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ORIGINAL ARTICLE

Experimental feeding affects the relationship between hematocrit and body mass in Spotless Starling (*Sturnus unicolor*) nestlings

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Abstract Hematocrit, the proportion of blood volume occupied by packed red blood cells, is frequently used as an estimate of phenotypic condition. Some studies in birds, however, suggest that hematocrit might not always be a good estimate of condition. We tested the reliability of hematocrit as an estimate of condition by investigating the relationship between hematocrit and two other estimates of phenotypic condition (body mass controlled for body size and T-cell-mediated immune response) in nestlings of the Spotless Starling (*Sturnus unicolor*) under different environmental conditions. Half of each brood received an experimental food supplement while the other half was kept as a control. Hematocrit was positively related to relative body mass only in control nestlings whereas the relationship between hematocrit and cellular immune response was far from significant in both groups of nestlings. As expected, experimental food supply weakened the relationship between hematocrit and relative body mass, but this effect was not mediated by a decrease in the variation of phenotypic condition among nestlings. Instead, the effect of food supplementation was condition-dependent, reducing hematocrit in heavier than average nestlings, and increasing hematocrit in lighter than average nestlings. These results suggest that hematocrit might not be a reliable estimate of phenotypic condition under certain nutritional circumstances.

Keywords Condition dependence · Experimental feeding · Hematocrit · Nestlings · Phenotypic condition

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Introduction

Hematocrit, the proportion of blood occupied by red blood cells, generally reflects blood oxygen-carrying capacity and, therefore, aerobic metabolism, because red blood cells contain hemoglobin, the oxygen transport protein. A large number of natural factors have been shown to be related to hematocrit in birds, for example, age, reproductive status, altitude, muscular activity, temperature, parasites, and diet (Fair et al. 2007, and references therein). The use of hematocrit as an estimate of phenotypic condition has methodological advantages, but it is particularly controversial in birds. On the one hand, hematocrit measures are easy to obtain compared to other physiological and hematological parameters revealing condition. Moreover, low hematocrit levels as a consequence of disease or malnutrition have been well documented (e.g., Hoi-Leitner et al. 2001; Hurtrez-Boussès et al. 1997; Whitworth and Bennett 1992). On the other hand, some studies have shown that hematocrit does not vary with phenotypic condition when condition is within the normal range (e.g., Amat et al. 2009; Dawson and Bortolotti 1997a, b), and that other hematological parameters (e.g., mean corpuscular volume or hemoglobin levels) are better predictors of fitness and parasite load (Bearhop et al. 1999; O'Brien et al. 2001). A recent study (Cuervo et al. 2007) suggested that hematocrit of nestling Barn Swallows (*Hirundo* *rustica*) was more weakly related to fitness than other traits such as body mass, either absolute or relative to body size. That study concluded that hematocrit was only weakly related to phenotypic condition, which would help to explain the lack of consistent results between studies.

A widely used test of reliability of hematocrit as a phenotypic condition estimate is to study the relationship between hematocrit and other estimates of phenotypic condition. This approach is based on the assumption that a strong relationship would reflect the goodness of hematocrit as such an estimate (e.g., Dawson and Bortolotti 1997a, b). However, most estimates of phenotypic condition are nutrition-dependent (e.g., Alonso-Álvarez and Tella 2001; Potti et al. 1999), and, thus, the relationships between different estimates of condition might be affected by individual nutritional status. Therefore, before concluding that two estimates of phenotypic condition are closely related, this relationship should be validated in a wide range of nutritional circumstances. This has rarely been done. In this study, we investigated the relationship between hematocrit and two other estimates of phenotypic condition under different environmental conditions in nestlings of a passerine bird, the Spotless Starling (*Sturnus unicolor*). The two other estimates of phenotypic condition were body weight relative to body size, which represents underlying energy reserves (e.g., Morrison et al. 2007), and T-cell-mediated immune response, estimated with the phytohemagglutinin (PHA) test. PHA is a plant mitogen which, when injected subcutaneously, provokes measurable skin swelling, which is a nutrition-conditioned trait (e.g., Alonso-Álvarez and Tella 2001) commonly used as an estimate of the cellular immune response (Kennedy and Nager 2006). In order to check these relationships in different nutritional situations while controlling for genetic and maternal effects, half the nestlings in each brood were given food supplements while the other half were not.

Our food supplement experiment allowed us to test not only whether hematocrit is a good estimate of condition in Spotless Starling nestlings but also whether its reliability as such an estimate depends on variation in phenotypic condition. As mentioned above, some studies (e.g., Dawson and Bortolotti 1997a, b) suggest that hematocrit is not a good estimate of condition within a normal condition range. This leads to the prediction that variation in phenotypic condition in the sampled individuals may affect the relationship between hematocrit and other estimates of condition. We assumed that food-supplemented nestlings would experience an improvement in nutritional state and, consequently, we expected an improvement in phenotypic condition (for confirmation of this prediction in relation to PHA response, see Soler et al. 2007, 2008). Moreover, a decrease in variation in phenotypic condition in food-supplemented nestlings was also expected, because experimental feeding would be more beneficial for individuals in poor condition than for individuals in good condition and, consequently, variation in condition would be reduced. As a result, we predicted that the relationship between hematocrit and other estimates of phenotypic condition would be weaker in food-supplemented than in control nestlings.

Methods

Study area and species

The study was carried out during the breeding season (April–June) in 2005 and 2006 near Guadix, in southeastern Spain (37°18′N, 3°11′W). Spotless Starlings bred in artificial nest-boxes placed in the area at the beginning of 2005. Nest-boxes were checked every second day for laying date, clutch size, hatching date and brood size. The Spotless Starling is a polygynous (Veiga et al. 2001) colonial passerine in which both males and females feed the nestlings (Cramp and Perrins 1994; Veiga et al. 2002), and mainly females incubate. As females lay one egg per day and begin incubation before clutch completion, 1-day hatching asynchrony is common (Cramp and Perrins 1994). Typical clutch size in our population is 4–5 eggs (Soler et al. 2008).

Experimental feeding

Three days after the first egg hatched, when nestlings were 2–3 days old, they were ranked within each brood by weight, and the heaviest nestling was assigned randomly to one of two treatments, experimental feeding or control. The other nestlings in the nest were assigned alternately to each treatment following the ranking order. Thus, in every nest, half of the brood was fed experimentally while the other half was kept as a control. At this stage, all nestlings were marked on their tarsi with permanent marker for individual identification. Experimental feeding consisted of a 0.2-ml dose of a calorie-rich (5 calories per gram) soft food enriched with vitamins and minerals (Nutri-Calorías; Schering-Plough Animal Health, Buena, NJ, USA) every second day until nestlings were 10–11 days old, i.e., five times. Control nestlings received 0.2 ml of mineral water five times instead. The dose and frequency of the food treatment were calculated based on the product instructions for a nestling of 50 g (De Neve et al. 2004). All nestlings were re-marked every time they were given food or water. A description of this experiment can also be found in Soler et al. (2007, 2008).

Assessment of nestling phenotypic condition

When nestlings were 13–14 days old, they were ringed, weighed with a Pesola spring balance to the nearest 0.5 g, and the tarsus was measured with a digital caliper to the nearest 0.01 mm. Relative weight (body weight controlled for a measure of body size) represents underlying energy reserves and is more commonly used as an estimate of phenotypic condition in birds than raw weight (e.g., Morrison et al. 2007). Relative weight was estimated as the residuals after regressing body weight on tarsus length, a measure of body size (adjusted $r^2 = 0.083$, $F_{1,128} = 12.62$, $P < 0.001$). Immediately after measurements, a blood sample was taken in a 75-µl heparinized capillary tube after puncture of the brachial vein. Blood samples were kept on ice in a cooler until taken to the laboratory and centrifuged at 12,000 r.p.m. for 5 min. Hematocrit was then calculated using a hematocrit reader as the percentage of tube length occupied by red blood cells in relation to tube length occupied by all blood components. Three blood samples were taken for 81 randomly chosen nestlings, and hematocrit was highly repeatable within individuals ($r = 0.99$, $F_{80,161} = 382.14$, $P < 0.0001$).

At the same time as ringing and blood collection, all nestlings were injected subcutaneously in the right wing web with 0.2 mg of PHA (Sigma, St Louis, MO, USA) dissolved in 0.04 ml of physiological saline solution (Bausch & Lomb, Rochester, NY, USA). In the left wing web, 0.04 ml of physiological saline solution was injected as a control. The thickness of both webs was measured to the nearest 0.01 mm with a digital pressure-sensitive micrometer (Mitutoyo, model ID-CI012 BS) before and 24 h after injection. PHA response was estimated as the change in thickness of the right wing web minus the change in thickness of the left wing web. The micrometer spring was removed before measurements, so the force exerted on nestling's wing web was the weight of the spindle (20 g $\approx$ 0.20 N). As gauge head diameter was 1 cm, pressure amounted to 2.50 kPa. The mean of 3 wing web measurements was used, because repeatability was very high (Soler et al. 2007).

Statistical analyses

Since we were interested in comparing experimental versus control nestlings in the same set of nests, only nests where at least one nestling for each experimental treatment survived until the age of blood collection were included in the study. Likewise, although in 2006 we also performed a partial cross-fostering experiment in which approximately half the brood was exchanged between nests immediately after hatching, only non-cross-fostered nestlings were included here. As cross-fostered nests and nestlings were randomly chosen, our sample is not biased. This study includes data from a total of 212 nestlings from 65 nests (37 in 2005 and 28 in 2006).

Average values per nest were calculated for each experimental treatment, and all statistical analyses were performed on these means. None of the continuous variables had a distribution differing significantly from a normal distribution (Kolmogorov–Smirnov test for normality, $P \geq 0.15$), so a linear mixed model was used to determine which variables were related to hematocrit, with treatment (feeding vs control) as a fixed factor, year and nest identity (nested within year) as random factors, and relative weight and PHA response as covariates. The interactions between feeding treatment and relative weight, feeding treatment and PHA response, feeding treatment and year, year and relative weight, and year and PHA response were also included in the model. In a backward stepwise procedure, we eliminated the variable or interaction with the highest non-significant P value and re-run the test until all terms included in the model were associated with a P value of less than 0.1. Feeding treatment was not removed from the model even if it was associated with a large non-significant P value, because the interaction between feeding treatment and relative weight explained a significant portion of the hematocrit variance. To represent graphically the relationship between hematocrit and relative weight controlling also for inter-nest variability, we first assessed the two residuals (relative weight values) of each nest, one for food-supplemented nestlings and the other for control nestlings. Secondly, inter-nest variability was controlled by forcing the mean residual for each nest to zero while keeping the difference between the two residuals within the nest unchanged.

The possible effect of the feeding treatment on relative weight or PHA response was also tested using linear mixed models, with the treatment (feeding vs control) as a fixed factor and year and nest identity (nested within year) as random factors. Since year was not significantly related to relative weight or PHA response, the analyses were repeated excluding year from the models. Differences between variances were tested with variance ratio tests (Zar 1984, pp. 122–125). All statistical tests were two-tailed with a 0.05 significance level, and performed with the Statistica V7.1 software.

Results

Nest identity, relative weight and the interaction between feeding treatment and relative weight explained a significant proportion of the variance in hematocrit (Table 1). The significant interaction between feeding treatment and relative weight (Table 1) means that the slopes associated to the relationships between hematocrit and relative weight were different in the two experimental groups. While the slope was positive for control nestlings, this pattern was

Table 1 General mixed model showing variables with a significant effect on the hematocrit of Spotless Starling (*Sturnus unicolor*) nestlings

	Mean square	*df*	*F*	*P*
Nest identity	61.37	65	8.47	<0.001
Feeding treatment	1.69	1	0.23	0.631
Relative weight	36.42	1	5.03	0.029
Feeding treatment × relative weight	47.58	1	6.57	0.013
Error	7.24	61		

Year, PHA immune response, and the interactions between year and feeding treatment, year and relative weight, year and PHA immune response, and PHA immune response and feeding treatment were removed from the model after backward stepwise procedure. Nest identity was considered as a random effect. Final model: adjusted $r^2 = 0.824$, $F_{68,61} = 9.91$, $P < 0.001$

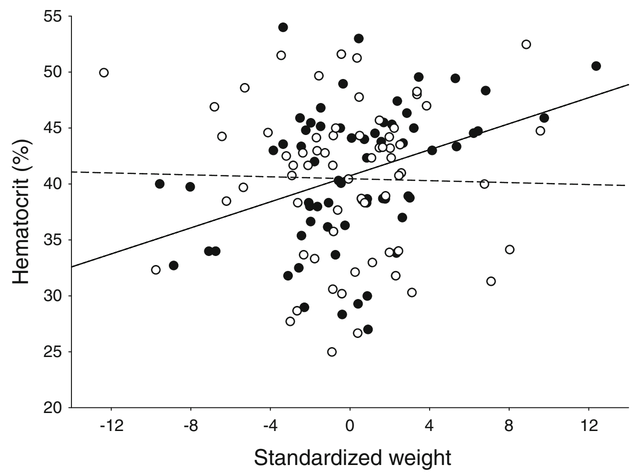

Fig. 1 Relationship between hematocrit and standardized weight (weight controlled for tarsus length and for inter-nest variability) in food-supplemented (*open circles*, *broken line*) and control (*closed circles*, *solid line*) Spotless Starling (*Sturnus unicolor*) nestlings. Regression equations: food-supplemented nestlings, $y = 40.469 - 0.043x$; control nestlings, $y = 40.733 + 0.582x$

absent in food-supplemented nestlings (Fig. 1). If the interaction was removed from the model, the relationships between hematocrit and relative weight ($F_{1,62} = 5.32$, $P = 0.024$) or feeding treatment ($F_{1,62} = 0.21$, $P = 0.65$) remained qualitatively identical, suggesting that these results do not depend on the inclusion of the interaction in the model.

As expected, the experimental feeding treatment had a significant effect on PHA response ($F_{1,63} = 7.23$, $P = 0.009$), with experimentally fed nestlings showing stronger PHA response than control ones (experimentally fed nestlings: least squares mean ± SE = 0.69 ± 0.02 mm; control nestlings: least squares mean ± SE = 0.62 ± 0.02 mm). The experimental food supply did not have a significant effect on relative weight ($F_{1,63} = 0.40$, $P = 0.53$). These results corroborate previous findings in the same Spotless Starling population (Soler et al. 2007, 2008). Contrary to our expectations, however, experimental feeding did not affect the variance in parameters used as estimates of nestling phenotypic condition. Differences in hematocrit, relative weight and PHA response variances between experimentally fed and control nestlings were not statistically significant (food-supplemented nestlings: variance of relative weight = 54.16, PHA response variance = 4.19 mm, hematocrit variance = 45.38%; control nestlings: relative weight variance = 48.47, PHA response variance = 5.24 mm, hematocrit variance = 37.71%; variance ratio tests, $F_{64,64} \leq 1.25$, $P > 0.20$ in the 3 cases).

Discussion

This study investigated the relationship between hematocrit and two other estimates of phenotypic condition in Spotless Starling nestlings with and without an experimental food treatment. We found that the relationship between hematocrit and relative weight (body mass controlled for body size) was positive and significant, but only in nestlings that received no experimental food supply. The fact that hematocrit was related to relative weight in the control group of nestlings indicates that hematocrit indeed reflected condition in nestlings of the studied population. However, the relationship between the two parameters was not significant in food-supplemented nestlings, which implies that hematocrit is not always a good estimate of phenotypic condition. This result supports previous studies (e.g., Amat et al. 2009; Dawson and Bortolotti 1997a, b) that suggested that hematocrit is not a good estimate of phenotypic condition under certain circumstances, for example certain nutritional states. The use of hematocrit as an estimate of phenotypic condition might be problematic because it is very difficult to know the nutritional state of individuals a priori. Our experiment reproduces, in food-supplemented and control nestlings, the disparity of results found in previous studies: a positive relationship between hematocrit and phenotypic condition in some cases, and no significant relationship in others (see references in "Introduction"). The fact that hematocrit was related to relative weight only under certain nutritional circumstances is also consistent with previous studies that suggest a weak relationship between hematocrit and phenotypic condition (e.g., Cuervo et al. 2007).

An interesting finding of this study was that food supplementation had a significant effect on the relationship between hematocrit and relative weight (see Fig. 1, and the interaction term in Table 1). As expected, when nestlings were food-supplemented, the relationship between hematocrit and weight became weaker and no longer significant.

We had predicted that the relationship between hematocrit and weight would be weaker in food-supplemented nestlings because experimental feeding should imply a reduction in the variation of phenotypic condition. However, the results did not confirm this point, because variances of condition-related variables (i.e., hematocrit, relative weight, and PHA response) did not differ significantly between food-supplemented and control nestlings. The explanation for these unexpected results might come from the fact that a basic assumption was not met: the effect of experimental feeding was not beneficial for all nestlings. As shown in Fig. 1, hematocrit was lower for food-supplemented than for control nestlings on the right half of the graph, i.e., when nestling relative weight was higher than average. Apparently, food supply had a negative effect on the hematocrit of those nestlings, while the effect on nestlings with low relative weight (left half of the graph) was positive (i.e., increased hematocrit).

A previous study (Soler et al. 2008) that included some of the nests investigated here found similar experimental effects, although for different variables. Blue-green egg coloration is a trait that in Spotless Starlings signals female quality and predicts nutritional environment during nestling development and, therefore, nestling phenotypic condition (Soler et al. 2008). In agreement with results presented here, the study by Soler et al. (2008) found that food-supplemented nestlings showed lower PHA response than control siblings in nests with dark blue eggs (i.e., good environmental conditions), while the opposite occurred in nests with pale blue eggs. A hypothetical scenario explaining condition-dependent effects of experimental food supply was also described (see Soler et al. 2008). Briefly, experimental food supply would cause satiation and, consequently, would decrease nestling solicitation. Thus, experimental nestlings would receive a lower proportion of natural food carried to the nest by parents than their control siblings. In general, this lower proportion of natural food will be compensated by the experimental food supply. However, in nests with high parental investment, i.e., with nestlings in good phenotypic condition, food provided by parents might be of better quality than food provided by researchers. In this particular case, experimental feeding might not fully compensate for the lower proportion of natural food, thereby worsening nestling condition. In any case, regardless of the mechanism responsible for this condition-dependent effect, if experimental feeding improved condition in some nestlings but worsened condition in others (depending on their phenotypic condition), a decrease in the variation of phenotypic condition among nestlings after food supplementation would no longer be expected.

Although an effect of the experimental food supply on nestling phenotypic condition was expected both in variance and mean, this does not mean that it would necessarily be found for all estimates of condition. For example, while experimental food supply in passerine nestlings usually affects their PHA response, a significant effect on weight is not common (De Neve et al. 2004; Saino et al. 1997; Soler et al. 2008; this study). Actually, although body mass of fledglings may predict probability of recruitment and, thus, fitness (e.g., Both et al. 1999), immune response seems to be a better predictor of survival in birds (Cichoń and Dubiec 2005; Moreno et al. 2005; Møller and Saino 2004). In this study, a significant positive relationship between hematocrit and relative weight was found in the group of nestlings without experimental food supply, but the relationship between hematocrit and T-cell-mediated immune response was not significant in either group of nestlings. The fact that some estimates of phenotypic condition are not related to each other should not be a surprise, because nutritionally-conditioned parameters may be regulated independently and the relationships between them are complex (e.g., Alonso-Álvarez and Tella 2001; Arriero 2009). In fact, there may even be trade-offs between different estimates of phenotypic condition, such as growth rate and immune response in passerine nestlings (Soler et al. 2003; Tschirren and Richner 2006).

Summarizing, hematocrit of Spotless Starling nestlings was not always significantly related to other estimates of phenotypic condition (body weight controlled for body size and cellular immune response) in the two nutritional situations tested (with and without experimental food supply). This means, firstly, that these relationships are nutrition-dependent and, secondly, that hematocrit might not be a good estimate of phenotypic condition under certain nutritional circumstances.

Zusammenfassung

Experimentelle Fütterung beeinflusst den Zusammenhang zwischen Hämatokrit und Körpermasse bei Nestlingen des Einfarbstars (Sturnus unicolor)

Hämatokrit, der Anteil der roten Blutkörperchen am Gesamtblutvolumen, wird häufig als Maß zur Bestimmung der phänotypischen Kondition benutzt. Dennoch belegen einige Studien an Vögeln, dass Hämatokrit nicht immer ein geeignetes Maß für Kondition ist. Wir testeten die Verlässlichkeit von Hämatokrit als Maß für Kondition, indem wir die Beziehung zwischen Hämatokrit und zwei anderen Maßen für phänotypische Kondition (Körpermasse korrigiert um Körpergröße und T-Zellen-vermittelnde Immunantwort) bei Nestlingen des Einfarbstars (*Sturnus unicolor*) unter verschiedenen Umgebungsbedingungen unersuchten. Die Hälfte jeder Brut erhielt einen experimentellen Futterzusatz, während die andere Hälfte als

Kontrolle diente. Hämatokrit war nur in der Kontrollgruppe positiv korreliert mit der relativen Körpermasse. Die Beziehung zwischen Hämatokrit und der zellulären Immunantwort war dagegen in keiner der Nestlingsgruppen signifikant. Wie erwartet verminderte das experimentelle Futterangebot die Beziehung zwischen Hämatokrit und der relativen Körpermasse. Dieser Effekt führte aber nicht zu einer Abnahme in der Variation der phänotypischen Kondition unter den Nestlingen. Stattdessen war der Effekt der Zusatzfütterung konditionsabhängig, reduzierte einerseits den Hämatokritwert in schwereren Küken und erhöhte andererseits den Hämatokritwert bei leichteren Küken im Verhältnis zu Küken mit durchschnittlichen Körpermassen. Diese Ergebnisse zeigen, dass Hämatokrit nicht unbedingt ein verlässliches Maß zur Bestimmung der phänotypischen Kondition unter bestimmten Ernährungsbedingungen darstellt.

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References

Alonso-Álvarez C, Tella JL (2001) Effects of experimental food restriction and body-mass changes on the avian T-cell-mediated immune response. Can J Zool 79:101–105

Amat JA, Rendón MA, Ramírez JM, Hortas F, Arroyo GM, Garrido A, Rendón-Martos M, Pérez-Hurtado A (2009) Hematocrit is related to age but not to nutritional condition in greater flamingo chicks. Eur J Wildl Res 55:179–182

Arriero E (2009) Rearing environment effects on immune defence in blue tit *Cyanistes caeruleus* nestlings. Oecologia 159:697–704

Bearhop S, Griffiths R, Orr K, Furness RW (1999) Mean corpuscular volume (MCV) as a measure of condition in birds. Ecol Lett 2:352–356

Both C, Visser ME, Verboven N (1999) Density-dependent recruitment rates in great tits: the importance of being heavier. Proc R Soc Lond B 266:465–469

Cichoń M, Dubiec A (2005) Cell-mediated immunity predicts the probability of local recruitment in nestling blue tits. J Evol Biol 18:962–966

Cramp S, Perrins CM (eds) (1994) *Sturnus unicolor* Spotless starling. In: The birds of the western palearctic, vol 8. Oxford University Press, Oxford, pp 260–269

Cuervo JJ, Møller AP, de Lope F (2007) Hematocrit is weakly related to condition in nestling barn swallows *Hirundo rustica*. Ibis 149:128–134

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

Dawson RD, Bortolotti GR (1997b) Variation in hematocrit and total plasma proteins of nestling American kestrels (*Falco sparverius*) in the wild. Comp Biochem Physiol 117A:383–390

De Neve L, Soler JJ, Soler M, Pérez-Contreras T (2004) Nest size predicts the effect of food supplementation to magpie nestlings on their immunocompetence: an experimental test of nest size indicating parental ability. Behav Ecol 15:1031–1036

Fair J, Whitaker S, Pearson B (2007) Sources of variation in haematocrit in birds. Ibis 149:535–552

Hoi-Leitner M, Romero-Pujante M, Hoi H, Pavlova A (2001) Food availability and immune capacity in serin (*Serinus serinus*) nestlings. Behav Ecol Sociobiol 49:333–339

Hurtrez-Boussès S, Perret P, Renaud F, Blondel J (1997) High blowfly parasitic loads affect breeding success in a Mediterranean population of blue tits. Oecologia 112:514–517

Kennedy MW, Nager RG (2006) The perils and prospects of using phytohaemagglutinin in evolutionary ecology. Trends Ecol Evol 21:653–655

Møller AP, Saino N (2004) Immune response and survival. Oikos 104:299–304

Moreno J, Merino S, Sanz JJ, Arriero E, Morales J, Tomás G (2005) Nestling cell-mediated immune response, body mass and hatching date as predictors of local recruitment in the pied flycatcher *Ficedula hypoleuca*. J Avian Biol 36:251–260

Morrison RIG, Davidson NC, Wilson JR (2007) Survival of the fattest: body stores on migration and survival in red knots *Calidris canutus islandica*. J Avian Biol 38:479–487

O'Brien EL, Morrison BL, Johnson LS (2001) Assessing the effects of hematophagus ectoparasites on the health of nestling birds: hematocrit vs hemoglobin levels in house wrens parasitized by fly larvae. J Avian Biol 32:73–76

Potti J, Moreno J, Merino S, Frías O, Rodríguez R (1999) Environmental and genetic variation in the haematocrit of fledgling pied flycatchers *Ficedula hypoleuca*. Oecologia 120:1–8

Saino N, Calza S, Møller AP (1997) Immunocompetence of nestling barn swallows in relation to brood size and parental effort. J Anim Ecol 66:827–836

Soler JJ, de Neve L, Pérez-Contreras T, Soler M, Sorci G (2003) Trade-off between immunocompetence and growth in magpies: an experimental study. Proc R Soc Lond B 270:241–248

Soler JJ, Avilés JM, Cuervo JJ, Pérez-Contreras T (2007) Is the relation between colour and immune response mediated by nutritional condition in spotless starling nestlings? Anim Behav 74:1139–1145

Soler JJ, Navarro C, Pérez-Contreras T, Avilés JM, Cuervo JJ (2008) Sexually selected egg coloration in spotless starlings. Am Nat 171:183–194

Tschirren B, Richner H (2006) Parasites shape the optimal investment in immunity. Proc R Soc Lond B 273:1773–1777

Veiga JP, Moreno J, Cordero PJ, Mínguez E (2001) Territory size and polygyny in the spotless starling: resource-holding potential or social inertia? Can J Zool 79:1951–1956

Veiga JP, Moreno J, Arenas M, Sánchez S (2002) Reproductive consequences for males of paternal vs territorial strategies in the polygynous spotless starling under variable ecological and social conditions. Behaviour 139:677–693

Whitworth TW, Bennett GF (1992) Pathogenicity of larval *Protocalliphora* (Diptera: Calliphoridae) parasitizing nestling birds. Can J Zool 70:2184–2191

Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice-Hall, Englewood Cliffs