidence showing that prolonged starvation increases the capacity of bird liver slices to generate urea (Lemonde 1959). Plasma uric acid concentration has been used as an index of protein catabolism, because birds excreted mostly uric acid as an end product of metabolized nitrogen (Griminger and Scanes 1986). Urea is a minor pathway for protein degradation in birds but the activity of liver arginase (the enzyme on which urea production in birds depends) has increased after a prolonged fast, and also the rise of urea during protein catabolism may be explained by a greater arginine availability. The time a bird keeps in using up its fat reserves and the moment it starts using its own muscle tissues as an energy source basically varies according to the individual's initial condition and the species' capacity for storing fat reserves. It has been shown in Emperor Penguins, *Aptenodytes forsteri* (Groscolas 1982, 1986; Robin et al. 1988), that there are still lipid stores when β-hydroxybutyrate levels in blood begin to decrease. A critical level in the lipid composition of adipose tissue or its distribution in the body may exist to keep these aquatic birds in a good thermal isolation condition that still allows them to dive in cold water. Perhaps small penguins like Rockhoppers would be more prone to start protein catabolism, showing a more rapid increase in urea level after fasting in a short time due to severe limitation on extra fat stores.

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## Compliance with ethical standards

**Conflict of interest** The authors declared no conflict of interest in this study.

**Ethical approval** Procedures used in this study comply with the current laws for working on the Falklands Islands, as well as with institutional guidelines for the care and use of animals (Spanish Council (CSIC) Ethical Committee). Permits to work in the study area and to take blood samples of Rockhoppers Penguins were granted by Falkland Government (number of license: R12/2014), as well as by the owners' of the Saunders Island.

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