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SHORT COMMUNICATION

Hematocrit is related to age but not to nutritional condition in greater flamingo chicks

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Abstract We measured the hematocrit from greater flamingo chicks *Phoenicopterus roseus* over 4 years to test whether this blood parameter was related to the nutritional condition of chicks, as there are controversial results on whether hematocrit may be used as an index of body condition. We also tested whether hematocrit increased with chick age, as there would be an age-related increase of oxygen demand due to exercising. We found no evidences that hematocrit was related neither to the nutritional nor to the body condition of chicks. Hematocrit increased with chick age, which may be related to the increased requirements of chicks for oxygen delivery during development.

Keywords Body condition · Food quality · Hematology · Packed red cell volume · *Phoenicopterus roseus*

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Introduction

The hematocrit, or packed red cell volume of blood (PCV), is affected by several factors such as season, molt status, migration, ambient temperature, etc. (Morton 1994; Dawson and Bortolotti 1997; Fair et al. 2007). Because a deficient nutrition, especially with respect to protein, leads to anemia, and then to low PCV, this blood parameter has been used to assess body condition (e.g., Johnston and Bilstein 1990; Merilä and Svensson 1995; Sánchez-Guzmán et al. 2004). Nevertheless, other studies found no relationship between PCV and body condition (e.g., Peterson 2002; Villegas et al. 2002), which led some authors to question the usefulness of PCV as an index of body condition (Dawson and Bortolotti 1997; Cuervo et al. 2007).

Greater flamingos *Phoenicopterus roseus* breeding in southern Spain foraged in distant areas 130–400 km from a breeding lake and spent 4–6 days in foraging sites between consecutive visits to the breeding site to feed their chicks (Rendón-Martos et al. 2000; Amat et al. 2005). Under these conditions, the chicks fasted a few days between consecutive feedings (Amat et al. 2007). It has been shown that dehydration during fasting increases plasma cholesterol (Campbell et al. 1994). Because young flamingos in southern Spain do not disperse from the natal site until late summer, they could suffer from dehydration if intervals between parental visits to feed them are long and ambient temperatures are high, and this may be accentuated in wetlands that dry out during summer before chick dispersal, as in the lake where we conducted our study. Dehydration may also be affected by ambient temperatures, PCV being higher when temperatures are warmer (Dawson and Bortolotti 1997), what may be due to the loss of body water when heat-stressed birds thermoregulate through evaporative cooling. Based on cholesterol levels in plasma, we inferred that

greater flamingo chicks with higher levels of cholesterol were experiencing longer fasting, and were in lower body condition, than those with lower plasma cholesterol levels (Amat et al. 2007). Because hydration affects plasma volume, PCV could vary with hydration state (e.g., Vleck et al. 2000; Peterson 2002) and hence with plasma cholesterol. Accordingly, we would expect a positive relationship between PCV and cholesterol level in plasma if PCV is affected by body condition.

Greater flamingo chicks progressively start to exercise their muscles as they grow older, mainly by running and flapping, and when 11 weeks old they also start to perform short flights (Johnson 1992). Therefore, because PCV increases with exercise, this blood parameter should increase with chick age (Gessaman et al. 1986; Puerta et al. 1989; 1992; Villegas et al. 2002).

Here, we report measurements of PCV of greater flamingo chicks over four breeding seasons during which the chicks were not fed with the same frequency and received food of different quality (Amat et al. 2007). We used data collected simultaneously on body condition and on several plasma chemistry parameters, as well as on the feeding state of the chicks (Amat et al. 2007), to test whether (1) PCV was related to the nutritional condition of flamingo chicks and whether (2) PCV increased with chick age. There is some previous information on hematological parameters of free-ranging greater flamingo chicks, but it is based on a limited number of samples collected during a single year and no control of fasting (Puerta et al. 1992).

Materials and methods

The study was conducted at Fuente de Piedra lake in southern Spain (36°06′ N, 4°45′ W). Starting in 1986, about 10% of greater flamingo chicks in the crèche have been captured every year in a single day during a drive for banding purposes. Every year, body measurements (body mass as well as bill, wing, and tarsus lengths) are recorded from every banded chick. From flamingo chicks that were banded in 1998–2001, we collected between 05:00 and 06:00 h (GMT) 1 ml of blood from the brachial or jugular veins in heparinized tubes. From these tubes, we transferred a small amount into microcapillary tubes for PCV determinations. The capillary tubes were sealed with plasticine at the bottom. Depending on year, the number of samples collected varied from 49 to 69.

PCV was recorded as red blood cell volume/[red blood cell volume+plasma volume] and expressed as percentage. The red blood cell and plasma volumes were measured on the capillaries with digital calipers to the nearest 0.01 mm under a magnification glass on three samples per bird after 8-min centrifugation at 11,500 rpm on a portable Bayer Diagnostic micro-hematocrit centrifuge, model M1101 (Bayer Diagnostic, München, Germany). We averaged these measurements for each bird.

Four categories of crop profiles were considered: from 0 when the crop profile was slightly concave (i.e., empty) to 3 when the crop profile was turgid (i.e., full of food) (see Amat et al. 2007). The crop profiles were indicative of the feeding state of chicks, i.e., whether they were fed the night before the samples were collected or they were fasting. We used the first principal axis (PC1) of a principal component analysis on several plasma chemistry variables (from Amat et al. 2007) to obtain a measure of the food quality received by the chicks, as PC1 was positively associated with glucose, triglycerides, uric acid, total proteins, calcium, phosphorus, and magnesium (Amat et al. 2007). Body condition was estimated as the residuals of an orthogonal regression of log-transformed body masses on log-transformed tarsus lengths (Green 2001). PCV values were arcsine transformed to approximate a normal distribution and were used in statistical analysis, although untransformed results are presented for comparative purposes. We used condition-

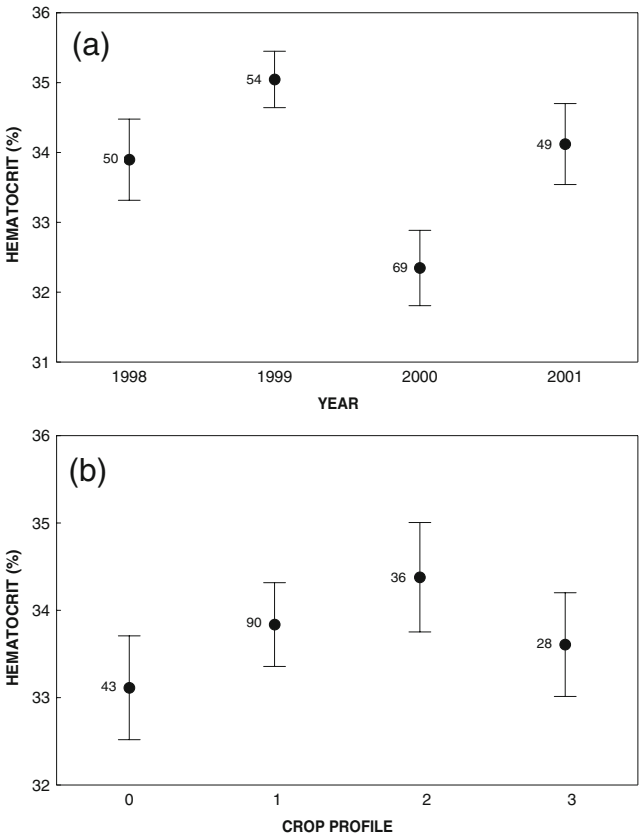

Fig. 1 Variations in hematocrit (±SE) of greater flamingo chicks according to year (**a**) and crop profile (**b**). The crop profiles were allocated to four categories, which varied from concave (crop profile 0) to turgid (crop profile 3) depending on the amount of food that chicks had in their crops. *Numbers beside dots* represent sample sizes

related variables (PC1, cholesterol, body condition index) and chick age (tarsus length) as covariates in an analysis of variance (ANOVA) to test for differences among years and crop profile categories (factors) in PCV.

Results

PCV varied among years (Fig. 1a; ANOVA $F_{3,\ 136}$=3.45, P=0.019), but not with crop profile of chicks (Fig. 1b; $F_{3,\ 136}$=0.41, P=0.747). The interaction year×crop profile was not significant ($F_{9,\ 136}$=0.59, P=0.804). PCV increased with tarsus length ($F_{1,\ 136}$=4.68, P=0.032). Neither body condition ($F_{1,\ 136}$=0.57, P=0.450) nor PC1 ($F_{1,\ 136}$=0.78, P=0.379) nor plasma cholesterol ($F_{1,\ 136}$=1.35, P=0.247) had an effect on PCV.

Discussion

The mean PCV value measured during our study on greater flamingo chicks (33.7%±SD 4.1) was within the range previously reported in another study for free-living and captive flamingo chicks (30−43%) (Puerta et al. 1992). These authors found differences between free-living and captive chicks, and attributed them to age differences between both groups. Similarly, we found that PCV increased with chick age, which may be related to the increased requirements of chicks for oxygen delivery during development (see "Introduction"). We also found annual variations in PCV, which may have been related to interannual variations in predation pressure by yellow-legged gulls *Larus michahellis* on chicks, since during harassment by gulls, flamingo chicks in crèches may run over considerable distances (personal observation), and then the demands for oxygen, and hence PCV, may increase.

The results from this study indicate that PCV is of little value to detect intraspecific differences in the nutritional and body conditions of greater flamingo chicks. Even the yearly differences cannot be explained by annual variations in food quality and body condition of chicks, since these were lower in 1998 than in 1999–2001 (Amat et al. 2007), but in 1998 mean PCV was not particularly low (Fig. 1). Similarly, Perry et al. (1986) found that PCV was not affected by diet quality in canvasbacks *Aythya valisineria*. Although it has been found that PCV increased with the length of fast (Vleck et al. 2000), we did not find evidence that fasting during a few days affected PCV in flamingo chicks, as this blood parameter was not affected by the crop profiles of chicks. It may be that the fasting periods experienced by flamingo chicks are not so severe as to induce an increase in PCV.

Because PCV is a rather plastic parameter, it may be difficult to control for all variables that potentially may affect it to ascertain whether there is some relationship between PCV and body condition (Peterson 2002; Fair et al. 2007).

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References

Amat JA, Rendón MA, Rendón-Martos M, Garrido A, Ramírez JM (2005) Ranging behaviour of greater flamingos during the breeding and post-breeding periods: linking connectivity to biological processes. Biol Conserv 125:183–192 doi:10.1016/j.biocon.2005.02.018

Amat JA, Hortas F, Arroyo GM, Rendón MA, Ramírez JM, Rendón-Martos M et al (2007) Interannual variations in feeding frequencies and food quality of greater flamingo chicks (*Phoenicopterus roseus*): evidence from plasma chemistry and effects on body condition. Comp Biochem Physiol A 147:569–576 doi:10.1016/j.cbpa.2007.02.006

Campbell NRC, Wickert W, Magner P, Shumak SL (1994) Dehydration during fasting increases serum-lipids and lipoproteins. Clin Invest Med 17:570–576

Cuervo JJ, Møller AP, de Lope F (2007) Haematocrit is weakly related to condition in nestling barn swallows *Hirundo rustica*. Ibis 149:128–134 doi:10.1111/j.1474-919X.2006.00610.x

Dawson RD, Bortolotti GR (1997) Are avian hematocrits indicative of condition? American kestrel as a model. J Wildl Manage 61:1297–1306 doi:10.2307/3802129

Fair J, Whitaker S, Pearson B (2007) Sources of variation in haematocrit in birds. Ibis 147:535–542 doi:10.1111/j.1474-919X.2007.00680.x

Gessaman JA, Johnson JA, Hoffman SW (1986) Hematocrits and erythrocyte numbers for Cooper's and sharp-spinned hawks. Condor 88:95–96 doi:10.2307/1367761

Green AJ (2001) Mass/lengths residuals: measures of body condition or generators of spurious results? Ecology 82:1473–1483

Johnson A (1992) Les flamants de Camargue. Parc Naturel Régional de Camargue.

Johnston JW, Bilstein KL (1990) Dietary salt as a physiological constraint in white ibis breeding in an estuary. Physiol Zool 63:190–207

Merilä J, Svensson E (1995) Fat reserves and health state in migrant goldcrest *Regulus regulus*. Funct Ecol 9:842–848 doi:10.2307/2389981

Morton ML (1994) Hematocrits in montane sparrows in relation to reproductive schedule. Condor 96:119–126 doi:10.2307/1369069

Perry MC, Obrecht HH III, Williams BK, Kuenzel WJ (1986) Blood chemistry and hematocrit of captive and wild canvasbacks. J Wildl Manage 50:435–441 doi:10.2307/3801100

Peterson CC (2002) Temporal, population, and sexual variation in hematocrit of free-living desert tortoises: correlational tests of

causal hypotheses. Can J Zool 80:461–470 doi:10.1139/z02-021

Puerta ML, Muñoz-Pulido R, Huecas V, Abelenda M (1989) Hematology and blood chemistry of black and white Storks. Comp Biochem Physiol 94A:201–204 doi:10.1016/0300-9629(89)90535-5

Puerta ML, García del Campo AL, Abelenda M, Fernández A, Huecas V, Nava MP (1992) Hematological trends in flamingos, *Phoenicopterus ruber*. Comp Biochem Physiol 102A:683–686 doi:10.1016/0300-9629(92)90723-4

Rendón-Martos M, Vargas JM, Rendón MA, Garrido A, Ramírez JM (2000) Nocturnal movements of breeding greater flamingos in southern Spain. Waterbirds 23(Special Publication 1):9–19

Sánchez-Guzmán JM, Villegas A, Corbacho C, Morán R, Marzal A, Real R (2004) Response of the haematocrit to body condition changes in northern bald ibis *Geronticus eremita*. Comp Biochem Physiol A 139:41–47 doi:10.1016/j.cbpb.2004.06.018

Villegas A, Sánchez JM, Costillo E, Corbacho C (2002) Blood chemistry and hematocrit of the black vulture (*Aegypius monachus*). Comp Biochem Physiol A 132:489–497 doi:10.1016/S1095-6433(02)00097-1

Vleck CM, Vertalino N, Vleck D, Bucher TL (2000) Stress, corticosterone, and heterophyl to lymphocite ratios in free-living Adélie penguins. Condor 102:392–400 doi:10.1650/0010-5422(2000)102[0392:SCAHTL]2.0.CO;2