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ORIGINAL ARTICLE

Plasma biochemistry and haematology of crested coots (*Fulica cristata*) and common coots (*Fulica atra*) from Spain

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Abstract We provide the first reference values of the plasma biochemistry and haematology of Palaearctic coots of the genus *Fulica*. We analysed blood samples from 29 birds of the threatened crested coot *Fulica cristata* reared in captivity and 21 common coots, *Fulica atra*, from a wild environment. We report haematocrit levels, leucocyte formula, metabolites (uric acid, urea), substrates (cholesterol, triglycerides) and enzymes (lactate dehydrogenase, creatine kinase). No differences were found between sexes within species. Except for monocytes and basophiles, significant inter-species differences were found for all blood and biochemical parameters. In general, mean values were higher for wild common coots. These results might suggest the better state of captive birds with regard to wild ones, likely due to differences in environmental conditions. Nevertheless, we cannot rule out the possibility that some of the inter-species differences reported in this study can be for phylogenetic reasons, so that the results reported here should be taken as reference values for Palaearctic coots.

Keywords Captive breeding · *Fulica cristata* · *Fulica atra* · Haematological parameters · Plasma biochemistry · Waterbirds

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Introduction

The crested coot (*Fulica cristata*), also known as the red-knobbed coot for the two small protuberances in its forehead which resemble a pair of horns, is one of the species at risk of extinction in the Iberian Peninsula. The waterbird is classified as being "in danger of extinction" and is protected by the Bird Directive of the European Union and the Berne Convention. It is also preserved by ornithologists of SEO/Bird Life (declaring it Bird of the Year in 2002), identifying as its principle threats: shooting, the loss and degradation of its habitat in wetlands, the excessive cattle load in these ecosystems, and fishing. All these elements are in the marshes of the Guadalquivir (around the Doñana Biological Station), where this bird survives with great difficulty (Amat and Raya 2004; Amat and Green 2010; Máñez et al. 2010)

The Spanish population of the threatened crested coot (*F. cristata*) is currently estimated as less than 100 birds, with few pairs breeding in the southern area (Díaz et al. 1996; BirdLife Int. 2000; Viedma 2002). On the other hand, the wintering population of common coots (*Fulica atra*) totals 150,000 birds, with thousands of breeding pairs (Díaz et al. 1996). Owing to this situation, a currently ongoing action plan aims at recovering the Spanish population of crested coots, mainly by releasing birds reared in captivity (BirdLife Int. 2004; Ballesteros et al. 2008). Nevertheless, to date, no study has focused on the haematology and biochemistry of this species, even though such studies can yield valuable insights into the nutritional and physiological status of individuals and their possible pathologies (García-Rodríguez et al. 1987a; Ferrer et al. 1994; Dawson and Bortolotti 1997; Rubio et al. 1999; Galkina et al. 2005; Cafarchia et al. 2006), immunological (Lvov et al. 2008) and parasite (Plutzer and Tomor 2009) studies. Moreover, conducting such studies in captive birds of threatened species is particularly relevant if those birds are to be released into the wild, as in this case.

Haematological samples are easy to obtain from birds, so most studies have focused on this tissue in order to make inferences on the physiological condition of individuals of single species, particularly birds of prey (García-Rodríguez et al. 1987b; Ferrer and Dobado-Berrios 1998; Villegas et al. 2002). On the other hand, comparative studies on the biochemistry and haematology of closely related species are scarce, and most of them have focused on the analysis of birds from several orders (Gee et al. 1981). We conducted a comparative analysis on the haematology and plasma biochemistry of crested coots reared in captivity and wild common coots from Spain. Since both species have similar ecological requirements (see Díaz et al. 1996), this study may provide important clues on the potential adaptability of crested coots released into wild conditions, as suggested by the basal values obtained from wild common coots. Moreover, although some studies have been conducted on the haematology of the American coot (*Fulica americana*) in the Nearctic (Newman et al. 2000), to our knowledge this is the first time that a comparative blood analysis has been performed on wild and captive *Fulica* species in the Palaearctic.

Material and methods

Animals

A total of 50 birds were used: 29 crested coots (13 males and 16 females) and 21 common coots (8 males and 13 females). Crested coots reared in captivity in the "La Cañada de los Pájaros", declared Natural Concerted Reserve of the Department of Environment in 1991 (the first one in Andalusia), are included in the RENPA (Network of Natural Species of Andalusia), in the Inventory of Wet Areas of Andalusia and in the Andalusian Plan for Wetlands, situated in southwestern Spain (lat, 37°14′52.58″N; long, 6°8′0.6″W) at 10 km north from Doñana Park. Common coots from natural surroundings in this area (see Ildefonso 2000).

In "La Cañada de los Pájaros", crested coots are maintained in semi-freedom, which closely resembles wild conditions. Nevertheless, birds were fed ad libitum with food based on floating breeding feed (gross protein, 20.5 %; gross fat, 5.3 %; gross cellulose, 4.5 %; gross ash, 7.25 %; methionine, 0.37 %; vitamins, amino acids, and oligoelements), rice and wheat (Ildefonso 2000). There was also a supplementary supply of natural food typical of the ecosystem in the lakes where they are raised, mainly composed of water hyacinths, water fern, the fish *Gambusia hollbrooki*, insects, as well as zooplancton and phytoplancton. Diet for free-ranging common coots was not controlled.

Samples from the *F. cristata* were taken in September, and those of *F. atra*, in February. Sample taking began at approximately 1100 hours and finished at about 1500 hours in both species, to minimise variation in blood parameters due to the circadian rhythm (García-Rodríguez et al. 1987b). The following protocol was followed for the sexing of each bird: since they comprised several ages and there is no sexual dimorphism in those species, the sexual differentiation *being* made according to the voice, shriller in the case of the males and deeper in the females (Kornowski 1957). Next, weighing was done on a Precisa 3000D scale (Pag Oerlikon Ag, Zurich, Switzerland). Then, approximately 2 ml of blood was extracted per animal, using the metatarsian vein. For the venipuncture, disposable needles of 0.5×16 and syringes of 2 cm^3 were used. Blood was collected in 1.5-ml sterile tubes with heparin-lithium anticoagulant and was centrifuged in the first 5 min of its extraction with a P Selecta (Ref.: S-240,J.R. Selecta SA, Barcelona) field centrifuge. The rest of the blood from the same syringe was used to fill a capillary tube and centrifuged in an M 1100 centrifuge (Labnet International) to determine the haematocrit value and to perform a blood smear to count the leucocytes. The plasma was taken in a portable icebox to the laboratory, in which the plasma was frozen (−80 °C) up to the analysis date.

The studies were performed in accordance with the ethics standards of the Committee on Animal Experimentation of Cordoba University.

Haematocrit and leucocyte formula

The haematocrit value was obtained by the microhaematocrit method (Jain 1993a). The differential counts of leucocytes (lymphocytes, heterophils, eosinophils, basophils, and monocytes) were made with an Olympus model BH-2 microscope (Olympus Optical CO-LTD, Japan) and with a Sony Trinitron, model Kx14CP1 screen incorporated. The staining used for it was that of May–Grünwald–Giemsa specially prepared for bird blood.

Biochemical analysis

Once the plasma was unfrozen, a total of six biochemical parameters were analysed (two metabolites: uric acid and urea; two substrates: cholesterol and triglycerides; and two enzymes: lactate dehydrogenase (LDH) and creatine kinase (CK)). In all cases, the SERAPAK® protocols were followed, and its reagents used for the chemical reaction. The absorbance was measured with an Ames model Quik Lab spectrophotometer. For each series of determinations, a standard and a blank were used.

Data analysis and inter-species comparisons

Average biochemical and haematological values were compared between sexes within species, and between species,

with one-way analyses of variance (ANOVA; Zar 1999). Due to the low sample size, the power of the statistical tests ($1-\beta$, Zar 1999) was fairly low for some analyses. Therefore, the P level of each test was assessed with Monte Carlo simulations (Manly 1997). For each analysis, 10,000 randomized samples (null models) were generated from the original data, and the P value was then estimated as the proportion of times that the empirical value was lower than the statistics estimated from the randomized samples (Manly 1997). When normality and homoskedasticity assumptions are met, the power of formal parametric tests and randomization procedures is fairly equivalent; when this is not the case, the randomization technique gives a larger power (Manly 1997). To control for the type-I error dispersion when performing multiple simultaneous tests, individual values were adjusted with the sequential Bonferroni technique (Rice 1989). Thus, prior to the analyses, data were $\log_{e-}$ transformed in order to stabilize their variance (Zar 1999), which further increased the robustness of the analyses. Unless otherwise stated, values are mean±standard deviation. Power was estimated with the software Gpower 2.0 (Faul and Erdfelder 1992), and the Monte Carlo simulations were carried out in EcoSim 7.0 (Gotelli and Entsminger 2001). $P \leq 0.05$ was taken as the minimum significance level.

Results

In the results, significant differences were found in the mass of males (897.26±164.22 g, $N=12$) and females (800.05± 108.93 g, $N=17$) ($F_{1,\ 27}=6.175$, $P=0.018$). Values ranged between a minimum of 542.2 g in females and a maximum of 1,086.7 g in males. For this reason, biochemical and haematological values were contrasted between sexes within species (two levels), and no significant differences were found either in the common coot or in the crested coot for any of the parameters. Therefore, a comparative analysis was performed between both species by pooling males and females within species.

Means±standard deviations of the haematological and biochemical parameters are shown in Tables 1 and 2, respectively, for both coots. The coefficients of variation for each of the parameters are also shown in the table.

Among the haematological parameters, the haematocrit, heterophil, and eosinophil percentages are significantly higher in common coots than in crested coots, while the lymphocyte percentage is higher in crested coots than in common coots. The basophil and monocyte percentages do not show any significant differences between species although the coefficients of variation of both parameters, together with the lymphocyte percentage, display a great variation within one same species.

All the biochemical parameters are significantly higher in common coots than in crested coots, with the highest coefficients of variation being detected in both species for the enzymes LDH and CK.

Discussion

Weighing

Significant differences were found in the body weights for males and females. This had already been reported by several authors (Fairall 1981; Cramp and Simmons 1994; Brinkof 1997). However, the higher weight of the males compared to the females did not signify any significant differences between sexes for any of the haematological or biochemical parameters analysed.

Haematological analysis

F. atra showed a higher haematocrit value than *F. cristata*. These values were similar to those reported by Bond and Gilbert (1958) for the common coot (46 %) and by Newman et al. (2000) for the American coot (42 %), with significant differences existing between the two coots analysed in the current work. It is interesting to note the low coefficients of variation in both species. Likewise, the haematocrit values lie within those given by Sturkie (2000) for other bird species (hen, turkey, pheasant, red-tailed falcon, great-horned owl, quail, diving duck, Andean-ruddy duck) with values of between 29 % (hen) and 58.5 % (pigeon). Huchzermeyer (1994) also verified how haematocrit values can enormously vary physiologically within one same species, describing values of between 30 and 61 % for the ostrich (*Strutio* sp.) although Campbell and Coles (1989) considers that a haematocrit of below 35 % corresponds to a state of anaemia.

Significant inter-species differences were found for mean lymphocyte, heterophil, and eosinophil values, but, on the contrary, the basophils and monocytes did not show any significant differences. This could be due to the leucocyte formula undergoing large variations because of many factors such as age, nutritional status, ambient temperature, etc. (Sturkie 2000). In fact, the coefficient of variation of both parameters was particularly high. For that reason, some authors question the use of the leucocyte count in birds (Lucas and Jamroz 1961; Polo et al. 1992). Second, these two parameters usually have a very low range of variation in most species (0 to 5 % in basophils and 0 to 10 % in monocytes), which might perhaps be an additional explanation for the inexistence of significant differences between species. In short, the leucocyte formula of both coots is of the lymphocyte type (Table 1), as occurs with the hen (Jain

Table 1 Inter-species comparison of mean percentages in several haematological parameters in crested and common coots

Blood parameters	Crested coot[a]	Common coot[a]	*P* value[b]	$1-\beta$[c]
Haematocrit (%)	37.5±3.13 (29), 8.34 %	41.04±5.8 (21), 14.13 %	**0.018**	0.88
Lymphocytes (%)	78.51±9.88 (29), 12.58 %	67.85±11.54 (20), 17 %	**0.000**	0.96
Heterophils (%)	18.06±7.66 (29), 42.41 %	26.7±10.41 (20), 38.98 %	**0.002**	0.91
Eosinophils (%)	0.79±1.23 (29), 155.69 %	1.95±2.62 (20), 134.35 %	**0.028**	0.59
Basophils (%)	0.75±1.55 (29), 206.66 %	0.55±1.23 (20), 223.63 %	0.588	0.08
Monocytes (%)	2.20±3.77 (29), 171.36 %	2.2±3.56 (20), 161.81 %	0.975	0.05

[a] Values shown are mean±standard deviation (sample size), coefficient of variation

[b] *P* value of the ANOVA was obtained after conducting 10,000 Monte Carlo simulations with the original dataset (see Manly 1997). Significant values are shown in bold type

[c] $1-\beta$ power of the test (probability of rejecting the null hypothesis when it is in fact false (Zar 1999)

1993b) and in most birds except for predators (Polo et al. 1992). Nevertheless, note that Newman et al. (2000) obtained a mostly heterophyllic leucocyte formula for the American coot (51 %).

Plasma biochemistry

The mean values of uric acid obtained in this work for both coots (Table 2) lie within the range reported by Campbell and Coles (1989) for most birds (2–15 mg/dl), by Gee et al. (1981), who reported a range of 0.9–12.4 mg/dl for species of the Gruiformes order (coots, cranes, and rails), and by Rodríguez et al. (2004) for red-legged partridge (*Alectoris rufa*), which ranged between 6.8 and 10.6 mg/dl. However, the highly significant inter-species difference for this parameter in spite of the large coefficient of variation for both coots (Table 2) should be noted. Similarly, the range indicated by Newman et al. (2000) for the American coot (6–9 mg/dl) is within the margin shown in this work for the common coot. On the other hand, the values for the crested coot are similar to those obtained by García-Rodríguez et al. (1987a) for the buzzard (*Buteo buteo*) in a fasting period (3 mg/dl), although the individuals in this sample have not undergone any fasting.

Significant differences in urea were also found between species, although in this case, the crested coot showed an average value comparable to that found in other bird species, mainly raptors. For these species, values ranged from 7 mg/dl in the buzzard to 14.9 mg/dl in the vulture (Balasch et al. 1976). Additionally, values were very similar to those obtained by Ferrer et al. (1994) in chinstrap penguins (*Pygoscelis antarctica*) in Antarctica. Nevertheless, the urea values of the common coot found in this study (43.3 mg/dl) were far higher than values found for most birds. Nevertheless, García-Rodríguez et al. (1987a) indicate that the buzzard can reach 50 mg/dl of urea when going through a period of food shortage. Since samples were collected during the winter, a season characterized by scant food availability and variety for free-roaming coots (Perrow et al. 1997; Schmieder et al. 2006), large urea values, as well as cholesterol levels (see below), might suggest a deficient feeding of free-ranging coots, the same as occurred in the buzzard.

Mean cholesterol values (Table 2) differed very significantly between species with a similar mean for common coots to that indicated by Newman et al. (2000) for the American coot (264 mg/dl). Mean values of plasma cholesterol which reach, or exceed, those we found for the *F. atra*

Table 2 Inter-species comparison of mean values in several biochemical parameters in crested and common coots

Biochemical parameters	Crested coot[a]	Common coot[a]	*P* value[b]	$1-\beta$[c]
Uric acid (mg/dl)	2.22±1.14 (28), 51.35 %	7.43±2.47 (21), 33.24 %	**0.000**	1.00
Urea (mg/dl)	8.74±3.35 (19), 38.32 %	43.3±17.6 (20), 40.64 %	**0.000**	1.00
Cholesterol (mg/dl)	157.25±44.45 (24), 28.26 %	251.61±52.85 (21), 21 %	**0.000**	1.00
Triglycerides (mg/dl)	116.42±46.49 (27), 39.93 %	172.23±68.06 (21), 39.51 %	**0.001**	0.95
Lactate dehydrogenase (IU/L)	575.66±451.97 (23), 78.51 %	985.73±394.22 (14), 39.99 %	**0.004**	0.78
Creatine kinase (IU/L)	189.16±274.06 (16), 144.88 %	1841.93±1450.19 (12), 78.73 %	**0.000**	0.99

[a] Values shown are mean±standard deviation (sample size), coefficient of variation

[b] *P* value of the ANOVA was obtained after conducting 10,000 Monte Carlo simulations with the original dataset (see Manly 1997). Significant values are shown in bold type

[c] $1-\beta$ power of the test (probability of rejecting the null hypothesis when it is in fact false (Zar 1999)

are only observed in some species like pigeons, 407–468 mg/dl (Lofland and Clarkson 1960), and in the herring gull (*Larus argentatus*), 331.9 mg/dl in well-fed animals and 391.8 mg/dl in starving birds (Jeffrey et al. 1985). Starving has been related to an increase in the level of blood cholesterol due to the mobilization of fats existing in the organism (Christie 1979; Galvin 1980; Black 1981). The higher value of plasma cholesterol in the common coot than that of the crested one might be due to food shortage, corroborated by the equally high level of urea described previously.

The triglyceride levels (Table 2) obtained in the current study are similar to those reported by Gee et al. (1981) and Livezey (1998) for *gruiform* birds (ranging between 102 and 190 mg/dl) and, in general, to values found for other bird groups. Jeffrey et al. (1985) verify how, in the seagull, the triglyceride levels dropped as the birds changed from "ad libitum" feeding (164.7 mg/dl) to a 4-day fast (149.4 mg/dl). Rodríguez et al. (2004) also found this fact in the red-legged partridge. The higher values found for *F. atra* (172.23 mg/dl) compared to 116.42 mg/dl for *F. cristata*, in spite of the latter being a larger bird, may be due to what was reported by Jenni-Eirmann and Jenni (1991, 1992) who observed that triglyceride values are higher in migratory birds since they constitute the important source of energy mobilized from the fat deposits in order to permit them to migrate. In this sense, Nuernberg et al. (2011), when analysing the composition of fatty acids in the intramuscular fat of wild birds, among them *F. atra*, observed that the content of polyunsaturated fatty acids was higher in those animals than that indicated for the domestic farm animals.

On analysing the enzyme activity of lactate dehydrogenase (LDH), significant differences could also be noted between both species with higher mean values than those found by Newman et al. (2000) for the American coot (377 IU/L), although this author found a great variability even within the same species depending on the sample collection date, sex, handling conditions, days in captivity, etc. In our work, there was a very high dispersion in the results obtained; for instance, the crested coot reached a maximum of 1,835.75 IU/L and a minimum of 92.85 IU/L, and the common coot between 1,678.60 and 307.14 IU/L. Campbell and Coles (1989) pointed out that haemolysis can cause an increase in the plasma values of LDH since the latter is found inside erythrocytes which, when bursting, release it. In some birds, like Psittacidae, haemolysis does not produce any increase in the LDH value (Ivins et al. 1985; Galvin 1980), but nothing is known in this regard about coots. There was a high degree of haemolysis in the samples of the birds studied, generally lower in *F. cristata* than in *F. atra*. This could therefore be the reason why the results for the latter could be higher than those of the former bird. It could also explain the wide range of values obtained for both species since each sample had a different degree of haemolysis, thus affecting the result in a different way.

Significant inter-species differences were also found in CK average values (Table 2) despite the large standard deviations found in both coots, above the average in the crested coot, which indicates a great dispersion of the data, coinciding with mean data supplied by Newman et al. (2000) ranging between 669±987 and 1,981±1,411 IU/L in the American coot. For domestic birds in general, Campbell and Coles (1989) report values ranging between 100 and 200 IU/L. On the other hand, CK levels in raptors range from 115 to 2,495 IU/L, with mean values of 300 IU/L (see Hernández et al. 1990; Polo et al. 1992; Knuth and Chaplin 1994; Rubio et al. 1999). There could be several reasons for an increase in CK levels in birds, but the most likely one is intensive physical exercise (Tripp and Schmitz 1982; Knuth and Chaplin 1994) especially if their training is deficient.

In conclusion, the common coot showed higher average blood values than the crested coot, and this could be the result of the great environmental differences between birds reared in captivity and birds captured from the wild. Indeed, captive *F. cristata* seemed to be in a better physical condition than wild *F. atra*, which further suggests that their release into nature could be highly successful. Moreover, several birds reared in captivity and released to the wild are currently breeding successfully in several Spanish wetlands (Viedma 2002). Nevertheless, we cannot rule out the possibility that some of the inter-species differences reported in this study can be due to phylogenetic reasons (Sturkie 2000), so the results reported here should be taken as reference values for Palaearctic coots.

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