TRADE-OFF BETWEEN IMMUNOCOMPETENCE AND GROWTH IN MAGPIES: AN EXPERIMENTAL STUDY

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ABSTRACT

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, since resource allocation to different activities (or organs) during growth is not easy to manipulate, this trade-off has only been experimentally tested by studying the effects of non-pathogenic antigens. Here, 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 limited resources allocation 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 others pathogens on nestling growth.

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 (e.g. Lindström 1999) that influence nestling condition and the ability of their immune system to resist pathogen attacks (e.g. Christe *et al*. 1998; González *et al*. 1999). However, development of a good immune system, as well as its maintainance and use, is costly in terms of energy and nutrients that otherwise could be used to further develop other phenotypic traits (Klasing & Leshchinsky 1999; Norris & Evans 2000; Lochmiller & Deerenberg 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; Ilmonen *et al*. 2000; Moreno *et al*. 2001; 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 hypothesised based on results from experimental parasitism of swallow (*Hirundo rustica*) nests with ectoparasites, which resulted in higher growth rates, but lower T-cell mediated immune response of experimental nestlings (Saino *et al*. 1998). Moreover, adult sand martins (*Riparia riparia*) did not respond to an ectoparasite treatment in terms of immunoglobulin concentration, while 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 costs of using the system have been experimentally increase (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 show that trade-off in a population of great tits (*Parus major*), by experimentally injecting a novel antigen (PHA) to nestlings and explore 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 reallocated from those used for growth.

Here, we aimed to investigate 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 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 immunocompetence and general health status in chicken and other domestic animals (National Organic Standards Board Technical Advisory Panel Review for the USDA National Organic Program, May 21, 2001, Methionine), mainly because insufficiency leads to profound growth retardation and compromise glutathione synthesis in the presence and absence of a high rate of cell division. Functioning of T-cells depends on intracellular glutathione concentration and may also be affected by sulphur amino acid insufficiency (Redmond *et al*. 1998; Grimble & Grimble 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 supplementation for experimental nestlings, it induces an exaggerate production of immune cells (lymphocyte repertoire), which is costly in term of energy but not in term of amino acids consumption (Klasing & Leshchinsky 1999). Then, our experiment reduces availability of resources for other kinds of cell production. Therefore, since methionine enhance T-cell mediated immune function, the hypothesis 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.

MATERIALS AND METHODS

Study area and species

The experiment was carried out in the springs of 1997 and 1998 in La Calahorra, and Hueneja respectively. Magpie subpopulations at those localities are about 5 km far from each other and located at the Hoya de Guadix (37°18'N, 3°11'W, southern Spain), a high altitude plateau, approximately 1000 m above sea level, where about 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 nest (for a more detailed description, see Soler 1990).

Magpies occur throughout large parts of the Holartic 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, clutch size ranging from 3 to 10 eggs (Birkhead 1991), and 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 suffer frequently from brood parasitism by the great spotted cuckoo (*Clamator glandarius*) (e.g. Soler & Soler 2000), but parasitized nests were not used in the present study.

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 in the nest with different waterproof colors on tarsus and feet to allow individual recognition. Heaviest nestlings were randomly assigned to one of the 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 two days old, we administered each of the experimental nestlings with 0.02 g of DL-methionine (Sigma, M-9500) in a pill during four consecutive days. Dose was determined taking into account 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 facilitate swallowing. Control nestlings were given an empty pills and water. We weighed all nestlings on all four visits to estimate relative growth as the percentage of weight gained during the four days of treatment divided by nestling weight at the first visit. Three to five days later, we checked again all nests to band nestlings with color rings. About 4 days before fledging, when chicks were about 17-18 days old, nestlings were ringed with an aluminium band, and their tarsus length 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 was measured with a ruler (accuracy 1mm).

Immunocompetence and level of parasitism

A phytohemagglutinin-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 about 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 hours 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, since 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 three

minutes, 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 microscope and then calculated how many fields were need to screen 10.000 red blood cells. Then, by using a light microscope under oil immersion (x1000), we counted parasitized cells as well as leukocytes in all those microscope fields. Avian species are known to respond to parasitism and infectious diseases by increasing the concentration of leukocytes (Davis 1981; Hawkey *et al*. 1983; Averbeck 1992). We used numbers of lymphocytes detected in the blood smears to estimate health status of nestlings.

Sample size and statistical analyses

After excluding nests that failed to rear nestlings in both experimental and control treatments, sample size was reduced from 77 to 69 nests (35 in 1997 and 34 in 1998). Experimental nests used in 1998 were from a different area than those used in 1997. Although adult magpies were not colour ringed, the probability of using the same magpie pair during the two years is greatly reduced due 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 due to the great number of magpie nestlings with no blood parasites. Thus, to compare the parasite load of nestlings, we only used nests in which at least a blood parasite was detected in one of the nestlings' blood smear. Since 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 ($R = 0.845$, $F_{(1,245)} = 612.2$, $P < 0.00001$). 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 $R = 0.856$, $F_{(2,244)} = 333.6$, $P < 0.00001$; Partial regression coefficients: tarsus length = 0.654, $t_{(244)} = 11.51$, $P < 0.00001$; wing length = 0.234, $t_{(244)} = 4.12$, $P = 0.00005$). 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 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 made as covariates. Degrees of freedom were estimated using the Satterthwaite method and the analyses were performed using the "Variance Components" module in the computer program Statistica' 98 edition (StatSoft, Inc.). We were then able to estimate 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, since we are interesting on detected differences between control and experimental nestling 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).

RESULTS

As expected from previous experimental results in poultry, supplemental methionine affected 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 stronger T-cell mediated immune response but lower growth rate than control nestlings (Fig. 1), both variables being explained by treatment (i.e. experimental or control) after controlling for variation among nests (Table 1). In addition, since the interaction between treatment and nest was not significant, the experiment apparently had similar effect across all magpie nests.

TABLE 1 AND FIGURE 1 ABOUT HERE

To explore the possible link between nestling health status, parasite load, body condition, T-cell immune response, and growth we performed analyses only using nests from 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 during four days at the beginning of the nestling period suffered less from parasitism and had lower lymphocyte counts than control nestlings (Fig. 2). Moreover, and in accordance with previous results, treatment significantly explained growth rate and T-cell immune response when using only data from 1997 (Table 2).

TABLE 2 AND FIGURE 2 ABOUT HERE

With respect to body condition index (see Material and Methods) and other biometrical variables of nestlings close to the age of fledging, we did not find any significant effect of treatment (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 last result could indicate that experimental nestlings experienced lower growth rate in the first half of the nestling period (due to supplemental methionine), but a higher growth rate in the second half of the nestling period. However, percentage of gained weight after the last day of methionine supplementation to the day of PHA injections (a period of about 11 days) divided by the nestling weight when 17-18 days old was similar in control (marginal mean = 341.7, SE = 8.6) than in experimental (marginal mean = 329.7, SE = 8.5) nestlings (ANCOVA, age and number of nestlings as covariables, treatment effect: $F_{(1, 25.7)} = 0.39$, $P = 0.54$). Then, perhaps the effect of supplemented methionine on nestling growth is mainly during the period of pills' supplementation, being less important afterward. Other possible explanation is related to the results of methionine supplemented nestlings were less parasitized, but demonstrated larger immune response to PHA injection, and were of better health status than control chicks (Table 2). Those results implying that experimental nestling suffered less from parasitism and diseases that could compensate for the lower growth rate imposed by the experimental methionine supplementation. Then, experimental and control nestlings reaching a similar body mass when fledgling.

TABLE 3 ABOUT HERE

DISCUSSION

Evidence for the role of methionine as a stimulant of the immune system comes from experiments carried out on poultry. Chicken 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 amino acids positively influences growth (Tsiagbe *et al*. 1987; Edwards & Baker 1999; Waibel *et al*. 2000). However, we found that the effect of our methionine supplementation experiment on growth was the opposite and, then, dose of methionine employed in this study cannot be considered as nutrient that can be used for body growth. Rather, that negative relation, together with the result of larger immune response of experimental nestling, implies that our experiment modified rules of resource allocation during growing, and affect differentially to growth and immune system. Therefore, our findings of a correlated negative response of growth rate following 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 increment of resources allocated to one trait necessitates a decrement of resources available for other traits (van Noordwijk & de

Jong 1986). Thus, reduced availability of energy/resources can substantially magnify a trade-off, while increased nutrient availability can diminish or eventually completely mask it (e.g. Nijhout & Emlen 1998; Zera & Brink 2000). In our experimental approach, we did not manipulate nutrient availability but, by experimental methionine supplementation, we manipulated the amount of nutrients allocated to the development of the immune system, which was confirmed by higher T-cell mediated immune responses 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 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 2000), but also with development of the immune system (Klasing & Leshchinsky 1999). In agreement with the hypothesis 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).

In this study, we found a significant effect of supplementary methionine on T-cell mediated immune response and growth, with experimental nestlings having

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 excess of certain amino acids can reduce feed intake and depress amino acid utilization in poultry (e.g. 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 with supplementation of methionine and lysine in their diet.

Moreover, we found support for a link between differential resource allocation to the immune system and health of nestlings. In spite of experimental and control nestlings sharing the same environment (i.e. nest and parents), experimental nestlings suffered less from blood parasites and had lower concentration of leukocytes in peripheral blood. Those results strongly suggest a link between immunocompetence and health status because we experimentally manipulated the first and, then, pointing out related benefits of an exaggerated immune response. However, those benefits can be counteracted by a relative lower growth rate that would imply a larger probability of nest predation (e.g. 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, benefits of the specific growth rate of nestlings surpass benefits of an exaggerated immunocompetence in natural condition. Since resolution of the trade-off between immunocompetence and growth is likely different for different species, depending on the level of parasitism and predation pressures suffering by nestlings, it can be predicted a larger naturally depressed immune system in species suffering more from those predation pressures.

Surprisingly, differences in growth rate induced during the methionine treatment did not result in different size at fledging. Experimental and control nestlings fledged with similar body mass, size and condition (Table 4). However, growth rate of experimental and control nestlings after experimental supplementation with methionine

did not differ significantly (see results). There are, at least three different explanations for those results. The first one implies that the effect of methionine on nestling growth was reduced after the four days of supplementation and, since mass gained during those four days represent only around 25% of the nestling body mass when 17-18 days old, significant statistical differences in growth rate disappeared when nestlings are close to fledge. The second explanation is related to the possibility of experimental nestlings being able to change the pattern of energy/resource allocation to growth, compensating the size difference observed during methionine treatment. The third explanation concerns 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 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 of 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 rate during the first phase of the nestling period because of redistribution of resources to immune function, but benefited also from a higher parasite protection. On the contrary, control nestlings grew faster, mainly during the first days of their life, but were less protected by their immune system. Thus, higher exposure to parasite 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 scenario would however require 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 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 for the associated costs (i.e. predation and parasitism at the nest) of a lower growth rate.

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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 covariates. F and R represent fixed and random effects, respectively. Degrees of freedom of the error term were computed using the Satterthwaite method.

	Effect	df effect	MS effect	df error	MS error	F	P
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 x Nest	R	64	283.78	107	685.12	0.41	0.9999
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 x Nest	R	64	0.171	107	0.147	1.16	0.24

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 covariates. F and R represent fixed and random effects, respectively. Degrees of freedom of the error term were computed using the Satterthwaite method. Data are from nests of 1997 from which values of all dependent variables were known.

	Effect	df effect	MS effect	df error	MS error	F	P
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 x Nest	R	22	145.1	45	204.6	0.71	0.81
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 x Nest	R	22	0.244	45	0.189	1.29	0.23
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 x Nest	R	22	225.7	45	620.2	0.36	0.99
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 x Nest	R	22	38.7	45	110.70	0.35	0.99

Table 3: Population marginal means ± Standard error of biometrical and body condition variables. Data are from 1997 and sample sizes are the number of nests with experimental or control nestlings. Treatments effect from a two factor ANCOVAs 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 covariates. F and R represent fixed and random effects, respectively. Degrees of freedom of the error term were computed using the Satterthwaite method. Data are from nests of 1997. Including data from 1998 did not change the results.

	Control (N = 24) Mean ± SE	Methionine (N = 23) Mean ± SE	df effect	df 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

FIGURE LEGENDS

Fig 1: Population marginal means of relative growth (A) and T-cell mediated immune response (B) for experimentally methionine fed and control nestlings. Sample sizes are nests with experimental or control nestlings. Error bars are S.E.

Fig 2: Population marginal means of relative growth (%) (A), T-cell mediated immune response (mm) (B), blood parasite load (C), and lymphocyte count (D) for experimentally methionine fed and control nestlings. Data are from 1997 and sample sizes are nests with experimental or control nestlings. Error bars are S.E.

Fig.1

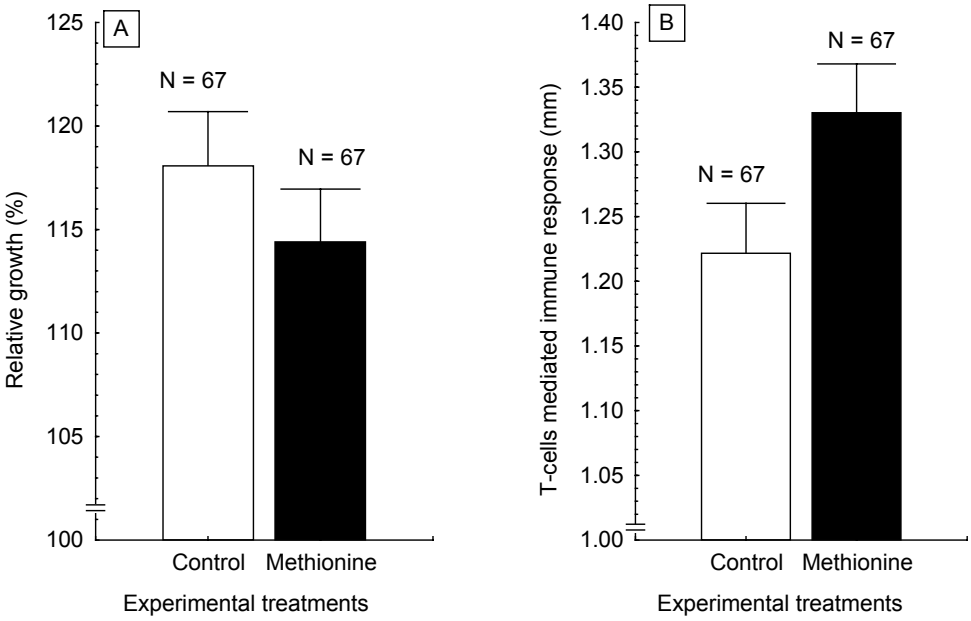
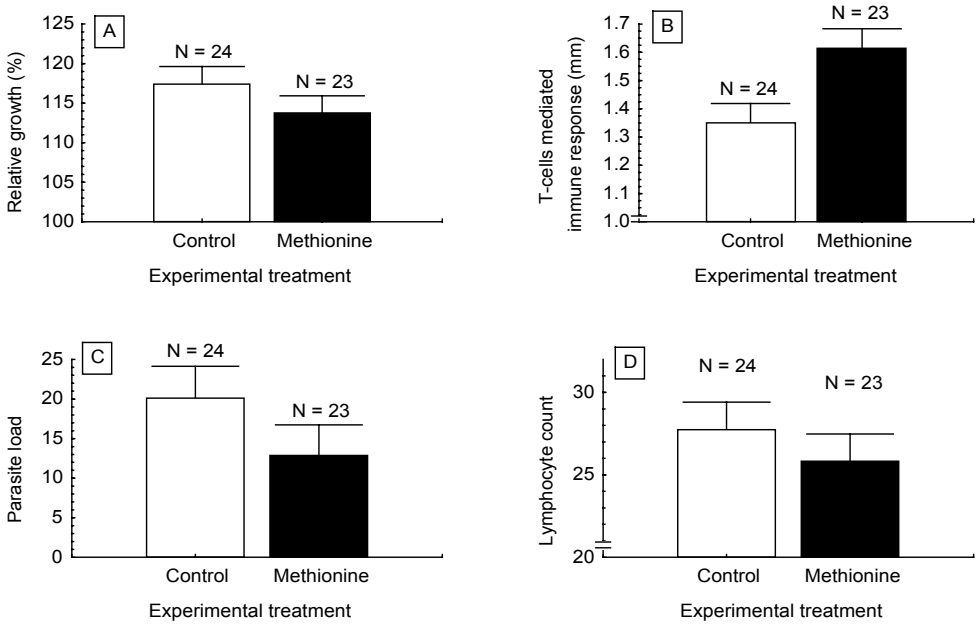

A
125
120
115
110
105
100
Relative growth (%)
N = 67
N = 67
Control
Methionine
Experimental treatments
B
1.40
1.35
1.30
1.25
1.20
1.15
1.10
1.05
1.00
T-cells mediated immune response (mm)
N = 67
N = 67
Control
Methionine
Experimental treatments

Fig. 2


A
Relative growth (%)
125
120
115
110
105
100
N = 24
N = 23
Control
Methionine
Experimental treatment
B
T-cells mediated immune response (mm)
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
N = 24
N = 23
Control
Methionine
Experimental treatment
C
Parasite load
25
20
15
10
5
0
N = 24
N = 23
Control
Methionine
Experimental treatment
D
Lymphocyte count
30
25
20
N = 24
N = 23
Control
Methionine
Experimental treatment