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Trade-off between immunocompetence and growth in magpies: an experimental study

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A trade-off between immunity and growth has repeatedly been suggested, mainly based on laboratory and poultry science, but also from experiments where parasitism intensity was manipulated in field bird populations. However, as resource allocation to different activities (or organs) during growth is difficult to manipulate, this trade-off has only been experimentally tested by studying the effects of non-pathogenic antigens. By providing some nestling magpies (*Pica pica*) with methionine, a sulphur amino acid that specifically enhances T-cell immune response in chickens, we investigated this trade-off by directly affecting allocation of limited resources during growth. Results were in accordance with the hypothetical trade-off because nestlings fed with methionine showed a lower growth rate during the four days of methionine administration, but a larger response when fledglings were challenged with phytohaemagglutinin (a measure of the intensity of T-lymphocyte-mediated immune responsiveness) than control nestlings. Surprisingly, we found that control and experimental nestlings fledged with similar body mass, size and condition, but experimental nestlings suffered less from blood parasites (*Haemoproteus*) and had fewer lymphocytes (a widely used measure of health status) than control nestlings, suggesting a negative effect of blood parasites or other pathogens on nestling growth.

Keywords: immune response; life history; parasite resistance; *Pica pica*; nestling growth; trade-offs

1. INTRODUCTION

Post-fledging survival is a crucial determinant of reproductive success in birds (Clutton-Brock 1988), and it is known to be affected by conditions experienced during nestling development (Lindström 1999) that influence the condition of nestlings and the ability of their immune systems to resist pathogen attacks (Christe *et al.* 1998; González *et al.* 1999). However, development of a good immune system, as well as its maintenance and use, is costly in energy and nutrients that, otherwise, could be used to develop other phenotypic traits further (Klasing & Leshchinsky 1999; Lochmiller & Deerenberg 2000; Norris & Evans 2000; but see Klasing 1998). Life-history theory predicts that natural selection favours the evolution of physiological mechanisms that ensure optimal allocation of limited resources to competing activities (Stearns 1992).

Trade-offs between the immune response and other important activities such as parental feeding (Råberg *et al.* 2000) and reproductive effort in general (Nordling *et al.* 1998; Moreno *et al.* 1999, 2001; Ilmonen *et al.* 2000; but see Williams *et al.* 1999) have been detected in nature (for a review, see Lochmiller & Deerenberg (2000)). A trade-off between growth and immunity has been proposed based on results from experimental parasitism of swallow (*Hirundo rustica*) nests with ectoparasites, which resulted in higher growth rates, but a lower T-cell-mediated immune response of experimental nestlings (see Saino *et al.* 1998). Moreover, adult sand martins (*Riparia riparia*) did not respond to an ectoparasite treatment in terms of immunoglobulin concentration, whereas nestlings did, suggesting that a developing immune system can be adjusted to environmental conditions (i.e. risk of parasitism) by trading developing immunity against growth (Szép & Møller 1999). Evidence of that important trade-off mostly comes from laboratory and poultry studies (Mangel & Stamps 2001) where the costs of using the system have been experimentally increased (Klasing & Leshchinsky 1999). Immune responses to non-pathogenic antigens have been shown to impair growth performance in domestic poultry (Klasing *et al.* 1987) and in Japanese quails (*Coturnix coturnix*) (Fair *et al.* 1999), two species with altricial rapidly growing chicks. Hörak *et al.* (2000) attempted to demonstrate that trade-off in a population of great tits (*Parus major*), by experimentally injecting a novel antigen (phytohaemagglutinin, PHA) into nestlings and exploring its effect on nestling growth. However, nestlings that grew poorly produced a weaker cutaneous response to PHA inoculation than well-growing nestlings suggesting that, although T-lymphocyte-mediated immune responsiveness is resource demanding, these resources are not re-allocated from those used for growth.

We investigated the trade-off between development of the immune system and growth in a wild population of magpies (*Pica pica*) by experimentally providing some of the nestlings in a nest with methionine, a sulphur amino

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acid that specifically enhances T-cell immune response in chickens (Tsiagbe *et al.* 1987; see below).

Diets with supplemental sulphur amino acids are commonly used to improve the immunocompetence and general health status in chickens and other domestic animals (National Organic Standards Board Technical Advisory Panel Review for the United States Department of Agriculture National Organic Program, 21 May 2001, Methionine). The main reason for this is that insufficiency leads to profound growth retardation and compromises glutathione synthesis in the presence and absence of a high rate of cell division. The functioning of T cells depends on the intracellular glutathione concentration and might be affected by sulphur amino acid insufficiency (Grimble & Grimble 1998; Redmond *et al.* 1998). In accordance with the influence of sulphur amino acids on the immune system, it has been experimentally shown that methionine addition in broilers' diet enhanced T-cell-mediated immune response (Tsiagbe *et al.* 1987; Swain & Johri 2000).

Although supplemental methionine can also be considered as a nutrient supplement for experimental nestlings, it induces an exaggerated production of immune cells (lymphocyte repertoire), which is costly in energy but not in amino acid consumption (Klasing & Leshchinsky 1999). Thus, our experiment reduces the availability of resources for other types of cell production. Therefore, because methionine enhances the T-cell-mediated immune function, the suggestion of a trade-off between growth and immunity predicts that nestlings supplemented with methionine should experience a larger immune response but a slower growth rate than control chicks.

2. MATERIAL AND METHODS

(a) *Study area and species*

The experiment was done in the spring of 1997 and 1998 in La Calahorra and Hueneja, respectively. Magpie subpopulations at those localities are *ca.* 5 km from each other and are located at the Hoya de Guadix (37°18′ N, 3°11′ W, southern Spain), a high altitude plateau, *ca.* 1000 m above sea level, where approximately 400 magpie pairs breed. The vegetation is sparse, including cultivated cereals (especially barley) and many groves of almond trees (*Prunus dulcis*) in which magpies prefer to build their nests (for a more detailed description, see Soler (1990)).

Magpies occur throughout large parts of the Holarctic region. They are territorial, sedentary and relatively long-lived for passerine birds, with a well-described biology (extensively reviewed in Birkhead (1991)). A single clutch is laid in spring, from March to May in their Western European range, with a clutch size ranging from 3 to 10 eggs (Birkhead 1991); nestling immune response decreases as the season progresses (Sorci *et al.* 1997). Some chicks regularly die from starvation, mainly in the first week after hatching, and the species is considered to adopt a brood reduction strategy (Slagsvold *et al.* 1992; Reynolds 1996). In our study area, magpies frequently suffer from brood parasitism by the great spotted cuckoo (*Clamator glandarius*) (Soler & Soler 2000), but parasitized nests were not used in this study.

(b) *Experimental procedure*

Two days after hatching of the last eggs (nests were visited daily), we established the within-nest nestling hierarchy by weighing each nestling on a portable digital balance (Sartorius Portable PT600, precision ± 0.01 g). We also marked all nestlings with different waterproof colours on tarsus and feet to allow individual recognition. The heaviest nestlings were randomly assigned to one of two treatments (methionine (M) or placebo (C)). Depending on the treatment of the heaviest chick, the second, third, etc., chicks were distributed alternately to one of the two treatments (e.g. first: M, second: C, third: M, fourth: C; or first: C, second: M, third: C, fourth: M). When the youngest nesting was 2 days old, we dosed each of the experimental nestlings with 0.02 g of DL-methionine (Sigma, M-9500) in pill form, for 4 consecutive days. The dose was calculated based on that administered to chickens (see Tsiagbe *et al.* 1987), and a mean body mass of magpie nestlings of 20 g. Water was also provided to aid swallowing. Control nestlings were given placebos and water. We weighed all nestlings on all four visits to estimate relative growth as the percentage of weight gained during the 4 days of treatment divided by nestling weight at the first visit. Three to five days later, we re-checked all nests to band nestlings with colour rings. Approximately 4 days before fledging, when chicks were *ca.* 17–18 days old, nestlings were ringed with an aluminium band, and their tarsus length was measured with a digital calliper to the nearest 0.01 cm. They were weighed with a Pesola spring balance (accuracy 0.5 g), and wing and tail length were measured with a ruler (accuracy 1 mm).

(c) *Immunocompetence and level of parasitism*

A phytohaemagglutinin-P (PHA-P, Sigma Chemical Co.) injection was used to evaluate the *in vivo* T-cell-mediated immune response of nestlings (Cheng & Lamont 1988). When nestlings were *ca.* 17–18 days old, they were subcutaneously injected in the right wing web with 0.5 mg of PHA dissolved in 0.1 ml of physiological saline solution (Bausch & Lomb Co.). The left wing web was injected with 0.1 ml of saline solution. The thickness of each wing web was measured at the injection site with a digital pressure-sensitive micrometer (Mitutoyo, model ID-CI012 BS, accuracy 0.01 mm) before, and 24 h after, the injection. We estimated the T-cell-mediated immune response or wing web index as the change in thickness of the right wing web (PHA injection) minus the change in thickness of the left wing web (Lochmiller *et al.* 1993). We repeated measurements of each wing web three times and, as they were highly repeatable ($R > 0.85$, see also Sorci *et al.* (1997)), the mean value was used in subsequent analyses.

In 1997, we took one blood smear from the brachial vein of each nestling (17–18 days old). Smears were subsequently air-dried, fixed in absolute ethanol for 3 min and dyed with Giemsa stain. Blood smears were then investigated for haematozoa (*Haemoproteus* sp.) and white blood cells. We first estimated how many red blood cells were contained in a field under a microscope and then calculated how many fields were needed to screen 10 000 red blood cells. Using a light microscope under oil immersion (×1000), we counted both parasitized cells and leucocytes in all those microscope fields. Avian species are known to respond to parasitism and infectious diseases by increasing the concentration of leucocytes (Davis 1981; Hawkey *et al.* 1983; Averbeck 1992). We used the number of lymphocytes detected in the blood smears to estimate the health status of nestlings.

(d) *Sample size and statistical analyses*

After excluding nests that failed to rear nestlings in both experimental and control treatments, the sample size was

reduced from 77 to 69 nests (35 in 1997 and 34 in 1998). Experimental nests used in 1997 and 1998 were from different areas. Although adult magpies were not colour-ringed, the probability of using the same magpie pair in both years is greatly reduced owing to adult breeding philopatry (Birkhead 1991).

All variables were approximately normally distributed (Kolmogorov–Smirnov test for continuous variables, $p > 0.2$) except the number of blood parasites because of the great number of magpie nestlings with no blood parasites. Thus, to compare the parasite load of nestlings, we only used nests in which a blood parasite was detected in at least one of the nestlings' blood smears. As comparisons were made between nestlings of the same nest, and variance in parasite load of experimental and control nestlings was homogeneous (Levene's test for homogeneity of variances, $F = 0.246$, $p = 0.62$), problems related to non-normality of data are not important.

Body mass and tarsus length were strongly positively correlated ($R = 0.845$, $F_{(1,245)} = 612.2$, $p < 0.0001$). However, residuals from this regression were positively correlated with two other body size indicators: wing length ($R = 0.149$, $F_{(1,245)} = 5.53$, $p = 0.028$) and tail length ($R = 0.140$, $F_{(1,244)} = 4.90$, $p = 0.028$). Therefore, we controlled body mass for both tarsus and wing length (multiple $R = 0.856$, $F_{(2,244)} = 333.6$, $p < 0.0001$; partial regression coefficients: tarsus length = 0.654, $t_{(244)} = 11.51$, $p < 0.0001$; wing length = 0.234, $t_{(244)} = 4.12$, $p < 0.0005$). Residuals from this regression were not significantly correlated with tail length ($R = 0.012$, $F_{(1,244)} = 0.04$, $p = 0.85$) and, therefore, these residuals are likely to represent an appropriate index of body condition (Green 2001).

To test for differences between experimental and control nestlings in T-cell-mediated immune response, relative growth, parasitism, health status and body condition index (dependent variables), we used a two-factor analysis of covariance (ANCOVA) design where nest and experiment (i.e. experimental and control nestlings) were random factors, and number of nestlings in the nest and nestling age at PHA injection were defined as covariates. Degrees of freedom were estimated by using the Satterthwaite method and the analyses were done using the 'Variance Components' module in the computer program STATISTICA '98 edition (StatSoft, Inc.). We then estimated the influence of methionine treatment on nestlings while taking into account variation among nests, using nests as independent data points (see, for instance, degrees of freedom in table 1). To explore the link between parasitism, health status, T-cell immune response and growth, as we were interested in detected differences between control and experimental nestlings sharing environmental conditions (i.e. nest), we only used magpie nests from 1997 in which at least one nestling was detected with blood parasites and from which we had data for all variables (25 magpie nests).

3. RESULTS

As expected from previous experimental results in poultry, supplemental methionine affected the immune response of magpie nestlings (table 1). In accordance with the prediction of a trade-off between investment in development of the immune system and growth, we found that experimental nestlings showed a stronger T-cell-mediated immune response but lower growth rate than control nestlings (figure 1), both variables being explained by treatment (i.e. experimental or control) after controlling for variation among nests (table 1). In addition, as the interaction between treatment and nest was not significant, the experiment apparently had a similar effect across all magpie nests.

To explore the possible link between nestling health status, parasite load, body condition, T-cell immune response and growth we only analysed nests for which we had information for all variables (data from 1997). The analyses revealed that both parasite load and lymphocyte counts were explained by treatment (table 2). Nestlings experimentally fed with methionine pills for four days at the beginning of the nestling period suffered less from parasitism and had lower lymphocyte counts than control nestlings (figure 2). Moreover, and in accordance with previous results, treatment was a significant factor in growth rate and T-cell immune response when using only data from 1997 (table 2).

For body condition index (see § 2) and other biometrical variables of nestlings close to the age of fledging, treatment had no significant effect (table 3). Experimental and control nestlings sharing the same nest fledged with similar body weight, tarsus, wing and tail length, and body condition index (table 3). This latter result could indicate that experimental nestlings experienced a lower growth rate in the first half of the nestling period (owing to supplemental methionine), but a higher growth rate in the second half of the nestling period. However, the percentage of weight gained after the last day of methionine supplementation to the day of PHA injections (a period of *ca.* 11 days) divided by the nestling weight when 17–18 days old was similar in control (marginal mean = 341.7, s.e.m. = 8.6) than in experimental (marginal mean = 329.7, s.e.m. = 8.5) nestlings (ANCOVA, age and number of nestlings as covariables, treatment effect: $F_{(1,\,25.7)} = 0.39$, $p = 0.54$). Thus, the effect of supplemented methionine on nestling growth occurs, perhaps, mainly during the period of pill supplementation, being less important afterwards. Another possible explanation is related to the result that methionine-supplemented nestlings were less parasitized, but demonstrated a larger immune response to PHA injection, and were of better health than control chicks (table 2). Those results imply that experimental nestlings suffered less from parasitism and diseases that could compensate for the lower growth rate imposed by the experimental methionine supplementation. Experimental and control nestlings reach a similar body mass when fledging.

4. DISCUSSION

Evidence for the role of methionine as a stimulant of the immune system comes from experiments done on poultry. Chickens fed with methionine during the growth period mounted a stronger T-cell-mediated immune response when adults (Tsiagbe *et al.* 1987; Swain & Johri 2000), although no effect was found on growth. These experiments, however, were performed under laboratory conditions with food provided *ad libitum*. Therefore, a possible trade-off between immunocompetence and growth could have been masked by the surplus of energy/resources available to developing organs or physiological activities (see Mangel & Stamps 2001). It is also known that *ad libitum* diets with supplemental sulphur

Table 1. Results of two-factor ANCOVAs with relative nestling growth and T-cell-mediated immune response as dependent variables, experimental treatment (supplemental methionine or control nestlings) and experimental nest as random factors, and number of nestlings and nestling age as co-variates. F and R represent fixed and random effects, respectively. Degrees of freedom of the error term were computed by using the Satterthwaite method. MS, mean square.

	effect	d.f. effect	MS effect	d.f. error	MS error	*F*	*p*
(*a*) relative growth							
age	F	1	66.72	67.08	3644.94	0.02	0.89
number of nestlings	F	1	18590.58	62.53	4433.27	4.19	0.045
treatment	R	1	1719.248	52.57	333.83	5.15	0.027
nest	R	65	3975.856	49.08	262.70	15.13	0.0000001
treatment × nest	R	64	283.78	107	685.12	0.41	0.9999
(*b*) T-cell immune response							
age	F	1	0.453	70.19	0.404	1.12	0.29
number of nestlings	F	1	0.014	59.66	0.469	0.03	0.87
treatment	R	1	0.934	54.82	0.184	5.06	0.028
nest	R	65	0.431	58.56	0.172	2.50	0.0002
treatment × nest	R	64	0.171	107	0.147	1.16	0.24

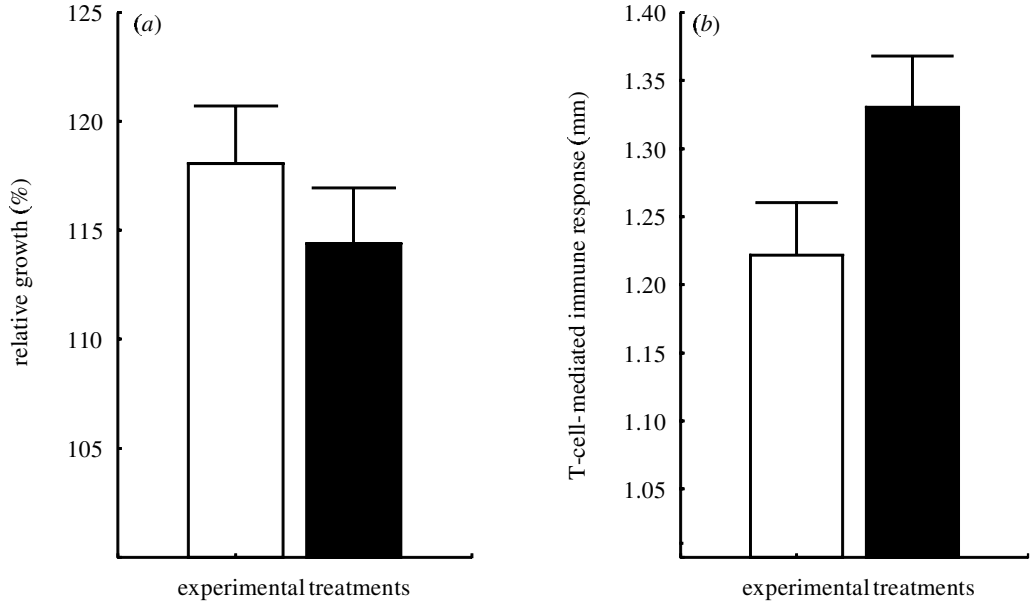

Figure 1. Population marginal means of (*a*) relative growth and (*b*) T-cell-mediated immune response for experimentally methionine-fed (black bars) and control (open bars) nestlings. Sample sizes are nests with experimental or control nestlings. Error bars are s.e.m., $N = 67$.

amino acids positively influence growth (Tsiagbe *et al.* 1987; Edwards & Baker 1999; Waibel *et al.* 2000). However, we found that the methionine supplementation experiment on growth produced the opposite effect and the dose of methionine used in this study cannot therefore be considered as a nutrient that can be used for body growth. Rather, that negative relation, with the greater immune response of experimental nestlings, implies that our experiment modified the rules of resource allocation during growth and affected, differentially, the growth and immune systems. Therefore, our findings of a correlated negative response of growth rate after the enhancement of the immune system with methionine support the idea of a trade-off between development of the immune system and growth.

Life-history trade-offs have been thought to result from competition among different organismic functions for limited internal resources (see Zera & Harshman 2001). If internal resources are limited, an increase of resources allocated to one trait necessitates a decrease of resources available for other traits (van Noordwijk & de Jong 1986). Thus, reduced availability of energy/resources can substantially magnify a trade-off, whereas increased nutrient availability can diminish or, eventually, completely mask it (Nijhout & Emlen 1998; Zera & Brink 2000). In our experimental approach, we did not manipulate nutrient availability but, by experimental methionine supplementation, we did manipulate the amount of nutrients allocated to the development of the immune system, which was confirmed by a higher T-cell-mediated immune response in methionine-supplemented magpie nestlings. Therefore, we directly manipulated priority rules, shaped by ecological factors and governing the relative allocation of nutrients to organ processes (see Zera & Harshman 2001). Thus, with similar amounts of resources (i.e. experimental and control nestlings shared the same nest), experimental

Table 2. Results of two-factor ANCOVAs with relative nestling growth, T-cell-mediated immune response, blood parasite load, and lymphocyte count as dependent variables. Experimental treatment (supplemental methionine or control nestlings) and experimental nest are random factors, and number of nestlings and nestling age are co-variates. F and R represent fixed and random effects, respectively. Degrees of freedom of the error term were computed by using the Satterthwaite method. Data are from nests of 1997 from which values of all dependent variables were known.

	effect	d.f. effect	MS effect	d.f. error	MS error	*F*	*p*
(*a*) relative growth							
age	F	1	189.9	23.33	3073.5	0.06	0.80
number of nestlings	F	1	4779.6	22.88	3538.0	1.35	0.26
treatment	R	1	1210.8	34.47	243.7	4.97	0.032
nest	R	23	3349.3	21.31	144.4	23.19	0.0000001
treatment × nest	R	22	145.1	45	204.6	0.71	0.81
(b) T-cell immune response							
age	F	1	0.329	27.34	0.185	1.78	0.19
number of nestlings	F	1	0.405	19.98	0.183	2.21	0.15
treatment	R	1	1.543	18.82	0.250	6.17	0.023
nest	R	23	0.185	21.62	0.245	0.76	0.74
treatment × nest	R	22	0.244	45	0.189	1.29	0.23
(*c*) blood parasite load							
age	F	1	1541.8	25.22	1523.8	1.01	0.32
number of nestlings	F	1	1285.7	22.46	1670.6	0.77	0.39
treatment	R	1	1223.4	15.26	217.5	5.62	0.031
nest	R	23	1596.0	20.65	221.2	7.21	0.00001
treatment × nest	R	22	225.7	45	620.2	0.36	0.99
(*d*) lymphocyte count							
age	F	1	261.9	25.02	299.2	0.88	0.36
number of nestlings	F	1	28.3	22.52	329.8	0.09	0.77
treatment	R	1	238.0	15.64	38.12	6.24	0.024
nest	R	23	314.7	20.60	37.93	8.30	0.000004
treatment × nest	R	22	38.7	45	110.70	0.35	0.99

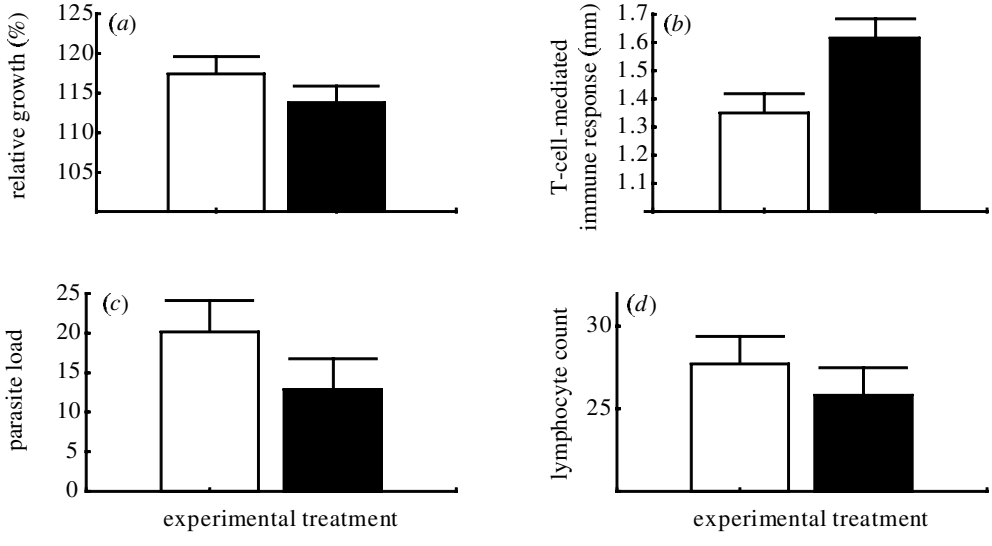

Figure 2. Population marginal means of (*a*) relative growth (%), (*b*) T-cell-mediated immune response (mm), (*c*) blood parasite load, and (*d*) lymphocyte count for experimentally methionine-fed (black bars; $N = 23$) and control (open bars; $N = 24$) nestlings. Data are from 1997 and sample sizes are nests with experimental or control nestlings. Error bars are s.e.

nestlings differentially allocated resources to the immune system.

Although it has been argued that the energetic cost of immune function might be relatively low (Klasing 1998; Owens & Wilson 1999), empirical and experimental evidence on trade-offs between immune response and other life-history traits are abundant (for a review see Lochmiller & Deerenberg (2000)). A trade-off between growth rate and inmunocompetence is predicted because substantial nutritional and energetic demands are associated with immune activation and the maintenance of an efficient immune system (Lochmiller & Deerenberg

Table 3. Population marginal means ± s.e.m. of biometrical and body condition variables. Data are from 1997 and sample sizes are the number of nests with experimental or control nestlings. Treatment effects from a two-factor ANCOVA with relative body mass, tarsus length, wing length, tail length and body condition index of nestlings as dependent variables. Experimental treatment (supplemental methionine or control nestlings) and experimental nest are random factors, and number of nestlings and nestling age are co-variates. F and R represent fixed and random effects, respectively. Degrees of freedom of the error term were computed by using the Satterthwaite method. The inclusion of data from 1998 did not change the results.

	control ($N = 24$) mean ± s.e.m.	methionine ($N = 23$) mean ± s.e.m.	d.f. effect	d.f. error	F	p
body mass (g)	141.2 ± 2.9	143.9 ± 2.9	1	19.15	0.0004	0.98
tarsus length (mm)	46.9 ± 0.4	47.4 ± 0.4	1	20.26	0.07	0.80
wing length (mm)	78.7 ± 1.4	80.6 ± 1.4	1	20.14	0.14	0.72
tail length (mm)	22.3 ± 1.1	22.7 ± 1.0	1	19.54	0.003	0.96
body condition index	0.95 ± 1.12	0.32 ± 1.10	1	22.80	0.21	0.65

2000), and with development of the immune system (Klasing & Leshchinsky 1999). In agreement with the theory of a trade-off between development of the immune system and growth, strains of chickens and turkeys artificially selected for high growth rates are more susceptible to a variety of pathogens than strains selected for other traits, such as high rate of egg production (see Mangel & Stamps (2001), and references therein).

We found a significant effect of supplementary methionine on T-cell-mediated immune response and growth, with experimental nestlings having a stronger immune response and lower growth. The lower growth rate of experimental nestlings could be due to an unbalanced source of amino acids in their diet because it is known that an excess of certain amino acids can reduce feed intake and depress amino acid utilization in poultry (Pack 1995). However, this does not seem to apply to methionine because Slominski *et al.* (1999) found higher weight and faster mass gains of chickens on a diet supplemented with methionine and lysine.

Moreover, we found support for a link between differential resource allocation to the immune system and the health of nestlings. Despite experimental and control nestlings sharing the same environment (i.e. nest and parents), experimental nestlings suffered less from blood parasites and had a lower concentration of leucocytes in peripheral blood. Those results strongly suggest a link between immunocompetence and health status because we experimentally manipulated the first and found effects on the second, thereby experimentally detecting benefits of an exaggerated immune response. However, those benefits can be counteracted by a relatively lower growth rate, which would imply a larger probability of nest predation (Martin 1995), or suffering the cost of parasites in the nests for a long period (Saino *et al.* 1998). Therefore, it could be that, in magpies, the benefits of the specific growth rate of nestlings surpass the benefits of an exaggerated immunocompetence under natural conditions. As resolution of the trade-off between immunocompetence and growth is probably different for different species, depending on the level of parasitism and predation pressures suffered by nestlings, a larger naturally depressed immune system can be predicted in species suffering more from those predation pressures.

Surprisingly, differences in growth rate induced during the methionine treatment did not result in different sizes at fledging. Experimental and control nestlings fledged with similar body mass, size and condition (table 3). However, the growth rate of experimental and control nestlings after experimental supplementation with methionine did not differ significantly (see § 3). There are at least three different explanations for those results. First, there is an indication that the effect of methionine on nestling growth was reduced after 4 days of supplementation and, because mass gained during those four days represents only *ca.* 25% of the nestling body mass when 17–18 days old, significant statistical differences in growth rate disappeared when nestlings approached fledging. Second, there is the possibility of experimental nestlings being able to change the pattern of energy/resource allocation to growth, compensating for the size difference observed during methionine treatment. Third, there are the possible effects of parasitism and diseases on nestling growth (see Møller 1995). Control and experimental nestlings shared the same environment (i.e. nest and parents), and the probability of parasitism should therefore have been similar for the two groups. Nevertheless, control nestlings were more likely to harbour blood parasites than experimental chicks from the same nest, and it is possible that parasites gradually slowed down the growth of their hosts. According to this scenario, methionine-supplemented nestlings suffered from significantly lower growth rates during the first phase of the nestling period because of redistribution of resources to immune function, but benefited from a higher parasite protection. However, control nestlings grew faster, mainly during the first days of their life, but were less protected by their immune system. Thus, a higher exposure to parasites and pathogens of control nestlings might have reduced their growth rate during the later phase of the nestling period to the point of that imposed by the experimental methionine supplementation. This possibility, however, requires further experimental work.

In conclusion, we found support for a trade-off between immunity and growth by experimentally feeding magpie nestlings with methionine, which increases resource allocation to the immune system. Moreover, the experimentally exaggerated T-cell-mediated immune response of experimental nestlings correlated with their better health status, but this benefit may be counteracted by the associated costs (i.e. predation and parasitism at the nest) of a lower growth rate.

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REFERENCES

Averbeck, C. 1992 Haemathology and blood chemistry of healthy and clinically abnormal great black-backed gulls (*Larus marinus*) and herring gulls (*Larus argentatus*). *Avian Pathol.* **21**, 215–223.

Birkhead, T. R. 1991 *The magpies. The ecology and behaviour of black-billed and yellow-billed magpies*. London: T. & A. D. Poyser.

Cheng, S. & Lamont, J. 1988 Genetic analysis of immunocompetence measures in a white leghorn chicken line. *Poultry Sci.* **67**, 989–995.

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

Clutton-Brock, T. H. 1988 *Reproductive success*. University of Chicago Press.

Davis, P. J. 1981 Immunity to Coccidia. In *Avian immunology* (ed. M. E. Rose, L. M. Payne & B. M. Freeman), pp. 361–385. Edinburgh: British Poultry Science Ltd.

Edwards, H. N. I. & Baker, D. H. 1999 Maintenance sulfur amino acids requirements of young chicks and deficiency of their use for accretion of whole-body sulfur amino acids and protein. *Poultry Sci.* **78**, 1418–1423.

Fair, J. M., Hansen, E. S. & Ricklefs, R. E. 1999 Growth, developmental stability and immune response in juvenile Japanese quails (*Coturnix coturnix japonica*). *Proc. R. Soc. Lond.* B **266**, 1735–1742. (DOI 10.1098/rspb.1999.0840.)

González, G., Sorci, G., Møller, A. P., Ninni, P., Haussy, C. & de Lope, F. 1999 Immunocompetence and condition-dependent sexual advertisement in male house sparrows (*Passer domesticus*). *J. Anim. Ecol.* **68**, 1225–1234.

Green, A. J. 2001 Mass/length residuals: measures of body condition or generators of spurious results? *Ecology* **82**, 1473–1483.

Grimble, R. F. & Grimble, G. K. 1998 Immunonutrition: role of sulfur amino acids, related amino acids, and polyamines. *Nutrition* **14**, 605–610.

Hawkey, C. M., Samour, J. H. & Ashton, D. G. 1983 Normal and clinical haematology of captive cranes (Gruiformes). *Avian Pathol.* **12**, 73–84.

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

Ilmonen, P., Taarna, T. & Hasselquist, D. 2000 Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. *Proc. R. Soc. Lond.* B **267**, 665–670. (DOI 10.1098/rspb.2000.1053.)

Klasing, K. C. 1998 Nutritional modulation of resistance to infectious diseases. *Poultry Sci.* **77**, 1119–1125.

Klasing, K. C. & Leshchinsky, T. V. 1999 Functions, costs, and benefits of the immune system during development and growth. In *Proc. 22nd Int. Ornithol. Congr. Durban* (ed. N. J. Adams & R. H. Slotow), pp. 2817–2835. Johannesburg: BirdLife, South Africa.

Klasing, K. C., Lauring, L., Peng, R. & Fry, M. 1987 Immunologically mediated growth depression in chicks: influence of feed intake, corticosterone and interleukin-1. *J. Nutr.* **117**, 1629–1637.

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

Lochmiller, R. L. & 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. & Boren, J. C. 1993 Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. *Auk* **110**, 503–510.

Mangel, M. & Stamps, J. 2001 Trade-offs between growth and mortality and the maintenance of individual variation in growth. *Evol. Ecol. Res.* **3**, 583–593.

Martin, T. E. 1995 Avian life history evolution in relation to nest sites, nest predation, and food. *Ecol. Monogr.* **65**, 101–127.

Møller, A. P. 1995 Parasitism and the evolution of host life history. In *Host–parasite evolution. General principles and avian models* (ed. D. H. Clayton & J. Moore), pp. 105–127. Oxford University Press.

Moreno, J., Sanz, J. J. & Arriero, E. 1999 Reproductive effort and T-lymphocyte cell-mediated immunocompetence in female pied flycatchers *Ficedula hypoleuca*. *Proc. R. Soc. Lond.* B **266**, 1105–1109. (DOI 10.1098/rspb.1999.0750.)

Moreno, J., Sanz, J. J., Merino, S. & Arriero, E. 2001 Daily energy expenditure and cell-mediated immunity in pied flycatchers while feeding nestlings: interaction with moult. *Oecologia* **129**, 492–497.

Nijhout, H. F. & Emlen, D. J. 1998 Competition among body parts in the development and evolution of insect morphology. *Proc. Natl Acad. Sci. USA* **95**, 3685–3689.

Nordling, D., Andersson, M., Zohari, S. & Gustafsson, L. 1998 Reproductive effort reduces specific immune response and parasite resistance. *Proc. R. Soc. Lond.* B **265**, 1291–1298. (DOI 10.1098/rspb.1998.0432.)

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

Owens, I. P. F. & Wilson, K. 1999 Immunocompetence: a neglected life history trait or conspicuous red herring? *Trends Ecol. Evol.* **14**, 170–172.

Pack, M. 1995 Excess protein can depress amino acid utilization. *Feed Mix* **3**, 24–25.

Råberg, L., Nilsson, J. A., Ilmonen, P., Stjernman, M. & Hasselquist, D. 2000 The cost of an immune response: vaccination reduces parental effort. *Ecol. Lett.* **3**, 382–386.

Redmond, H. P., Stapleton, P. P., Neary, P. & Bouchier-Hayes, D. 1998 Immunonutrition: the role of taurine. *Nutrition* **14**, 599–604.

Reynolds, P. S. 1996 Brood reduction and siblicide in black-billed magpies (*Pica pica*). *Auk* **113**, 189–199.

Saino, N., Calza, S. & Møller, A. P. 1998 Effects of a dipteran ectoparasite on immune response and growth trade-offs in barn swallow, *Hirundo rustica*, nestlings. *Oikos* **81**, 217–228.

Slagsvold, T., Husby, M. & Sandvik, J. 1992 Growth and sex ratio of nestlings in two species of crows: how important is hatching asynchrony? *Oecologia* **90**, 43–49.

Slominski, B. A., Simbaya, J., Campbell, L. D., Rakow, G. & Guenter, W. 1999 Nutritive value for broiler of meals derived from newly developed varieties of yellow seeded canola. *Anim. Feed Sci. Technol.* **78**, 249–262.

Soler, J. J. & Soler, M. 2000 Brood–parasite interactions between great spotted cuckoos and magpies: a model system for studying coevolutionary relationships. *Oecologia* **125**, 309–320.

Soler, M. 1990 Relationships between the great spotted cuckoo *Clamator glandarius* and its corvid host in a recently colonized area. *Ornis Scand.* **21**, 212–223.

Sorci, G., Soler, J. J. & Møller, A. P. 1997 Reduced immunocompetence of nestlings in replacement clutches of the European magpie (*Pica pica*). *Proc. R. Soc. Lond.* B **264**, 1593–1598. (DOI 10.1098/rspb.2000.1053.)

Stearns, S. C. 1992 *The evolution of life histories*. Oxford University Press.

Swain, B. K. & Johri, T. S. 2000 Effect of supplemental methionine, choline and their combinations on the performance and immune response of broilers. *Br. Poultry Sci.* **41**, 83–88.

Szép, T. & 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.

Tsiagbe, V. K., Cook, M. E., Harper, A. E. & Sunde, M. L. 1987 Enhaced immune responses in broiler chicks fed methionine supplemented diets. *Poultry Sci.* **66**, 1147–1154.

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

Waibel, P. E., Carlson, C. W., Brannon, J. A. & Noll, S. L. 2000 Limiting amino acids after methionine and lysine with growing turkeys fed low protein diets. *Poultry Sci.* **79**, 1290–1298.

Williams, T. D., Christians, J. K., Aiken, J. J. & Evanson, M. 1999 Enhanced immune function does not depress reproductive output. *Proc. R. Soc. Lond.* B **266**, 753–757. (DOI 10.1098/rspb.1999.0701.)

Zera, A. J. & Brink, T. 2000 Nutrient absorption and utilization by wing and flight muscle morphs of the cricket *Gryllus firmus*: implications for the trade-off between flight capability and early reproduction. *J. Insect Physiol.* **46**, 1207–1218.

Zera, A. J. & Harshman, L. G. 2001 The physiology of life history trade-offs in animals. *A. Rev. Ecol. Syst.* **32**, 95–126.

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