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The energetic cost of humoral immunity in the Collared Dove, *Streptopelia decaocto*: is the magnitude sufficient to force energy-based trade-offs?

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Summary

1. Energy saving is often suggested as the basis of a resource trade-off between immunocompetence and other fitness-relevant traits. This suggests that the energetic cost of an immune response is significant and sufficient to force trade-offs. To date, few studies have investigated the energetic cost of the humoral component of the immune system in birds and furthermore, existing results are contradictory.

2. We addressed this question through two experiments. In experiment 1, the basal metabolic rate (BMR) of Collared Doves, *Streptopelia decaocto*, challenged with sheep red blood cells (SRBC) was compared with the BMR of control birds. The energetic cost of immunity on host life-history strategies was compared with another physiological activity, thermoregulation, in experiment 2 to assess its significance.

3. Experiment 1 showed that antibody production against SRBC increased BMR of birds, with a peak of energy expenditure 7 days after immune activation (+8·5% BMR). In addition, we found that among birds fed *ad libitum*, those mounting a stronger immune response lost significantly more mass than controls or birds mounting a low immune response. In experiment 2, we found the cost of thermoregulation to be 5·27% BMR $°C^{-1}$.

4. If results from experiment 1 primarily suggested that an energy-based trade-off was expected between immune functions and other fitness-related traits, experiment 2 showed that the magnitude of this energetic cost corresponded to that used during low levels of thermoregulation. Consequently, we suggest that energy saving is not the central mechanism of a trade-off mediating immunocompetence. We provide some evidence that the degradation of body condition should be considered as an important additional cost of humoral immunity in the context of a resource-based trade-off.

Key-words: Basal metabolic rate, body condition, physiological trade-offs, SRBC, thermoregulation

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Introduction

Parasites and infectious agents represent some of the most important selection pressures driving the life histories of their hosts (Grenfell & Dobson 1995; Sheldon & Verhulst 1996; Clayton & Moore 1997). To prevent the proliferation of pathogens, numerous adaptations have been selected in hosts. Among them, the immune system is certainly the most evolved physiological mechanism by which hosts resist disease (Zuk & Stoehr 2002). Despite the efficiency of mechanisms involved in immunity, no organisms are resistant to the entire range of existing pathogens. This phenomenon leads us to the question of why immune systems with exhaustive competences have not been selected. One hypothesis considers that immunocompetence is costly, since investing in immune functions should affect other life-history traits that covary with individual fitness (Read & Allen 2000; Lochmiller & Deerenberg 2000; Schmid-Hempel 2003). If advantages of immune defences are clearly identified (e.g. fighting against intruding pathogens and parasites), the costs in turn are suggested in vertebrates and invertebrates through the negative effects of immune activation on fitness components such as breeding effort (Råberg *et al*. 2000; Rigby & Jokela 2000; Bonneaud *et al*. 2003) and laying date (Andersson 2001). Conversely, changes in

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components related to breeding effort affect immune activity and parasite load (Norris, Anwar & Read 1994; Richner, Christe & Oppliger 1995; Deerenberg *et al*. 1997; Nordling *et al*. 1998; Moreno, Sanz & Arriero 1999).

Although costs associated with immunity have been clearly demonstrated, direct mechanisms implying trade-offs between the immune system and other life-history traits remain equivocal. Among suggested mechanisms (e.g. immunopathology, oxidative stress; Råberg *et al*. 1998; Graham 2002), it is implicitly assumed that there is an energy-based trade-off between the immune system and other fitness-related activities, and there is some indirect and direct evidence to support this hypothesis. For instance, several studies have shown that quantity and quality of food resources modulate immune abilities (Lochmiller, Vestey & Boren 1993; Saino, Calza & Møller 1997; Klasing 1998) and the fitness cost of an immune response (Moret & Schmid-Empel 2000). However, the existence of an energy-based trade-off is unclear since some studies failed to show any changes in immune functions when manipulating the energetic budget in different species (see Owens & Wilson 1999). Moreover, existing studies investigating the effect of mounting an immune response on energy expenditure have given contradictory results. Increases in basal (BMR) or resting metabolic rate (RMR) were found in the mouse *Mus musculus* (Demas *et al*. 1997) and in Rainbow Trout, *Onchorhynchus mykiss* (Ackerman, Iwana & Thornton 2000). In full-grown birds, a similar result was found in the House Sparrow, *Passer domesticus*, after activating a cell-mediated immune response (Martin, Scheuerlein & Wikelski 2002), but results are more contrasted when considering the humoral component of the immune system. A significant increase in BMR was documented in the Great Tit, *Parus major*, when faced with novel antigens (Ots *et al*. 2001); however, similar results were not reported in the Greenfinch, *Carduelis chloris* (Hõrak *et al*. 2003), or the Blue Tit, *Parus caeruleus* (Svensson *et al*. 1998), despite the use of the same immune challenge in the former study. Therefore, the precise energetic cost of the humoral component of the immune system remains equivocal and needs further investigation. In addition, when assessing the effect of the deployment of immune defences on the energy budget and fitness in birds, the energy expenditure of an immune response is usually compared with the energy demand of other key biological functions (Martin *et al*. 2002). More extensively, under the assumption that the magnitude of the energetic cost of humoral immunity is sufficient to force trade-offs, all other physiological activities with equivalent energy expenditure are expected to affect fitness-related traits in a similar way to humoral immunity.

Consequently, the aims of this study were twofold. Firstly, we investigated the energetic cost of mounting a humoral immune response in a non-passerine bird species. The BMR of captive Collared Doves (*Streptopelia decaocto*) challenged with sheep red blood cells (SRBC) was compared with those of saline-injected controls and the relationship between the intensity of the immune response and the magnitude of the cost was tested. Furthermore, the effect of treatment on subsequent changes in body mass was investigated. In a second experiment, we compared the energetic cost of thermoregulation in Collared Doves with energy expenditure under immune challenge. Since low temperatures are known to negatively affect fitness-related traits such as clutch phenology, proportion of breeding females and egg weight (Järvinen & Ylimaunu 1986; Meijer *et al*. 1999; Nilsson & Svensson 1993; Yom-Tov & Wright 1993; Perrins 1996; Lessels, Dingemanse & Both 2002), we expected that if the energetic cost of humoral immunity was sufficient to force trade-offs, it would not be negligible with regard to the energy demand of thermoregulation at low ambient temperature.

Materials and methods

BIRD CAPTURE AND CARE

Collared Doves ($n = 35$) were trapped on their roosting sites between 20 January and 4 February 2003 in Charente-Maritime (Western France, 46° N, 0·5° W). Trapping sessions were carried out at night using a searchlight and a landing net. Each bird was fitted with an individual ring, weighed (±0·5 g) and aged (yearling *vs* adult) following Baker (1993). Doves were housed indoors at the Centre d'Etude Biologique de Chizé in compliance with the veterinary services of Deux-Sèvres. Conditions of captivity were controlled for humidity (50%), temperature (22 °C) and light (11L:13D from 07·30). Birds showing any signs of stress 5 days after capture (anorexia, prostration, $n = 11$), were released and excluded from our experiments. The remaining 24 birds were kept in $70 \times 80 \times 60$ cm^3 cages after randomization. Two birds were kept in each cage and fed *ad libitum* throughout the course of the study with cereals, mineral complements, grit and water. Doves were acclimatized for 1 month before the start of the experiments.

BMR MEASUREMENTS

Basal metabolic rate (BMR) was assessed from oxygen consumption measured in an open circuit respirometer (DePocas & Hart 1957). Atmospheric air was pumped through a calorimetric chamber. The flow rate of air entering was set to 1·19 l min^{-1} and measured using an infrared debimeter (Model 2044, Platon Flow Control Ltd, Basingstoke, UK). After leaving the chamber, the air was dehumidified by channelling through two successive drying systems: first, through an aluminium tube immersed in a tank containing alcohol that was frozen by a compressor, and secondly, through two successive water scrubbers (W.A. Hammond Drierite Co., Xenia, Ohio, USA). Oxygen concentration was measured with a paramagnetic analyser (Model 1100, resolution: ± 0·02%, Servomex Ltd, La Plaine

Saint-Denis, France) and corrected for changes in atmospheric pressure during the course of the measurement. Oxygen consumption was calculated following equations from Hill (1972) and on the basis of the 5-min period of lowest O_2 consumption. We assumed an energetic equivalence of 20·1 kJ l^{-1} O_2 (Carey 1996).

The calorimetric chamber was a Plexiglas box (dimensions: $18 \times 18 \times 28{\cdot}5$ cm^3), placed in a climate-controlled chamber allowing a precise control of the ambient temperature (±0·01 °C). During the measurements, birds were kept in total darkness to measure overnight BMR. Measurements started at 21·00 and ended at 5·30 the following morning. The duration of one measure was 7200 s. To ensure that birds were postabsorptive (Blem 2000), they were deprived of food for 16 h before measurements.

EXPERIMENT 1: BMR, BODY MASS AND IMMUNE RESPONSE

We measured the BMR of immunized (n = 12) and control (n = 12) birds at 22 °C, within their thermoneutral zone (lower limit: 19·5 °C, Gavrilov & Dolnik 1985). Body mass was measured before and after the metabolic measurements and the mean body mass was used for subsequent analyses. Metabolic measurements were carried out on the day before immunization (day – 1) and on days 3 and 7 after immunization. Birds were intra-abdominally injected (day 0) with a phosphate-buffered saline solution (PBS, Sigma Diagnostics, L'Isle d'Abeau Chesne, France) containing 5×10^7 SRBC, 100 μl^{-1} (Biomérieux, Lyon, France). Control birds were injected with PBS only. Birds were weighed before injection and the injected dose was calculated accordingly (100 µl 20 g^{-1}, Aitken & Parry 1974). After each metabolic measurement, a blood sample was collected from the brachial vein (1000 µl) and centrifuged (4000 rpm for 15 min). Plasma was extracted and stored at –20 °C. To investigate the time course of the immune response, we also collected blood samples on days 10 and 15 after injection.

To avoid stress induced by change in environment (e.g. when transferring birds from one cage to another during the fasting period), the two birds measured each night came from the same cage. Each cage included one SRBC-challenged and one control bird. The same birds were kept in the same cages during the entire course of the experiment.

Antibody titres were assessed on days –1, 3, 7, 10 and 15 using the haemagglutination method protocol (Abbas, Lichtman & Pober 1994). The plasma was heated to 56 °C for 30 min to prevent the deterioration of red cells by complements, and serially diluted in PBS in U-shape microtitre plates (dilution: 1, 1/2, 1/4, … , 1/512, 1/1024). An equal volume of a SRBC solution (1%) in NaCl (0·15 M) was added to the different dilutions. After agitation, the plates were covered with parafilm and incubated at 37 °C for 60 min, and agglutination was visually determined. The titre was expressed as the maximal dilution showing a positive agglutination.

EXPERIMENT 2: THERMOREGULATION

For this experiment, we randomly selected 10 doves among the control group from experiment 1. Metabolic rate (MR) below the thermoneutral zone was measured for each bird at 15, 5 and –5 °C to assess the energetic cost of thermoregulation. Three hours before measurements, postabsorptive birds were kept in a climate-controlled chamber for acclimatization. During this period, the ambient temperature was gradually reduced from 22 °C to the chosen temperature. The protocol for MR measurements was similar to experiment 1. Birds were kept in total darkness to measure overnight BMR, measurements started at 21·00 and ended at 5·30 the following morning and, the duration of one measure was 7200 s. Body mass was recorded before and after measurements and mean body mass was used for analyses.

STATISTICAL ANALYSES

Means are given ±1 standard error (SE). BMR are expressed in kilojoules (kJ) or kilojoules per kilogram (kJ kg^{-1}) in compliance with the international unit system. Analyses were performed using Statistica 6·0 (StatSoft) and SAS (SAS Institute).

Experiment 1

We used general linear models (GLMs) to assess differences in initial bird condition (BMR and body mass on day – 1) between treatments (immunized *vs* control birds) and age classes (yearlings *vs* adults). Repeated-measures GLMs were performed to test the effects of treatment on BMR and body mass. We used the residuals of a linear regression of BMR on mass to remove the effect of body mass on BMR. Among the 24 birds, we failed to obtain a plateau of O_2 consumption at each step of the experiment for four birds. These birds were removed for further BMR analyses. For all repeated GLMs, Mauchley tests were performed to evaluate the hypothesis that the sphericity assumption held. When this assumption was rejected, P-values for univariate tests of within-subject factors were Greenhouse–Geisser adjusted.

For each immunized bird, the intensity of the immune response was assigned as *low* (LR birds) when the antibody titre was inferior to the mean, and *high* (HR birds) otherwise. The effects of the intensity of the response on changes in BMR and body mass (values in day 7 minus values in day – 1) were assessed using Kruskall–Wallis analyses of variance. Post-hoc comparisons were performed using Mann–Whitney tests and P-values were Bonferroni corrected for multiple comparisons. In addition, Spearman rank correlations were performed to assess the degree of association between antibody titre and changes in BMR and body mass.

Experiment 2

Mixed GLM with individual as a random factor was used to assess the relationship between metabolic rate (MR) and ambient temperature (T_a) below the thermoneutral zone. The following model was fitted to data:

$$MR = \text{intercept} + \beta(T_a), \qquad \text{eqn 1}$$

where MR is the metabolic rate expressed in kJ kg^{-1} h^{-1} and T_a is the ambient temperature expressed in °C (Weathers, Hodum & Blakesley 2001; Cortès, Tiradoa & Rosenmann 2003). When fitting the model, we assumed that BMR measured at 22 °C was equal to BMR value at the lower critical temperature (e.g. 19·5 °C, Gavrilov & Dolnik 1985). The energetic cost of thermoregulation (kJ kg^{-1} h^{-1} $°C^{-1}$) can be assessed as the slope (β) of the regression line when T_a predicted for a zero metabolism is equal to body temperature (McNab 1980). We failed to obtain an accurate metabolic measurement at $T_a = -5$ °C for two birds, so the corresponding values were dropped before carrying out analyses.

Results

BIRD CONDITION BEFORE EXPERIMENTATION

BMR and body mass measured on day – 1 did not differ between treatments and age classes. Furthermore, there were no significant interactions between treatments and age classes for both BMR and body mass (Table 1).

TIME COURSE OF THE IMMUNE RESPONSE

All SRBC-challenged birds ($n = 12$) produced antibodies against SRBCs, while no antibodies were detected in control birds. When considering SRBC-challenged birds only, we found a positive agglutination in 43% of birds on day 3 and in 100% on days 7 and 10 (Fig. 1). Mean antibody titre was the highest on day 7 (6·27 ± 2·58) and decreased after that date to reach a value of 1·96 (±1·77) on day 15, when antibodies were detected in 83% of birds (Fig. 1).

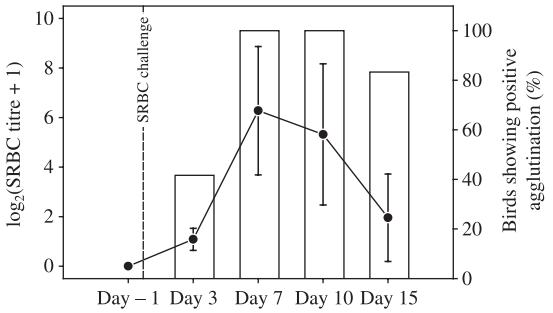

Fig. 1. Time course of the primary humoral immune response in SRBC-challenged birds ($n = 12$): ● mean antibody titre (± SE); open bars show the percentage of birds showing a positive agglutination (e.g. presence of specific antibodies). Vertical dashed line depicts day of injection. No antibodies against SRBC were detected in control individuals.

EFFECT OF IMMUNE RESPONSE ON BMR

The trend in BMR over the course of the experiment differed significantly between treatments. The BMR of SRBC-challenged birds increased from day – 1 to day 7, whereas the BMR of the control birds decreased over the same interval (Table 2, Fig. 2a). Age had no effect on trend in BMR between treatments (Table 2).

On average, the BMR of SRBC-challenged birds was 4·94% (±1·32) higher on day 7 than on day – 1. Conversely, the BMR of control birds was 3·61% (±1·73) lower. According to Martin *et al.* (2002) the effect of immunization on BMR should be considered to be the

Table 1. Results of GLMs testing for difference in initial condition of birds (BMR and body mass) between treatments and age classes. GLMs were performed separately for each dependent variable. Means are given ±SE and sample sizes are given in brackets

Effects	Groups	Means (± SE)	*F*	df	*P*
Treatment*					
BMR on day – 1†	Controls	3·33 ± 0·11 (10)	1·042	1,16	0·323
	SRBC-challenged	3·12 ± 0·07 (10)			
Body mass on day – 1‡	Controls	181·1 ± 3·7 (12)			
	SRBC-challenged	177·4 ± 3·0 (12)			
Age¶					
BMR on day – 1†	Yearlings	3·19 ± 0·09 (12)	2·284	1,16	0·150
	Adults	3·15 ± 0·08 (8)			
Body mass on day – 1‡	Yearlings	175·8 ± 3·1 (15)	3·348	1,20	0·082
	Adults	185·1 ± 2·6 (9)			
Treatment × age					
BMR on day – 1†			0·008	1,16	0·929
Body mass on day – 1‡			0·001	1,20	0·971

*Treatment = SRBC-challenged *vs* control birds.
†BMR measured before SRBC injection (expressed in kJ h^{-1}).
‡Body mass the day of first BMR measurement (expressed in grams).
¶Age = yearlings *vs* adults.

Table 2. Results of repeated GLMs performed to test the trend in mass-corrected BMR and body mass according to treatment and age

	BMR			Body mass		
Effects	df	*F*	*P*	df	*F*	*P*
Between subjects						
Treatment*	1	0·049	0·828	1	0·004	0·950
Age†	1	1·623	0·221	1	4·122	0·059
Age × treatment	1	0·553	0·468	1	0·011	0·918
Error	16			16		
Within subjects						
Day‡	2	0·161	0·852	2	14·464	< 0·0005
Day × treatment	2	7·858	0·002	2	1·1483	0·242
Day × age	2	0·912	0·412	2	0·442	0·647
Day × treatment × age	2	1·236	0·304	2	0·127	0·881
Error	32			32		

*Treatment = SRBC-challenged *vs* control birds.
†Age = yearlings *vs* adults.
‡Day = day – 1/day 3/day 7.

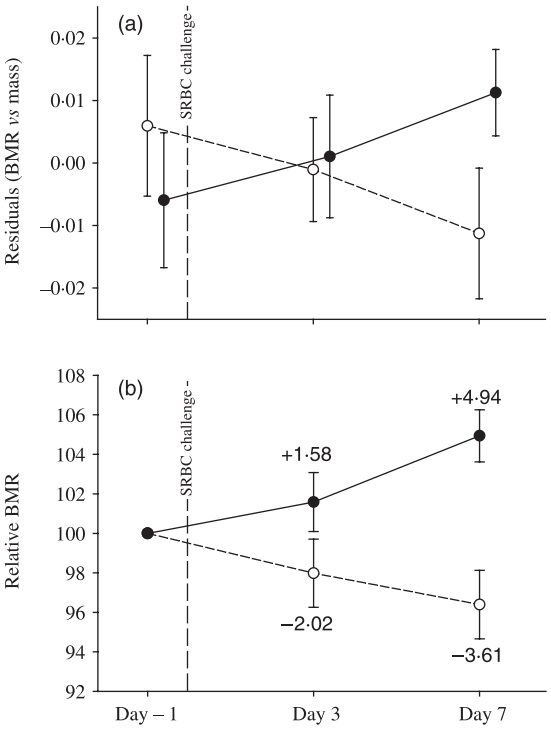

Fig. 2. Effect of immunization on basal metabolic rate (BMR) of Collared Doves with ● mean BMR (±SE) of SRBC-challenged birds (n = 10) and ○ mean BMR (±SE) of control birds (n = 10). Vertical dashed line depicts day of SRBC injection. (a) Mass-corrected BMR (residuals of a linear regression of BMR on mass). (b) Relative BMR expressed in per cent of BMR measured on day – 1.

mean difference between BMR of treatments. Thus for SRBC-challenged birds, we found a 3·60% increase in BMR on day 3 and an 8·55% increase on day 7 (Fig. 2b). For an individual with mean characteristics (body mass = 178·4 g, BMR = 76·13 kJ day^{-1}), we assessed the energetic cost of mounting an immune response to be 2·74 kJ day^{-1} on day 3 and to be 6·47 kJ day^{-1} on day 7. By using the mean of these values as an estimate

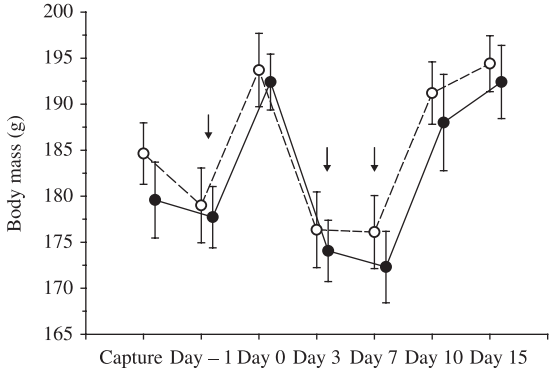

Fig. 3. Trend in body mass of Collared Doves throughout the course of the experiment 1, with ● mean body mass (±SE) of SRBC-challenged birds (n = 10) and ○ mean body mass (±SE) of control birds (n = 10). Black arrows depict fasting periods dictated by BMR measurements.

of the mean daily energy expenditure (4·61 kJ day^{-1}), we calculated the overall energetic cost to be 32·27 kJ during the 7 days following immune activation.

We found no evidence to suggest that the intensity of the immune response affected subsequent change in BMR. The increase in BMR between day 1 and day 7 did not differ between LR and HR birds (Fig. 4a, χ^2 = 0·011, df = 1, P = 0·917). However, we noticed that change in BMR over a shorter interval (between day 3 and day 7), tended to be higher in birds mounting a stronger immune response (Fig. 4b), with a positive but not significant relationship between change in BMR and antibody titre (r_s = 0·548, P = 0·10, n = 10).

EFFECT OF IMMUNE RESPONSE ON BODY MASS

Owing to periods of food deprivation (necessary to ensure that birds were postabsorptive), body mass declined throughout the days of BMR measurements (Table 2, Fig. 3). The loss of body mass was more pronounced in SRBC-challenged birds than in control birds from day – 1 to day 7 (means: –3·57 ± 1·03% *vs* –1·68 ± 0·58%, respectively), but this difference was not significant when considering the effect of treatment (Table 2).

However, when considering the intensity of the immune response, we found that change in body mass between day – 1 and day 7, differed significantly among controls, LR and HR birds (Fig. 4c, χ^2 = 7·626, df = 2, P = 0·022). HR birds lost significantly more mass than LR birds (χ^2 = 5·429, df = 1, P = 0·038), whereas change in body mass did not differ between LR birds and controls (χ^2 = 0·431, df = 1, P = 1). Overall, decline in body mass of SRBC-challenged birds over the 7-day period following immunization was positively correlated with antibody titre assessed on day 7 (r_s = −0·689, P = 0·013, n = 12).

Note that birds did not suffer during experiment 1 because body mass measured outside the fasting periods was higher on day 15 than on the day of capture (mean: +6·43 ± 1·38%).

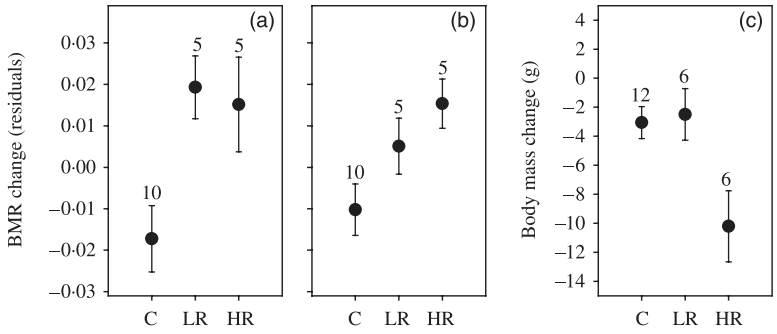

Fig. 4. Effect of the intensity of the humoral immune response on subsequent changes in BMR and body mass. Results are means ± SE. (a) Residuals (regression of BMR on mass) in day 7 minus residuals in day-1. (b) Residuals (regression of BMR on mass) in day 7 minus residuals in day 3. (c) Body mass (g) in day 7 minus body mass in day-1. C = control birds, LR = low immune response, HR = high immune response. Sample sizes are given above bars.

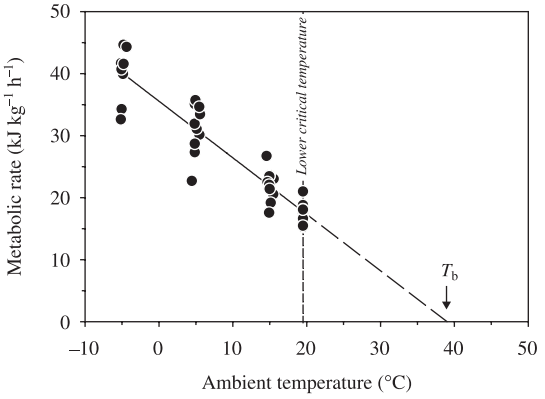

Fig. 5. Relationship between metabolic rate (MR) and ambient temperature (T_a) below the thermoneutral zone. Vertical dashed line depicts the lower critical temperature (19·5 °C, Gavrilov & Dolnik 1985). Solid line depicts the equation of the mixed GLM: MR = 35·715 – 0·924T_a. Dashed regression line shows the extrapolation of metabolic rate to 0, predicting a theoretical body temperature (T_b).

THE ENERGETIC COST OF THERMOREGULATION

The mixed GLM analysis showed that MR increased linearly with declining T_a below the thermoneutral zone ($t = -21{\cdot}44$, $P < 0{\cdot}0001$, Fig. 5). The relationship between MR and ambient temperature was described by the following equation:

$$\text{MR (kJ kg}^{-1}\text{ h}^{-1}) = 35{\cdot}715 - 0{\cdot}924T_a. \qquad \text{eqn 2}$$

This equation predicted a zero metabolic rate at body temperature, T_b = 38·66 °C (Fig. 5). Because this value was close to body temperature in Collared Dove (39·4 °C, Gavrilov & Dolnik 1985), the slope of the model was used to assess the energetic cost of thermoregulation: 0·924 kJ kg^{-1} h^{-1} °C^{-1} or 5·27% of BMR (mean BMR of the 10 birds used in the present analysis: 17·54 kJ kg^{-1} h^{-1} ± 0·53). Therefore, we calculated the daily cost of maintaining body temperature, at an ambient temperature 1 °C below the thermoneutral zone, to be 3·93 kJ for a bird with a body mass corresponding to our sample mean (177·25 g ± 4·19).

Discussion

To assess the energy expenditure required when mounting a primary humoral immune response, the BMR of SRBC-challenged Collared Doves was compared with the BMR of control birds. Our results show clearly that mounting a humoral immune response significantly increased energy expenditure in Collared Doves that had access to unlimited food. We found that BMR of SRBC-challenged individuals was 8·5% higher 7 days after injection. Interestingly, this result matched the value of 8·6% recorded in free-living Great Tits 7 days after a similar SRBC-challenge (Ots *et al*. 2001) and was in the range of 8–13% assessed for Blue Tits challenged with a diphtheria–tetanus vaccine (Svensson *et al*. 1998). Conversely, our results contrasted with the absence of BMR changes reported in Greenfinches following an SRBC-challenge (Hõrak *et al*. 2003). In both studies, birds were fed *ad libitum*, thus it is likely that food access does not account for this difference.

In agreement with results from Ots *et al*. (2001), no correlation was found between antibody titre on day 7 and change in BMR from day – 1 to day 7. Together, these results would suggest to the absence of a positive relationship between the intensity of antibody production and energetic cost. However, it is likely that experimental designs used in past and present studies are not ideally designed to investigate this question since they do not take into account for individual differences in the timing of the immune response. Furthermore, it is likely that for each individual, the exact time when antibody titre and energy expenditure peak are not accurately known. By analysing the change in energy expenditure and antibody titre over a period when antibody production occurred in all individuals (from day 3 to day 7), we found a positive tendency between the intensity of the response and the energetic cost, suggesting that such a relationship is expected. Therefore, further studies are needed to investigate the relationship between the intensity of a humoral immune response and the associated energetic cost because it could determine the magnitude of trade-offs between immunocompetence and other fitness-relevant traits, consequently acting as a selective pressure.

In the Collared Dove, indirect comparisons with other important physiological activities suggest that immune activation might divert the energetic budget allocated to other fitness-related traits. We calculated the overall energetic cost to be 32·27 kJ during the first week following immune defence deployment. However, there is evidence that the energetic cost extended the 7-day period because antibodies were detectable up to day 15 (Fig. 1, see also Roulin *et al*. 2000). Therefore, assuming a similar daily energy expenditure until day 15, we calculated the overall energetic cost of the immune response to be 69·15 kJ from day 0 to day 15,

which is equivalent to the estimated cost of producing an egg (70 kJ). Consequently, negative impacts on the energy budget could be more pronounced when birds must sustain an immune activity over a long time, especially when it is concurrent with other physiological activities such as moulting, chick feeding or egg-laying. Assuming that individuals face limited resources (Drent & Daan 1980, Ricklef & Wikelski 2002), our results suggest that trade-offs can be expected between immune functions and costly activities related to breeding effort or thermoregulation. In addition, seasonal variability in these trade-offs may explain seasonal changes in immune functions that have been reported in several occasions (see Nelson *et al*. 2002).

However, evidence that the energetic cost of an immune response is the central mechanism mediating such trade-offs relies upon indirect comparisons. If the cost of humoral immunity is essentially related to energy, an equivalent energy expenditure from another physiological activity should affect fitness-related traits in a similar way to an immune response (reduced breeding effort, Deerenberg *et al*. 1997; Råberg *et al*. 2000; Bonneaud *et al*. 2003; delayed laying, Andersson 2001). Thus the fitness cost of thermoregulation may be used as a comparison. The second experiment showed that the daily energetic cost of maintaining body temperature at an ambient temperature between 1 and 2 °C below the thermoneutral zone (5·2–10·4% of BMR) was in the same order as the daily cost of a humoral immune response. Numerous studies investigating direct relationships between temperature and fitness-related traits have shown negative effects of low temperature on clutch phenology, proportion of breeding females and egg weight (Järvinen & Ylimaunu 1986; Meijer *et al*. 1999; Nilsson & Svensson 1993; Yom-Tov & Wright 1993; Perrins 1996; Lessels *et al*. 2002). However, the effects of a low level of thermoregulation on life-history traits, such as laying date, seem to be equivocal (Nager & Van Noordwijk 1992). According to Nilsson (2002), this suggests that the energetic cost of the immune system is probably not the only central mechanism mediating immunocompetence and other fitness-related traits (*see also* Råberg *et al*. 2002).

An additional cost would reside in the degradation of body condition of an individual mounting a humoral immune response. SRBCs are thymus-dependent antigens; they involve T and B lymphocytes, antigen-presenting macrophages, and consequently, the release of soluble mediators such as pro-inflammatory cytokines (Roitt 1984). These include for example, interleukin-1 (Guenounou, Vacheron & Nauciel 1985, Krymskaya *et al*. 1994), which is known to cause several changes in physiological and behavioural states of the host, including anorexia, fever, muscle protein degradation and lipolysis (Johnson 2002). Indeed, loss of body mass was reported in chickens (Klasing *et al*. 1987) and free-living Great Tits (Ots *et al*. 2001) after an SRBC-challenge. Our results showed a similar effect of SRBC treatment in doves, but our results varied according to the intensity of the immune response. We found that only birds that mounted a stronger immune response incurred an important loss of mass (Fig. 4c). No similar results were reported in the Greenfinch by Hõrak *et al*. (2003), who suggested that housing conditions could have permitted birds to partly compensate for degradation of their body condition. We had no data to test this hypothesis, but under the assumption that birds have compensated, our results are conservative because doves were fed *ad libitum* and kept in aviaries, which limited energy expenditure related to searching for food and other activities.

In conclusion, our results indicate that the energetic cost of a humoral immune response is slight regarding to the energy demand of other physiological functions. Simply knowing the energetic cost of producing antibodies is a first step in the process to estimate the cost of humoral immunity and consequently, direct assessment of the impact of a slight increase in energy expenditure on fitness is the next step for future studies. Nevertheless, our results partly suggest that the combination of energy expenditure and loss of body mass might result in a significant resource-based trade-off, mediating immune functions and other fitness-related traits. However, this hypothesis is not exclusive in explaining the evolutionary process of the vertebrate immune system and its lack of exhaustive abilities. A potential cost of strong immunity would reside in the risk of tissue damage and auto-immune responses (Råberg *et al*. 1998; Svensson *et al*. 1998; Graham 2002) which are increased during heavy physical workloads (Wood *et al*. 1993). An alternative cost of the immunity would imply the production of free radicals and their deleterious effects on organisms (Beckman & Ames 1998; Finkel & Holbrook 2000). Therefore, the down-regulation of immune functions during heavy physical workloads such as breeding effort, would regulate the metabolic activity and consequently the production of free radicals (Ilmonen, Taarna & Hasselquist 2000; Nilsson 2002). However, the adaptive significance of the suppression of the immune system in this context is currently under debate (Råberg *et al*. 1998). Costs with potentially short- or long-term effects suggest the complexity of mechanisms regulating the immune system. Further experimental studies are needed to assess the cost of immunity through an integrative approach including energy, nutrients, auto-immunity and oxidative stress. No doubt, such studies will provide a better full understanding of the selective processes behind the evolution of vertebrate immune systems and their lack of exhaustive abilities.

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