Response of the haematocrit to body condition changes in Northern Bald Ibis *Geronticus eremita*


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Abstract

We study the usefulness of the haematocrit as a predictor of body condition in birds, using a captive population of the endangered species Northern Bald Ibis (*Geronticus eremita*). This population is 14% of the worldwide captive population, which is far greater than the known free-living population. The haematocrit, body mass and body condition index responded in the same sense to two different nutritional periods, and there was a statistically significant relationship between changes in condition index and haematocrit of individual birds between the two periods. We discuss the relationship of these parameters with subcutaneous fat and muscle mass, and analyse the individuals’ nutritional status in each of the periods studied. The conclusion was that the haematocrit is sensitive to variations in body condition since it responds to mass-loss processes corresponding to phases of mobilization of fat reserves, a situation prior to the mobilization of muscle proteins when there is a manifest deterioration of the individual’s aspect.

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1. Introduction

There have been numerous studies of the physical condition of birds (Johnson et al., 1985; Beintema, 1994; Brown, 1996; Dawson and Bortolotti, 1997a,b; Jenni-Eiermann and Jenni, 1998; Alonso-Álvarez et al., 2002), and of which variables might be used as measures of that condition with as little effort as possible. Biochemical and blood cell parameters have commonly been employed as useful non-invasive indicators of the health or nutritional status of many species (Jenni-Eiermann and Jenni, 1998). An example is the haematocrit defined as the percentage of the total blood volume occupied by erythrocytes, which has occasionally been used as an indicator of the health of both captive and free-living individuals (Brown, 1996; Horak et al., 1998). There has been discussion of the true utility of this parameter in the case of birds (Dawson and Bortolotti, 1997a; Bearhop et al., 1999). The haematocrit depends on the variation in plasma volume, the rate of erythrocyte production and destruction (a process in which haemolytic diseases or blood parasites may be involved), dehydration, toxins, and direct blood loss as a result of injury or blood-sucking ectoparasites (LeResche et al., 1974; Whitworth and Bennet, 1992; Dawson and Bortolotti, 1997a), and it may hence be used as an index of the ‘health’ of the oxygen transport system.

The haematocrit has been related to bird life history variables, with many studies noting its different responses to a variety of events or life processes, such as exercise (Snyder et al., 1981), increased brood size (Horak et al., 1998), egg-laying in the case of females (Jones, 1983), time of day (Lyons, 1996), passage of the seasons (Morton, 1994), altitude (Clemens, 1990), thermoregulation (Swanson, 1990), hypoxia (Sturkie, 1986), and para-
site load (Potti et al., 1999), inter alia. But, as is noted by Dawson and Bortolotti (1997a), there have been few avian studies of the relationship between haematocrit and the individual’s condition, having simply been assumed that birds in better physical condition or that are parasite free have a higher haematocrit than those in worse condition or with parasites.

Here we looked at the relationship between physical condition and haematocrit in a captive population of Northern Bald Ibis (Geronticus eremita) as part of a broader project studying the fitness of individuals of different species (Bucerotidae, Gruidae, Cracidae) that are candidates for use in reintroduction campaigns. G. eremita is an endangered species (Birdlife Intl., 2000) whose current distribution area is 580 km² and is present in only three breeding colonies (Birdlife, 2000). Estimates of the population size vary between 200 individuals (Birdlife Intl., 2000) and 400–450 individuals (Rose and Scott, 1994). The captive population is larger—about 900 individuals (International Zoo Yearbook, 1998). There is added value to the present study therefore in that it could prove to be an instrument of great utility in the management of these populations and of the species globally, especially as the study population is 14% of the total captive population.

2. Materials and methods

The study was performed during 2000 and 2001 with a captive population of 128 Northern Bald Ibis individuals in the ‘El Retiro’ Ornithological Park (Málaga, Spain). These birds came from other captive populations whose origin was within the species’ western free-living populations, probably Morocco. Individuals were housed in two 20-m long, 5-m wide and 3.5-m high outside aviaries constructed from wire mesh on a metal framework, with nest boxes, branches for perching and a shallow pool for drinking and bathing, they were kept under normal conditions of temperature and light, and were supplied with water ad libitum. In the first study year, the individuals were fed twice daily a diet consisting of a mixture of boiled rice, minced calf heart, grated carrot, and fish meal (6% proteins, 2.7% fat, 7.6% carbohydrates, 0.7% fibre, 0.1% calcium) and a commercial granulated pellet (15% total proteins, 6% fat, 5% cellulose, 14% ash, and vitamins A, D, and E as dietary supplement). In December 2000, administrative changes at the Park led to a change in diet; and they were fed twice daily with the granulated pellets only, the rest of the conditions remaining the same as before.

In March of 2000 and 2001 blood samples were taken from the same 70 adults. In order to avoid metabolic interference with the haematological parameters, blood samples were taken after a 12–15 h fast. At the time of sampling, the individuals were weighed on Pesola scales (precision±20 g), and seven linear measurements of size were taken with calipers (precision=0.05 mm): lengths of the hand, eighth primary, tail, head, culmen, tarsus, and keel. The subcutaneous fat reserves were determined using the 0–8 scale described by Kaiser (1993), and the size of the pectoral muscles on a scale of 0–3 (Pinilla, 2000). All birds had previously been examined and considered clinically normal by the centre’s veterinary team.

The blood samples were taken from the brachial vein and transferred to a collecting tube from which a small amount was immediately drawn off into a heparinized microcapillary tube for the haematocrit determination. All samples were kept in portable refrigerators at 4 °C for transport to the laboratory within 1 h of sampling. All samples were taken between 08:00 and 11:00 h.

The microcapillaries and collection tubes were centrifuged at 13,000×g for 15 min, and the haematocrit determined directly in a microhaematocrit reader. Plasma was stored at −20 °C (maximum 1 month) until analysis. Plasma levels of uric acid, total proteins, glucose, cholesterol, and triglycerides were assayed in a multi-parameter autoanalyser (Falcon 300, Menarini Diagnostics) using the reagents recommended by Menagent (Menarini Diagnostics). Since the volume of blood extracted varied between individuals, in some samples the amount of plasma obtained was insufficient to assay all the aforementioned parameters. The sample sizes are therefore not uniform. The cell fraction was used to sex the individuals following the protocol described by Fridolfsson and Ellegren (1999).

For the identification of blood parasites, each year a drop of blood from 14 birds was smeared over a microscope slide, air-dried, fixed in absolute methanol, and stained with Giemsa. Half of each smear was examined under 200× magnification searching for large extra-erythrocytic haematozoa (e.g., Trypanosoma), and in the other half 20 fields were scanned under 400× magnification for intra-erythrocytic haematozoa (e.g., Haemoproteus). The intensity of the extra-erythrocytic parasite load was quantified as the number of parasites per 100 fields, and the number of parasites per 2000 erythrocytes for intra-erythrocytic parasites, under 1000× magnification with oil immersion (Merino and Potti, 1995; Merino et al., 1997).

To ensure that the experiment was not influenced by the ambient temperature, a circumstance that has been indicated by certain workers, we compared the temperatures of the week of blood sampling and of the four previous weeks between the 2 years. There was not a statistically significant difference (P>0.1) in either comparison.

The linear measurements were subjected to a principal component analysis, and the first component (PC1) was used as a body structural size index. Mass of an animal is partly a function of its structural size, so that to obtain an index of body condition it is necessary to control for body size (Dawson et al., 2001). The residues of a linear regression with mass as the dependent variable and PC1 as the independent variable were used as an index of an individual’s physical condition (Villegas et al., 2002).
The mean values of the haematocrit, biochemical parameters, mass, condition index, subcutaneous fat reserves, and pectoral muscles were compared between years using Bonferroni-adjusted paired sample tests for the means. Correlation analysis was used to assess the relationship between haematocrit and condition index of individual birds. Whenever possible, parametric statistics were used to analyse the data, but when the data did not satisfy the assumptions of normality and homoskedasticity (Zar, 1999), non-parametric statistics were used. In all statistical analyses \( P<0.05 \) was taken as the statistical significance level.

3. Results

None of the measured variables showed significant differences between birds housed in the two aviaries in either year, therefore all birds were pooled for subsequent analysis. There were no differences in haematocrit between the sexes in either year (2000: \( t=1.76, df=55, P=0.08 \); 2001: \( t=0.44, df=55, P=0.66 \)). The mean for the first year was 46.32 (36.7–56) and for the second 42.91 (36–50), with the variability being greater in the first year (Table 1, Fig. 1) and with statistically significant differences between the two (paired \( t \)-test: \( t=5.25, df=56, P<0.001 \); Fig. 1). The mean body mass was greater in the first year, 1248 (980–1571) g, than in the second one, 1174 (880–1430) g, the differences being statistically significant (\( r=4.88, df=69, P<0.001 \); Fig. 1). The individuals’ condition index also differed between the two years, with a greater mean value in the first (year 2000: \( n=62, \bar{x}=45.2, SD=3.6, \text{Range}=36.7–56 \), \( t=3.27, df=69, P<0.01 \); Fig. 1). The trend was the same in the subcutaneous fat reserves (Wilcoxon test: \( T=180, n=67, P<0.001 \); Fig. 2), while the differences found in the case of pectoral muscle size were not significant (\( T=18, n=68, P>0.1 \); Fig. 2).

The biochemical variables presented different trends (Table 1). The mean values obtained in 2000 were greater for glucose, uric acid, total proteins, and cholesterol, whereas for the triglycerides they were higher in 2001. These differences were statistically significant in all cases except for glucose and total proteins (Table 1). None of the samples presented any blood parasites.

| Table 1 |
| Haematocrit, plasma metabolites levels and body mass in Northern Bald Ibis (G. eremita) during the two study years |
| Year 2000 | Year 2001 |
| \( n \) | Mean±SD | Range | \( n \) | Mean±SD | Range |
| Haematocrit (%) | 62 | 46.3±3.6 | 36.7–56.0* | 64 | 42.9±2.9 | 36.0–50.0 |
| Triglycerides (mg/l) | 53 | 865.0±431.0 | 370.0–2580.0* | 61 | 2011.0±638.0 | 790.0–4760.0 |
| Cholesterol (mmol/l) | 57 | 6.5±1.5 | 4.1–11.5* | 66 | 4.2±0.9 | 1.8–9.3 |
| Glucose (mmol/l) | 57 | 12.5±2.4 | 7.1–21.1 | 67 | 12.3±3.3 | 1.1–18.7 |
| Uric acid (mmol/l) | 56 | 0.6±0.3 | 0.2–1.3* | 68 | 0.4±0.2 | 0.1–1.2 |
| Total proteins (mg/l) | 57 | 32.0±5.0 | 9.0–41.0 | 68 | 31.0±4.0 | 24.0–50.0 |
| Body mass (g) | 70 | 1248.3±122.3 | 980.0–1571.0* | 70 | 1174.4±121.0 | 880.0–1430.0 |

* Differences between years according to Bonferroni adjusted paired-sample \( t \)-test.

Fig. 1. Haematocrit, body mass, and condition index in the Northern Bald Ibis (G. eremita) individuals during the two study years.

There was a significant and positive correlation between haematocrit and condition index in 2000, but not in 2001 (2000: \( r=0.37, n=62, P<0.01 \); 2001: \( r=0.14, n=64, P>0.1 \).
The changes in condition index of individual birds were positive and significantly related with changes in hematocrit values ($r=0.29$, $n=60$, $P=0.027$).

4. Discussion

The hematocrit values found lie within the range reported for this and other species of Ciconiiformes in captivity (Celdrán et al., 1994; Dutton et al., 2002). Many studies have discussed the variation in this parameter in response to a variety of circumstances, including an increase in hematocrit with exercise (Snyder et al., 1981). The cage size did not vary, however, between the two sampling dates, so that one must assume the exercise to have been similar in the two cases. The samples were taken sufficiently before the beginning of the breeding cycle for the onset of breeding behaviour (Sturkie and Griminger, 1976), brood size (Horak et al., 1983), or onset of moult (Morton, 1994). Changes due to age (Potti et al., 1999) mainly affect the first years of life, and in our case all the animals studied were at least 5 years old. Furthermore, there was no variation in the prevalence of parasites between the two years, so that differences in the destruction of red blood cells due to such a circumstance must be assumed to be negligible.

While some studies have previously concluded (Dawson and Bortolotti, 1997a) that birds only present notable variations from their normal hematocrit levels when they are in a limiting situation, in such poor physical condition that their status is already obvious from their aspect alone, the present results show that in this population there is a relationship between hematocrit and physical condition and body mass. This seems to show that the hematocrit responds to non-extreme changes in these last two parameters. Indeed, the prior veterinary examination of the ibises found no problems at all in the individuals used for the samples, and furthermore there were no differences in pectoral muscle size between the 2 years. The problem lies in the complexity involved in isolating all the factors that might determine an alteration in haematopoiesis. If this is stimulated by phenomena of tissue hypoxia (Rosse and Waldmann, 1966; Burton and Smith, 1972; Weinstein et al., 1985; Sturkie, 1986), then one would have to expect that periods of high ingestion-combustion, or the aforementioned increase in exercise (Snyder et al., 1981), would lead to at least slight variations in hematocrit. It is no less certain, however, that any of these processes prolonged over time should lead to an improvement in the individuals' condition since many of the aforementioned influencing factors (breeding phenology, clutch size, brood size, etc.) need the bird to already be in or to acquire good physical condition, in which the efficacy of oxygen transport is fundamental (Dawson and Bortolotti, 1997a). Thus, whereas a low hematocrit may be indicative of disease (Harrison and Harrison, 1986), it does not necessarily have to reflect a pathological disorder since it may simply be the result of poor nutritional status (Merilä and Svensson, 1995), including anaemic processes, as has been demonstrated in other bird species (Boiesmenu et al., 1992). Indeed, hematocrit levels fall during fasting (Boiesmenu et al., 1992), possibly because haematopoiesis partially depends on the aforementioned nutritional status (Merilä and Svensson, 1995).

The positive association of hematocrit with body mass, condition index, and subcutaneous fat reserves indicates that the variations in this parameter, once the effect of the variables mentioned above has been isolated, is directly related to the status of the individuals at the times of the two blood extractions. Body mass is a widely used measure indicating an individual's energy reserves (Johnson et al., 1985). While changes in mass may provide an adequate picture of the alterations that occur in these reserves for a given individual status (Blem, 1990; Jenni-Eiermann and Jenni, 1994; Horak et al., 1998), the condition index, which corrects the mass for the individual's body size, is a better
approximation of its status (Johnson et al., 1985). Also fat deposits are the main source of reserve energy for most birds, and constitute both a major buffer against environmental unpredictability (Merilä and Svensson, 1995), especially with respect to food availability, and a source of energy for activities involving exercise without pauses for feeding (Blem, 1990; Witter and Cuthill, 1993). How much fat is stored is interpreted as being the solution to an optimization problem in which the amount is determined by different selection pressures (Merilä and Svensson, 1995). When the individuals lose mass, these fat reserves, mainly triglycerides, are hydrolysed releasing glycerol and fatty acids into the plasma. These replace glucose as the fuel for respiration in certain tissues (Williams et al., 1999). Hence, the variations in body mass, condition index, and subcutaneous fat reserves that we found in the individuals of this study point to those of the first year being in better physical condition. Similar conclusions were reached by Svensson and Merilä (1996) who found higher haematocrit levels in Parus caeruleus with higher levels of subcutaneous fat, and by Potti et al. (1999), who found that Ficedula hypoleuca chicks in better physical condition (as measured by their mass and the development of the pectoral muscles) also had greater mean haematocrit values, suggesting that this parameter is sensitive to the conditions experienced by the chick during its growth.

The phenology of the use of an organism’s energy resources is in the order of carbohydrates, lipids, and finally proteins (Jenni-Eiermann and Jenni, 1998). Thus an individual with low lipid reserves may find the energy it needs in other resources such as muscle proteins (Martins and Wright, 1993). One must suppose therefore that the first body mass changes during processes of fasting or poor nutrition are the direct consequence of losses of fat reserves, and that only in very prolonged periods will catabolism of muscle proteins be used as an energy source. Our results would thus indicate that in the second year the animals were in a poorly nourished state, using catabolism of their stored fats as a supplementary energy source, but not that of muscle proteins since there were no differences in pectoral muscle mass between the two sampling periods.

Concentrations of plasma metabolites, as is the case with body mass or subcutaneous fat, reflect the physiological status since they are the direct consequence of intermediate metabolism (Jungermann and Möhler, 1980) and can therefore be used as indicators of that status, helping to provide a clearer picture of the relationships described above. We observed a variation in the same direction as the haematocrit values for the concentrations of uric acid and cholesterol, and in the opposite direction for triglycerides. Cherel et al. (1988) distinguish three metabolic states—feeding, fasting, and starvation—that may correspond to five physiological states (Jenni-Eiermann and Jenni, 1998). For each state, there is a corresponding increase in the concentrations of certain plasma metabolites. Thus, some parameters such as glucose vary little from one state to another and therefore have little or no predictive value. Uric acid, however, is the end product of protein catabolism (Robin et al., 1987; Lindgard et al., 1992), and an increase in plasma uric acid thus indicates the use of dietary proteins, or those of some other origin, while a decrease indicates a fasting phase because of the low protein catabolism (Jenni-Eiermann and Jenni, 1998). The origin of cholesterol is also from the diet, and it has likewise been related in experimental studies with high body condition indices and is claimed to be a good predictor in changes of body mass (Alonso-Álvarez et al., 2002). An increase in the plasma concentration of triglycerides is expected to be due to the predominance of “resorption” processes and a decrease in periods of fasting (Jenni-Eiermann and Jenni, 1998). Diet composition has also been shown to affect plasma triglycerides, with a greater concentration found in birds with rich fat diets (Polo, 1995). The fat content in the diet of the ibises was greater in 2001 than in 2000 (2.7 vs. 6%), which could explain the greater concentration of this metabolite in the second year. Moreover, the triglyceride concentration in our case includes amounts of free glycerol, so that the increase observed between the two years may be the result of the mobilization of fats. Similar findings have been reported in other studies (Totzke et al., 1999; Alonso-Álvarez and Ferrer, 2001) in analogous processes.

According to these results, one may conclude that the haematocrit can be used as a suitable indicator of physical condition in this species, since it responds to slight changes in the birds’ physiological status such as periods of poor nutrition. However, it is necessary to point out the fact that this study has been conducted in a captive population, with a fair degree of control over confounding variables, such as temperature, breeding and moult status, etc. In studies with free-living birds, however, the existence of all the uncontrollable factors that could be affecting birds, may mask the relationships between haematocrit and physical condition and limit the utility of this variable as an indicator of condition. Other measures, such as MCV (mean corpuscular volume), an indication of the change in the rate of production of red blood cells, can add to the information given by haematocrit alone, (Bearhop et al., 1999), and could be a better indicator of condition in these situations.

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