Relationship between avian growth rate and immune response depends on food availability

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Summary

Life history theory predicts that when resources are limited growing organisms are likely to trade an immune response for competing demands of growth. To test this we examined the effect of energy intake on body mass increments and an immune response in hand-reared sand martin (*Riparia riparia*) nestlings. We subjected the nestlings to three different feeding regimes, mimicking the range of food availability in the wild, and then evaluated nestlings' immune response to phytohaemagglutinin (PHA). Direction of correlation between the magnitude of PHA-induced swelling response and body mass increments depended on food availability: it was negative when food was scarce and positive when resources were plentiful. There was no significant correlation between the two traits under intermediate feeding conditions. We conclude that the relative cost of immune function in young birds depends on food availability and, therefore, may be modified by external factors such as weather conditions or hatching asynchrony.

Key words: body mass increments, food availability, immune response, life history, *Riparia riparia*, trade-off.

Introduction

Life history theory predicts that when resources are limited any investment in a costly trait is likely to reduce resources available for other vital functions (Roff, 2002). Such trade-offs are considered to be fundamental factors shaping the evolution of immune defence (Zuk and Stoehr, 2002). In recent years, one of the most intensively studied trade-offs is that between immune function and growth, particularly in birds (e.g. Hõrak et al., 2000; Merino et al., 2000; Soler et al., 2003; Mauck et al., 2005) (see also Pilorz et al., 2005). Nestlings of altricial birds are characterized by a very fast and resource-demanding growth (Starck and Ricklefs, 1998). However, young birds frequently face unfavourable environmental conditions, which reduce food availability, suppress normal development, and subsequently compromise their fitness (Gebhardt-Henrich and Richner, 1998; Lindström, 1999). Under such conditions limited resources are likely to be preferentially allocated to the development of structures and functions essential for survival and fledging (Schew and Ricklefs, 1998; Metcalfe and Monaghan, 2001).

The ability to mount a strong immune response is a significant determinant of fitness in young altricial birds (Christe et al., 1998; Christe et al., 2001; Merino et al., 2000; Møller and Saino, 2004; Cichoń and Dubiec, 2005; Moreno et al., 2005). Since immune function is costly (Lochmiller and Deerenberg, 2000; Norris and Evans, 2000; Klasing, 2004), one can expect it to drain essential resources away from other important traits, in particular growth of nestlings. However, studies aimed at demonstrating this relationship have yielded conflicting results. Although many authors reported a negative correlation between body mass increments and immune response (e.g. Merino et al., 2000; Soler et al., 2003; Brommer, 2004), other studies failed to demonstrate the expected trade-off (e.g. Hõrak et al., 2000; Whitaker and Fair, 2002). Here, we propose that this ambiguity stems from the lack of experimental control of the amount of resources traded between competing functions. The significance of any trade-off can only be effectively tested under conditions that preclude an increase of resource acquisition, which may cover the extra costs evoked by immune challenge. This is because an increased resource acquisition may weaken an otherwise inverse relationship between the traded traits, or even reverse it to a positive one (van Noordwijk and de Jong, 1986; Książek et al., 2003; Sandland and Minchella, 2003).

Aerial insectivores are frequently used as model organisms in studies on immune function (e.g. Saino et al., 1997; Saino et al., 2001; Saino et al., 2002; Christe et al., 1998; Merino et al., 1999; Merino et al., 2000; Szép and Møller, 1999; Szép and Møller, 2000). They frequently face periods of food shortage incurred by unfavourable weather conditions (Bryant, 1975; McCarty and Winkler, 1999; Lifjeld et al., 2002), which adversely affect their immune function (Christe et al., 2001; Lifjeld et al., 2002). In the present study, we investigated the effect of a gradient of food intake on the relationship between

body mass increments and the magnitude of response to a novel antigen in nestlings of sand martin (*Riparia riparia* Linnaeus 1758) – a small aerial insectivorous passerine. Sand martin nestlings have a considerable level of developmental plasticity, which enables them to tide over inclement weather conditions. They are able to slow the pace of development during food shortage, and resume it when feeding conditions improve (Brzek and Konarzewski, 2001; Brzek and Konarzewski, 2004). Thus, they may be particularly likely to trade their growth for enhanced immune response (Szép and Møller, 1999). Sand martin nestlings are therefore a suitable subject for testing the presumed trade-off between body mass increments and immune function.

In our study we measured the thickness of swelling induced by sub-cutaneous injection of phytohaemagglutinin (PHA) as a surrogate for immune function of the sand martin nestlings. Our experiment was carried out under laboratory conditions. This enabled us to analyse the direction of correlation between body mass increments and PHA-induced swelling response after controlling for food intake. We attempted to simulate the range of food availability encountered by nestlings in the wild. We predicted that the direction of correlation between body mass increments and the magnitude of immune response should vary with different levels of food provisioning. In particular, we expected that the correlation was most likely to be negative in nestlings subjected to lowest food intake, which forces them to trade limited resources between competing demands of body mass increments and immune function.

Materials and methods
Experimental procedures

Twenty-eight, 6-day-old sand martin nestlings were collected in June 2002 in two breeding colonies near Białystok, north-eastern Poland. To avoid the possible effects of relatedness and hatching order, we did not use the last-hatched young, collected no more than two nestlings from each nest, and always assigned them to different feeding regimes. All laboratory rearing and feeding protocols applied in the present study were validated in our previous experiments, as described in details elsewhere (Brzek and Konarzewski, 2001; Brzek and Konarzewski, 2004). Briefly, the nestlings were reared in artificial nests, made of transparent plastic. The experiment was carried out in a climatic chamber at 32°C, 90% relative humidity and 16 h:8 h light:dark regime.

We created seven artificial broods, each of four nestlings, assigned randomly to the broods. We assigned the broods to three different feeding regimes. Eight nestlings (two whole artificial broods) were fed with a limited amount of food, resulting in low body mass increments. This group will be referred to as FR (food-restricted). The other twelve nestlings (three whole broods) were fed without restriction (hereafter referred to as the *ad libitum*, or AL group). The remaining eight nestlings (two whole broods) were fed with an amount of food intermediate between that fed to FR and AL individuals (referred to as the I group). The nestlings from group AL were fed until they stopped begging every 45 min between 06.00 h and 22.00 h CEST (22 times per day). The remaining groups (FR and I) were fed 17 times per day, between 08.00 h and 20.00 h CEST, with deliberately smaller quantities of food, representing around 40 and 70% of *ad libitum* intake, respectively. All nestlings were hand-fed alternately with equal amounts of crickets and a special formula designed for the nestlings of insectivore birds: fresh, soft cheese, glucose, rice flour and maize flour, mixed in the mass proportions 300:30:15:12 (J. Desselberger, personal communication). This mixture was subsequently added to hard-boiled hens' eggs in the proportion 3:1 and enriched with the vitamin mixture Vitaral (Jelfa, Jelenia Gora, Poland). Fresh portions of food were prepared twice a day. Since we were interested in testing not only between-, but also within-regime effects, we did not attempt to feed the artificial broods with strictly equal amounts of food. This resulted in within-regime variation in food consumption, which we quantified daily for each individual nestling. Energy content of food was determined in a Berthelot-type calorimeter. The energy intake during the whole experiment averaged 74±2.9 (mean ± s.e.m.), 121±2.9 and 186±2.6 kJ in FR, I and AL groups, respectively. The energy intake of individual nestlings was strongly correlated between subsequent days (Pearson correlation, $r>0.95$ for all comparisons).

The experiment stretched over three consecutive days (72 h; Fig. 1). The day when the experiment began is hereafter referred to as day 1, and the following days are referred accordingly. Two AL nestlings started to exhibit abnormally low food intake and low body mass increments (at the level of FR group) during day 3 and were subsequently excluded from the experiment and immediately returned to their natal nests. The remaining nestlings were returned to their natal nests upon termination of the experiment.

PHA-induced swelling response

To estimate PHA-induced swelling response we applied the protocol validated by Smits et al. (Smits et al., 1999; Smits et al., 2001). The PHA trial started on day 2 of the experiment, when nestlings were 8-days old (Fig. 1). The nestlings were injected into a marked site on the right wing web with 0.2 mg of PHA (Sigma, Sp. zo.o., Poznan, Poland; L-8754), diluted in 0.04 ml phosphate-buffered saline (PBS). We measured the thickness of the wing web before injection and after 24 h with a spessimeter Teclock SM-112 (Teclock, Nagano, Japan; accuracy 0.01 mm). Each measurement was repeated three times, then the mean value was used for subsequent analyses. Repeatability of our measurements calculated as an intraclass correlation coefficient (Lessells and Boag, 1987) was $\tau=0.96$. The magnitude of PHA-induced swelling response was quantified as the difference between pre- and post-injection measures of the wing web thickness.

Statistics

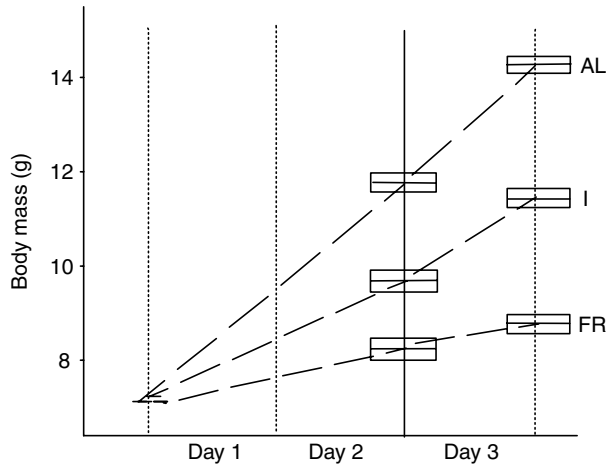Differences between experimental groups were analysed by

Fig. 1. Graphic scheme of applied experimental design. Body mass of sand martin nestlings at the beginning of the experiment, at the antigen injection (end of day 2, marked as solid vertical line), and at the conclusion of the experiment (end of day 3) are shown. AL, nestlings fed *ad libitum*; I, nestlings subjected to an intermediate feeding regime; FR, nestlings fed with a limited amount of food. Mean values (connected by broken lines for each feeding regime), and s.e.m. (vertical limits of boxes) are shown. Body mass changes during days 1–2 are referred to as body mass increments before injection, whereas body mass changes during day 3 are referred to as body mass increments after injection.

means of ANOVA or ANCOVA, with feeding regime as the main effect, initial body mass and/or body mass increment as covariates, and the respective interaction terms. In the present experiment nestlings were reared in artificial broods. Therefore, in all statistical models we also attempted to control for the possible effect of a common artificial brood environment. However, the inclusion of this effect produced spurious results in full ANCOVA models that contained feeding regime and the brood (randomly nested within feeding regime) as main effects and the respective interaction terms. Although the models were highly significant ($P<0.0001$), none of their components were statistically significant (in all cases $P>0.7$). We therefore recognized the full ANCOVA models as over-parameterized. Further statistical analyses of reduced models revealed that the nested effect reflected minor, but consistent between-brood differences in food intake, being the primary determinant of other studied traits. For these reasons in the final ANCOVAs testing the effects of interactions we elected not to include the nested effect of artificial broods, because it was collinear with the other analysed factors. To ensure that within-brood autocorrelation of values of studied traits did not confound our results we checked the significance of this effect by means of randomization tests (Manly, 1997) carried out on the residuals from the final ANCOVA models described above. We randomly shuffled these residuals among artificial broods (each time 1000 runs) and calculated the proportion of iterations for which calculated within-brood sum of squares was larger than that in the original data set. The tests revealed that the residuals were randomly distributed among artificial broods (e.g. $P=0.21$ for the model testing the effect of interaction between body mass increments and feeding regime on magnitude of immune response), which indicated to us that pseudoreplication did not significantly confound our statistical inference.

Individual *a priori* pair-wise means comparisons were tested by *t*-statistics corresponding to the two-sided *P* values. Variables were log-transformed to improve distribution of residuals. The standard level of significance $\alpha=0.05$ was applied. In the case of multi-group comparisons in Table 1, we adjusted the conventional level of significance by applying a Bonferroni correction. To do this, we divided $\alpha=0.05$ by 6, i.e. the number of inter-group tests performed. All tests were carried out using the SAS 9.1.3 statistical package.

Results

Body mass of nestlings did not differ between feeding regimes at the beginning of the experiment (Table 1; Fig. 1). However, applied feeding regimes resulted in highly significant differences in food intake during the course of our study (Table 1; *t*-test: $P<0.0001$ for all inter-group comparisons). These differences resulted in significant between-regime differences in body mass increments during the whole

Table 1. *Summary of results of ANOVA or ANCOVA of the effect of experimental manipulation on body mass, body mass change and the magnitude of PHA-induced swelling response*

Dependent variable	Feeding regime			Covariate		
	F	d.f.	*P*	*F*	d.f.	*P*
Initial body mass	0.05	2,25	0.95	–	–	–
Energy intake*	405.2	2,22	<0.0001	0.12	1,22	0.7
Total body mass increments*	409.8	2,22	<0.0001	9.47	1,22	0.0055
Body mass at PHA injection*	159.1	2,24	<0.0001	32.7	1,24	<0.0001
Body mass increments after injection[†]	27.6	2,22	<0.0001	5.10	1,22	0.034
Wing web thickness change*	13.1	2,22	0.0002	0.28	1,22	0.6

*Initial body mass used as covariate.

[†]Body mass at phytohaemagglutinin (PHA) injection used as covariate.

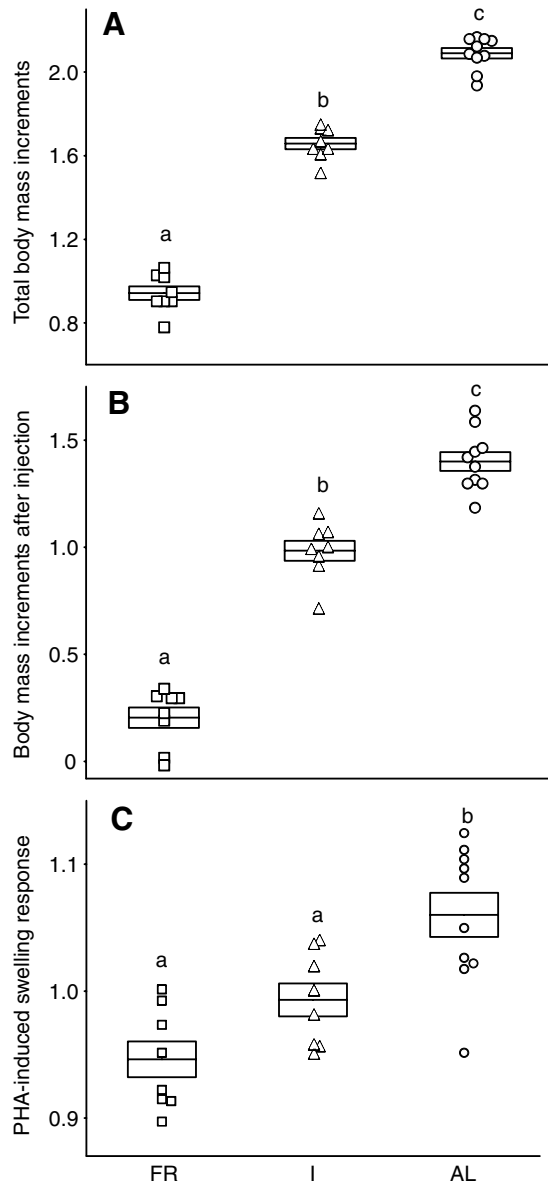Feeding regime × covariate interaction was non-significant for all dependent variables.

Fig. 2. (A) Total body mass increments (least square means corrected for initial body mass) in the course of our study; (B) body mass increments (least square means corrected for body mass at injection) during day 3, i.e. following the immune challenge, and (C) magnitude of phytohaemagglutinin (PHA)-induced swelling response (an estimate of immune function) in nestlings subjected to three feeding regimes. All variables are log-transformed. Horizontal lines denote mean values, boxes denote s.e.m. Squares, triangles and circles denote food-restricted group (FR), intermediate group (I) and *ad libitum* fed group (AL), respectively. Different letters above symbols indicate significant between-group differences ($P<0.05$), tested by pair-wise *t*-tests.

experiment (Table 1; Fig. 2A; *t*-test: $P<0.0001$ for all inter-group comparisons). They were already highly significant at the time of PHA injection (Table 1; Fig. 1; *t*-test: $P<0.0001$ for all inter-group comparisons). Feeding regime also brought about significant between-group differences in body mass

increments after antigen injection (Table 1; Fig. 2B; *t*-test: $P<0.003$ for all inter-group comparisons).

Feeding regime significantly affected the magnitude of PHA-induced swelling response (Table 1; Fig. 2C). This response was stronger in the AL group than in the two other feeding regimes (AL *vs* I group: $P=0.007$; AL *vs* FR group: $P<0.0001$). Likewise, nestlings from group I had a stronger swelling response than those from group FR, though this difference did not reach statistical significance ($P=0.059$). However, another ANCOVA test of the magnitude of PHA-induced swelling response as a dependent variable, with feeding regime as the main factor, and the total body mass change during the course of the whole experiment as a covariate, revealed a strong interaction between body mass change and feeding regime (Fig. 3A; $F_{2,20}=6.63$, $P=0.006$). This interaction was marginally non-significant, when the analysis was restricted to body mass change during the first 2 days of experiment, that is, before the immune challenge ($F_{2,20}=3.44$, $P=0.052$). By contrast, the interaction was far from significance when ANCOVA was carried out for body mass increments during day 3, i.e. 24 h following the immune challenge (Fig. 3B; $F_{2,20}=0.83$, $P=0.45$).

It is important to note that ANCOVA carried out with the total body mass changes as a covariate can be potentially confounded by non-overlapping ranges of their variation between feeding regimes (Fig. 3A). Therefore, we also analysed the correlations between the magnitude of swelling response and total body mass increments separately for each feeding regime. We found a significant, negative correlation in the FR group ($r=-0.72$, $P=0.046$). The same correlation was non-significant in group I ($r=-0.19$, $P=0.65$), and positive and significant in AL nestlings ($r=0.69$, $P=0.027$). These correlation coefficients were significantly different between feeding regimes ($0.01<P<0.005$). However, multiple comparison tests revealed that the only significant difference was that between the FR and AL groups ($0.01<P<0.005$), whereas in group I the correlation coefficient did not differ from those in other feeding regimes ($P>0.05$ for both comparisons). Finally, the correlation between the magnitude of PHA-induced swelling response and body mass increments calculated across all feeding regimes was positive and highly significant, both for the whole experimental period (Fig. 3A; $r=0.70$, $P<0.0001$) and for day 3 (Fig. 3B; $r=0.62$, $P=0.0008$).

Discussion

To our knowledge, this is the first test of the presumed trade-off between growth and immune function in altricial nestlings, carried out under conditions of laboratory-controlled energy intake. We found that the energy intake significantly affected both studied traits: body mass increments and the magnitude of swelling response to injected PHA, used here as a surrogate of immune function. This corroborates the results of earlier studies reporting that experimentally manipulating the level of nutrition affects the immune response of growing birds

(Lochmiller et al., 1993; Saino et al., 1997; Birkhead et al., 1999). However, we were also able to demonstrate that, depending on energy intake, the correlation between body mass increments and PHA-induced swelling response may take all possible directions: it was positive in the *ad libitum*-fed AL group, negative in the food-restricted FR nestlings, and non-significant in the intermediate I group. Particularly relevant here are the results reported by Snoeijs et al. (Snoeijs et al., 2005), who found a negative correlation between PHA-induced swelling response and tarsus growth in great tit nestlings exclusively fed by a single mother, and a positive correlation in the young reared by both parents. Our study strongly suggests that similar changes in immune function reported in many other field studies involving manipulation of the brood size or parental effort (e.g. Fargallo et al., 2002; Saino et al., 2002; Gwinner and Berger, 2006) are primarily underlined by food availability. This supports the general prediction that the immune-related trade-offs should be mainly expressed when resources are limited (Sandland and Minchella, 2003; Schmid-Hempel, 2003).

It is also important to note that the correlation between the magnitude of PHA-induced swelling response and body mass increments, calculated across all feeding regimes applied in our experiment, was strongly positive (Fig. 3). However, in the context of within-regime correlations, the cross-regime correlation must be interpreted as the epiphenomenon of the between-regime variation of food intake. This again highlights the necessity to take into account the resource availability in studies on immune-related trade-offs carried out under natural conditions.

The interaction between feeding regime and body mass increments, which underlies the correlations between the magnitude of PHA-induced swelling response and changes in body mass (Fig. 3A), was significant only for the whole period of our experiment (i.e. days 1–3), and non-significant for body mass increments after antigen injection (i.e. during day 3, Fig. 3B). Thus, the correlations depicted in Fig. 3A most likely reflect the trade-off (or the lack thereof) involving the costs of development and maintenance of immune function incurred prior to immune challenge (constitutive costs) (*sensu* Sandland and Minchella, 2003), rather than the immediate investment in mounting a response to injected PHA (i.e. inducible costs). When food is plentiful, as in the case of AL nestlings, no limitations on nutrient or energy availability compromise immune function (Klasing, 2004), and the positive correlation between body mass increments and an immune response presumably reflects the between-individual variation of genetic quality or condition (Hõrak et al., 2000; Hoi-Leitner et al., 2001). In contrast to AL nestlings, under-fed FR nestlings traded limited resources between competing demands of immune function and body mass increments, which resulted in the observed inverse correlation between those traits. However, immediate limitations resulting in this correlation are difficult to identify, and deserve further study. Although widely used, PHA-induced swelling response does not provide an unambiguous index of immune function,
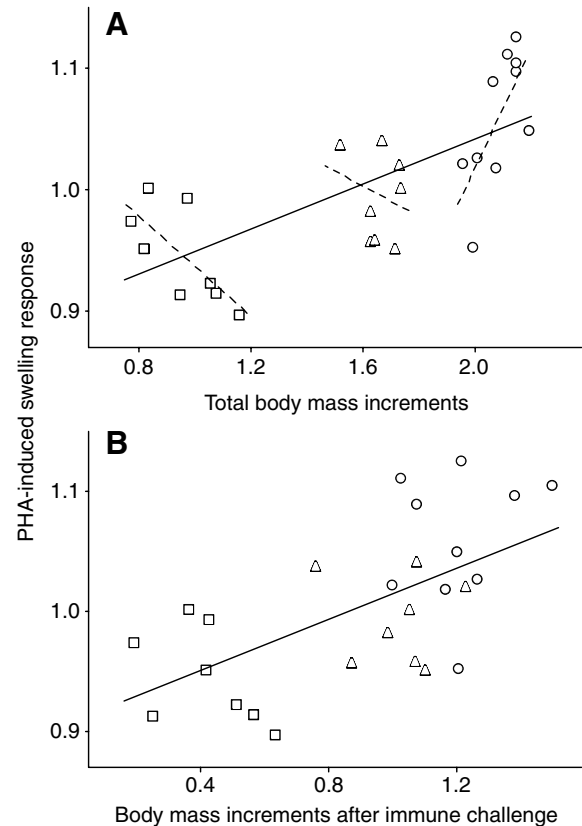

Fig. 3. (A) Relationship between the magnitude of phytohaemagglutinin (PHA)-induced swelling response and total body mass increments in the course of the whole experiment, and (B) relationship between the magnitude of PHA-induced swelling response and body mass increments during day 3, i.e. following the immune challenge in the food-restricted group (FR; squares), intermediate group (I; triangles), and *ad libitum* fed group (AL; circles). All variables are log-transformed. Broken lines denote within-group trends, whereas solid lines denote the trend across all experimental groups.

because it involves both innate and adaptive components of the immune response (Kennedy and Nager, 2006; Martin et al., 2006). The costs of development, maintenance and use of these components are different. Non-specific immune mechanisms are cheap to develop and maintain, but the nutritional costs of their use are high, whereas the opposite applies to the adaptive components of the immune response (Klasing and Leschinsky, 1999; Klasing, 2004). One can therefore hypothesize that an inverse correlation between the magnitude of PHA-induced swelling response and changes in body mass observed in FR nestlings may stem from three processes: (i) a suppression of costly adaptive components of the immune response, the development and maintenance of which strongly impinge on the available nutrients, which are also required for the competing demands of body mass increments; (ii) a suppression of costly acute phase response; (iii) a suppression of both components.

The first hypothesis predicts the presence of a significant trade-off between the magnitude of immune response and body

mass increments before antigen injection, whereas the second predicts the same after antigen injection. In our experimental design the existence of such trade-offs would be revealed by significant effects of the interaction between body mass increments and feeding regime on the magnitude of PHA-immune swelling. However, these interactions were not significant either before, or after antigen injection, even though the interaction calculated over the whole period of the experiment was highly significant (Fig. 3A). This suggests that neither hypotheses (i) nor (ii) may exclusively explain the observed pattern. Nevertheless, our results lead us to reject the hypothesis that the significant interaction resulted only from food shortage suppressing immediate investment in mounting the acute phase response. It is also possible that the growth retardation following PHA challenge may become apparent with some delay longer than 24 h. However, the nestlings in our experiment were in the linear phase of body mass growth (Turner and Bryant, 1979), when its retardation due to conflicting resource demands should be particularly evident. Moreover, several other studies demonstrated that higher rate of development of immune function negatively affects body mass growth or wing development (Soler et al., 2003; Brommer, 2004; Mauck et al., 2005).

Our results suggest that the undernourished sand martin nestlings develop higher immune function only at the expense of a part of their growth potential. Body size is often a decisive factor, which determines the outcome of sibling competition for food (Mock and Parker, 1997). Therefore, if the development of immune function drains some vital resources away from body mass increments, it may affect nestlings' competitive abilities (Saino and Møller, 2002). This effect is likely to be particularly strong in asynchronously hatched broods, where younger and smaller nestlings frequently face undernourishment (Mock and Parker, 1997), and may be burdened with larger parasite load than their bigger nest-mates (Christe et al., 1998; Simon et al., 2003). For example, Szép and Møller (Szép and Møller, 2000) reported that parasitic infection increases the variation of body mass in the sand martin broods. Thus, the trade-off between immune function and body mass growth may significantly affect survival of small nestlings in asynchronously hatched broods.

In conclusion, we demonstrated that the direction of correlation between magnitude of PHA-induced swelling response and body mass increments rate in young sand martins depends on food availability: they are inversely correlated when food is scarce, whereas both traits are positively correlated when the resources are plentiful. We therefore strongly advise that the design of the studies on immune function carried out under natural conditions should be based on a careful monitoring of the availability of resources essential for the immunity development and use.

References

Birkhead, T. R., Fletcher, F. and Pellatt, E. J. (1999). Nestling diet, secondary sexual traits and fitness in the zebra finch. *Proc. R. Soc. Lond. B Biol. Sci.* **266**, 385-390.

Brommer, J. E. (2004). Immunocompetence and its costs during development: an experimental study in blue tit nestlings. *Proc. R. Soc. Lond. B Biol. Sci.* **271**, S110-S113.

Bryant, D. M. (1975). Breeding biology of House Martins *Delichon urbica* in relation to aerial insect abundance. *Ibis* **117**, 180-216.

Brzęk, P. and Konarzewski, M. (2001). Effect of food shortage on the physiology and competitive abilities of sand martin (*Riparia riparia*) nestlings. *J. Exp. Biol.* **204**, 3065-3074.

Brzęk, P. and Konarzewski, M. (2004). Effect of refeeding on growth, development, and behavior of undernourished Bank Swallow (*Riparia riparia*) nestlings. *Auk* **121**, 1187-1198.

Christe, P., Møller, A. P. and de Lope, F. (1998). Immunocompetence and nestling survival in the house martin: the tasty chick hypothesis. *Oikos* **83**, 175-179.

Christe, P., de Lope, F., González, G., Saino, N. and Møller, A. P. (2001). The influence of environmental conditions on immune responses, morphology and recapture probability of nestling house martins (*Delichon urbica*). *Oecologia* **126**, 333-338.

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

Fargallo, J. A., Laaksonen, T., Pöyri, V. and Korpimäki, E. (2002). Intersexual differences in the immune response of Eurasian kestrel nestlings under food shortage. *Ecol. Lett.* **5**, 95-101.

Gebhardt-Henrich, S. and Richner, H. (1998). Causes of growth variation and its consequences for fitness. In *Avian Growth and Development* (ed. J. M. Starck and R. E. Ricklefs), pp. 324-388. Oxford: Oxford University Press.

Gwinner, H. and Berger, S. (2006). Parasite defence in birds: the role of volatiles. *Acta Zool. Sinica Suppl.* **52**, 280-283.

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

Hõrak, P., Ots, I., Tegelmann, L. and Møller, A. P. (2000). Health impact of phytohaemagglutinin-induced immune challenge on great tit (*Parus major*) nestlings. *Can. J. Zool.* **78**, 905-910.

Kennedy, M. W. and Nager, R. G. (2006). The perils and prospects of using phytohaemagglutinin in evolutionary ecology. *Trends Ecol. Evol.* **21**, 653-655.

Klasing, K. C. (2004). The cost of immunity. *Acta Zool. Sinica* **50**, 961-969.

Klasing, K. C. and Leshchinsky, T. V. (1999). Functions, costs, and benefits of the immune system during development and growth. In *Proceedings of the 22nd International Ornithological Congress, Durban* (ed. N. J. Adams and R. H. Slotow), pp. 2817-2832. Johannesburg: BirdLife South Africa.

Książek, A., Konarzewski, M., Chadzińska, M. and Cichoń, M. (2003). Costs of immune response in cold-stressed laboratory mice selected for high and low basal metabolic rates. *Proc. R. Soc. Lond. B Biol. Sci.* **270**, 2025-2031.

Lessells, C. M. and Boag, P. T. (1987). Unrepeatable repeatabilities: a common mistake. *Auk* **104**, 116-121.

Lifjeld, J. T., Dunn, P. O. and Whittingham, L. A. (2002). Short-term fluctuations in cellular immunity of tree swallows feeding nestlings. *Oecologia* **130**, 185-190.

Lindström, J. (1999). Early development and fitness in birds and mammals. *Trends Ecol. Evol.* **14**, 343-348.

Lochmiller, R. L. and Deerenberg, C. (2000). Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**, 87-98.

Lochmiller, R. L., Vestey, M. R. and Boren, J. C. (1993). Relationship between protein nutritional status and immunocompetence in Northern Bobwhite chicks. *Auk* **110**, 503-510.

Manly, B. F. J. (1997). *Randomization, Bootstrap and Monte Carlo Methods in Biology*. London: Chapman & Hall.

Martin, L. B., Han, P., Lewittes, J., Kuhlman, J. R., Klasing, K. C. and Wikelski, M. (2006). Phytohemagglutinin-induced skin swelling in birds: histological support for a classic immunoecological technique. *Funct. Ecol.* **20**, 290-299.

Mauck, R. A., Matson, K. D., Philipsborn, J. and Ricklefs, R. E. (2005). Increase in the constitutive innate humoral immune system in Leach's Storm-Petrel (*Oceanodroma leucorhoa*) chicks is negatively correlated with growth rate. *Funct. Ecol.* **19**, 1001-1007.

McCarty, J. P. and Winkler, D. W. (1999). Relative importance of environmental variables in determining the growth of nestling Tree Swallows *Tachycineta bicolor*. *Ibis* **141**, 286-296.

Merino, S., Martinez, J., Møller, A. P., Sanabria, L., de Lope, F., Perez, J. and Rodriguez-Caabeiro, F. (1999). Phytohaemagglutinin injection assay and physiological stress in nestling house martins. *Anim. Behav.* **58**, 219-222.

Merino, S., Møller, A. P. and de Lope, F. (2000). Seasonal changes in cell-mediated immunocompetence and mass gain in nestling barn swallows: a parasite-mediated effect? *Oikos* **90**, 327-332.

Metcalfe, N. B. and Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* **16**, 254-260.

Mock, D. W. and Parker, G. A. (1997). *The Evolution of Sibling Rivalry*. Oxford: Oxford University Press.

Møller, A. P. and Saino, N. (2004). Immune response and survival. *Oikos* **104**, 299-304.

Moreno, J., Merino, S., Sanz, J. J., Arriero, E., Morales, J. and 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.

Norris, K. and Evans, M. R. (2000). Ecological immunology: life history trade-offs and immune defence in birds. *Behav. Ecol.* **11**, 19-26.

Pilorz, V., Jäckel, M., Knudsen, K. and Trillmich, F. (2005). The cost of a specific immune response in young guinea pigs. *Physiol. Behav.* **85**, 205-211.

Roff, D. A. (2002). *Life History Evolution*. Sunderland, MA: Sinauer Associates.

Saino, N. and Møller, A. P. (2002). Immunity and begging. In *The Evolution of Begging* (ed. J. Wright and M. L. Leonard), pp. 245-267. Dordrecht: Kluwer Academic.

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

Saino, N., Incagli, M., Martinelli, R., Ambrosini, R. and Møller, A. P. (2001). Immunity, growth and begging behaviour of nestling Barn Swallows *Hirundo rustica* in relation to hatching order. *J. Avian Biol.* **32**, 263-270.

Saino, N., Ferrari, R. P., Romano, M., Ambrosini, R. and Møller, A. P. (2002). Ectoparasites and reproductive trade-offs in the barn swallow (*Hirundo rustica*). *Oecologia* **133**, 139-145.

Sandland, G. J. and Minchella, D. J. (2003). Costs of immune defence: an enigma wrapped in an environmental cloak? *Trends Parasitol.* **19**, 571-574.

Schew, W. A. and Ricklefs, R. E. (1998). Developmental plasticity. In *Avian Growth and Development* (ed. J. M. Starck and R. E. Ricklefs), pp. 288-304. Oxford: Oxford University Press.

Schmid-Hempel, P. (2003). Variation in immune defence as a question of evolutionary ecology. *Proc. R. Soc. Lond. B Biol. Sci.* **270**, 357-366.

Simon, A., Thomas, D. W., Blondel, J., Lambrechts, M. M. and Perret, P. (2003). Within-brood distribution of ectoparasite attacks on nestling blue tits: a test of the tasty chick hypothesis using insulin as a tracer. *Oikos* **102**, 551-558.

Smits, J. E., Bortolotti, G. R. and Tella, J. L. (1999). Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Funct. Ecol.* **13**, 567-572.

Smits, J. E., Bortolotti, G. R. and Tella, J. L. (2001). Measurement repeatability and the use of controls in PHA assays: a reply to Siva-Jothy and Ryder. *Funct. Ecol.* **15**, 812-817.

Snoeijs, T., Pinxten, R. and Eens, M. (2005). Experimental removal of the male parent negatively affects growth and immunocompetence in nestling great tits. *Oecologia* **145**, 165-173.

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

Starck, J. M. and Ricklefs, R. E. (1998). Patterns of development: the altricial-precocial spectrum. In *Avian Growth and Development* (ed. J. M. Starck and R. E. Ricklefs), pp. 3-30. Oxford: Oxford University Press.

Szép, T. and Møller, A. P. (1999). Cost of parasitism and host immune defence in the sand martin *Riparia riparia*: a role for parent-offspring conflict? *Oecologia* **119**, 9-15.

Szép, T. and Møller, A. P. (2000). Exposure to ectoparasites increases within-brood variability in size and body mass in the sand martin. *Oecologia* **125**, 201-207.

Turner, A. K. and Bryant, D. M. (1979). Growth of nestling sand martins. *Bird Study* **26**, 117-122.

van Noordwijk, A. J. and de Jong, G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.* **128**, 137-142.

Whitaker, S. and Fair, J. (2002). The costs of immunological challenge to developing mountain chickadees, *Poecile gambeli*, in the wild. *Oikos* **99**, 161-165.

Zuk, M. and Stoehr, A. M. (2002). Immune defence and host life history. *Am. Nat.* **160**, S9-S22.