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## Variations of body mass and immune function in response to food unpredictability in magpies

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#### Abstract

The effects of food unpredictability on body mass regulation have been studied in a few species of birds. It has been shown that small passerines can counteract variability of food resources by actively regulating their body reserves through an increase of mass. However, it was recently found that, with unpredictable food availability, quails and hooded crows do not adopt an adaptive fattening strategy but lose mass. At present, little is known about the effects of food unpredictability on other body parameters, such as immune functions and blood variables. In this study, we analysed the responses to food abundance and unpredictability by performing two experiments of controlled food administration in the magpie, a passerine intermediate in size between small passerines and crows. Body mass, leukocyte abundance, haematocrit, spleen mass and immune reaction to phytohaemagglutinin (PHA) were assessed during a 15-d period. In the first experiment, the food release was predictable (same amount each day), but the quantity of food was supplied, but with predictable or unpredictable (random) schedules. As previously reported for crows and quails, magpies did not adaptively increase their mass in response to food unpredictability. Furthermore, there was a decrease of haematocrit and immune response to PHA. It appears that a body mass decrease in the magpie can be induced by a reduction of either food abundance or food predictability, and it is accompanied by a worsening of health state. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Body condition; Food predictability; Health state; Immunocompetence

## 1. Introduction

Many bird species are able to regulate body reserves when they face periods of high energy requirements (Blem, 1990; Biebach, 1996), food scarcity (Stuebe and Ketterson, 1982) or food unpredictability (Cuthill et al., 2000). In these situations, birds usually store fat reserves, but they can also actively lose mass, for example in the presence of predators (Gosler et al., 1995). These facts deserve proximate and ultimate explanations (Sherman, 1988).

With regard to proximate causes, many physiological studies have focused on nutritional stress experiments (fasting and, more rarely, over-feeding), looking mainly at their effects on physiological parameters. Food deprivation experiments can induce altered protein metabolism in addition to mass loss (Garcia-Rodriguez et al., 1987). Blood

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parameters can also be altered, as found in gulls and raptors (Ferrer and Dobado-Berrios, 1998; Totzke et al., 1999). In the hooded crow, low levels of food have induced an increase in erythrocyte sedimentation (hereafter ES) rate, suggesting a worsening of health conditions (Acquarone et al., in press). As well as food quantity, the protein composition of the diet can influence the development of immunocompetent organs (for the bobwhite, see Lochmiller et al., 1993).

Studies considering ultimate explanations have attempted to predict mass changes in the light of the risksensitive foraging theory (McNamara and Houston, 1990; Brito e Abreu and Kacelnik, 1999). The effects of food unpredictability have mainly been studied in small passerines, which, as a rule, increase mass and store fat in response to this situation (Bednekoff and Krebs, 1995). A different response to food unpredictability was recently described in Japanese quail chicks (Boon et al., 1999) and in adult hooded crows (Acquarone et al., in press). Birds fed with the same quantity but with an unpredictable (random) schedule lost mass significantly more than regularly fed controls. This suggests a response that covers only their immediate energy needs, i.e. they neither anticipate a fasting period nor respond to feeding conditions experienced on previous days. In hooded crows subjected to a highly variable schedule of nutrition, we also observed an increased ES rate and a reduction of phytohaemagglutinin (PHA) index and haematocrit value (Acquarone et al., in press). These results show that food unpredictability can cause a non-adaptive reduction in mass accompanied by a worsening of health state. As demonstrated in several bird species, in the long term, a deterioration of immune function lowers the prospect of survival (Christe et al., 1998; Gonzales et al., 1999; Hõrak et al., 1999; Soler et al., 1999; Merino et al., 2000).

In this study of the magpie, we evaluated the energetic and humoral responses to food quantity and unpredictability; in this species, the immune defence was recently shown to be condition-dependent in free-living individuals (Blanco et al., 2001). We utilized an experimental approach to assess body and health conditions of 57 magpies subjected to two experimental protocols:

(a) to evaluate the effects of food quantity, four groups of magpies were fed regularly with different rations (50, 60, 70, 80 g);

(b) to evaluate the effects of food unpredictability, we compared two groups fed constantly with the same quantity of food (60 and 80 g) with two other groups supplied with the same 15-d average but with the quantity of food varied from day to day according to a random schedule.

## 2. Materials and methods

The study was carried out at Rivalta Scrivia (NW Italy) in the springs of 1999 and 2000. Fifty-seven magpies were captured with Larsen traps as part of the provincial administration's agricultural pest management programme (Acquarone et al., in press). No birds were trapped exclusively to collect scientific data. The personnel of the provincial administration were authorized to cull magpies by the National Wildlife Institute. Measurements were taken on two occasions. At the beginning and, 15 d later, at the end of experimental treatment, we measured (in vivo) each individual's mass, while blood samples and smears were collected for subsequent analyses. Post mortem, each bird's body was brought to the laboratory, where morphological variables and spleen mass were measured. In detail, the following data were obtained.

#### 2.1. Morphological variables and spleen mass

We measured: (i) body mass (0.1 g accuracy); (ii) wing length, with the flattened wing outstretched perpendicular to the body; (iii) tarsus length; (iv) bill length. Tarsus and bill

measurements were made to the nearest 0.1 mm with a calliper, while wing was measured to the nearest 0.5 mm with a flat ruler. The three body measures (ii-iv) were highly correlated with each other; thus, they were compacted in an index of structural size calculated as the first factor from a PCA. Factor 1 explained 70.6% of the variance in the original variables and was highly related to them (loadings: wing = 0.856, tarsus = 0.899, bill = 0.461). We examined the abdominal cavity of dead birds to extract the spleen, and its mass was measured by a precision balance with 0.1 mg accuracy. To provide a better condition index, spleen mass should be expressed relative to the structural size of an individual (Brown, 1996). Therefore, we fitted a regression between spleen mass and PCA1 size and used the residuals from this regression as size-corrected spleen mass values.

# 2.2. Leukocyte counts, erythrocyte sedimentation (ES) rate and haematocrit

We counted leukocytes and red blood cells in blood smears stained by the May–Grunwald–Giemsa method. Blood smears were scanned at  $630\times$  magnification following standard routines. In each microscopic field, we counted red blood cells and leukocytes classified as lymphocytes, monocytes, eosinophils, heterophils and basophils. In each smear, we counted 100 leukocytes and the corresponding red blood cells. We then calculated the relative frequency of leukocytes of each family with respect to the total leukocyte population (relative counts) and the number of leukocytes of each family per 10,000 red blood cells (absolute counts). This method provides significantly repeatable relative and absolute leukocyte counts (Saino et al., 1995). Absolute values were  $log_{10}$  transformed for statistical analysis.

To measure sedimentation rate, 70 µl blood samples in heparinized haematocrit capillary tubes were placed in vertical position for 4 h in a refrigerated container (4 °C). According to Saino and Møller (1996), the ES rate, i.e. the proportion of blood forming a sediment per hour, was expressed as: (volume of the part of the capillary not occupied by blood cells)/(blood volume in the capillary) × 0.25. The ES rate is a diagnostic of many acute and chronic diseases, including infections and rheumatic and inflammatory diseases (Merilä and Svensson, 1995).

Only in the "predictable 80 g" and "unpredictable 80 g" groups (group description is reported below) were blood samples then centrifuged for 4 min at 3000 rpm. Haematorit was expressed as volume of the part of the capillary occupied by blood cells/blood volume in the capillary.

### 2.3. Phytohaemagglutinin index

In both "predictable 80 g" and "unpredictable 80 g" groups, the thickness of the right wing web was measured with a spessimeter with an accuracy of 0.01 mm at the end of the experimental period. The wing web was then injected

with 0.25 mg of PHA (Sigma L-8754) in 0.05 ml of phosphate buffered saline (PBS). Twenty-four hours later, we again measured the thickness of the wing web at the inoculation site. Reaction to PHA was expressed as the change in thickness of the inoculated wing web. This wing web index is being routinely applied in avian study and is assumed to be proportional to the intensity of T-lymphocyte cell-mediated immunocompetence (Lochmiller et al., 1993; Smits et al., 1999).

## 2.4. Experimental protocol

After capture, all birds were kept in individual cages  $(100 \times 60 \times 80 \text{ cm})$  for 15 d and were fed with different diets, with water ad libitum. As food, we used a mixture of meat (33%), fruit (53%) and maize (14%). For each individual, we calculated the daily energy intake utilizing a standard table of conversion from grams to kilojoules (Italian Nutritional Institute: meat = 8.96 kJ/g, fruit = 1.42 kJ/g; maize = 14.86 kJ/g). Food was dispensed individually in poultry seed cups at 9 AM. Food consumption was ascertained daily. In the data analysis, we did not correct for food spillage because the birds invariably ate almost all the food; spilled food consisted only of calorically irrelevant peels of fruit.

We assigned the magpies to different treatment groups using a completely randomized procedure. Half of the individuals in each group were collected in the first year (1999) and the other half in the second year (2000). As we did not find any year-related difference for the measured variables, data from individuals assigned to the same group were pooled. Post-mortem gonadal inspection revealed that 60% of the magpies were females, which were equally distributed in the treatment groups. We established two experimental protocols.

Experiment (a): in order to evaluate the effects of food quantity, the birds were nourished with daily quantities of food that were constant but different in the four groups: 50 g (four individuals), 60 g (11 individuals), 70 g (seven individuals) and 80 g (15 individuals). The different quantities

of food delivered were chosen to provide a range of food abundances encompassing the predicted basal metabolic rate: 428 kJ, i.e. 77 g.

Experiment (b): in order to evaluate the effects of food unpredictability, we established two groups with variable schedules. Each bird was provided with a different quantity of food each day, the amount being determined by extraction of random numbers. Thus, the individuals pertaining to each group had different schedules but an identical mean food quantity and S.D.  $(60 \pm 29, 80 \pm 39 \text{ g/d})$  at the end of the study period. The "unpredictable 60 g" group included seven individuals. As a control, we utilized the 11 individuals from the "predictable 60 g" group of the first experiment. The "unpredictable 80 g" group included 13 individuals. As a control, we utilized the 15 individuals from the "predictable 80 g" group of the first experiment.

## 3. Results

#### 3.1. Experiment (a): food quantity effects

Table 1 reports the results of a multivariate analysis of variance (MANOVA) with change in mass, ES, heterophil and lymphocyte variations as dependent variables, the food abundance (50, 60, 70 or 80 g) as factor, and the initial mass, ES, heterophil and lymphocyte abundances as covariates.

At the end of the period of differential nutrition, the four experimental groups differed significantly in mass, while they did not show different changes in the ES, heterophil and lymphocyte values (Table 1). The mass loss was considerable for birds of the 50 g group, subjected to a harsh diet, but was moderate for birds of the 60 g and 70 g groups. In contrast, birds of the 80 g group, with abundant food, increased slightly in mass (Table 1).

Final spleen mass and size-corrected spleen mass did not differ among treatment groups (spleen mass:  $F_{3,33} = 0.54$ , P = ns; size-corrected spleen mass:  $F_{3,33} = 0.64$ , P = ns).

Table 1

Experiment (a): comparison of mean values of mass and haematological parameters in four groups fed with different quantities. Mean  $\pm$  S.D. for homogeneous samples are reported

Dependent variable variation	Mean ± S.D.	MANOVA independent variables									
		Quantity		Initial ES rate		Initial heterophils		Initial lymphocytes			
		F <sub>3,29</sub>	Р	$F_{1,29}$	Р	F <sub>1,29</sub>	Р	F <sub>1,29</sub>	Р		
ES rate	$+ 0.049 \pm 0.018$	0.90	ns	1.28	ns	3.76	ns	2.16	ns		
Heterophils	$-6.5 \pm 23.1$	0.35	ns	3.05	ns	2.51	ns	1.11	ns		
Lymphocytes Change in mass	$+$ 18.0 $\pm$ 79.2	1.32	ns	0.71	ns	2.57	ns	3.13	ns		
50 g 60 g 70 g 80 g	$-40.9 \pm 8.0$ $-3.1 \pm 8.4$ $-6.5 \pm 3.0$ $+2.5 \pm 6.6$	10.7	<0.001	0.30	ns	0.07	ns	1.01	ns		

#### 3.2. Experiment (b): food unpredictability effects

Table 2 reports the results of a two-way MANOVA with mass, ES, heterophil and lymphocyte variations as dependent variables, the food abundance (60 or 80 g) and variability as factors, and the initial mass, ES, heterophil and lymphocyte abundances as covariates.

At the end of the period of differential nutrition, the experimental groups differed significantly in mass, while they did not show different variations of the ES, heterophil and lymphocyte values (Table 2). Both the predictability and abundance of food significantly influenced the mass variation. The individuals of the unpredictable schedule groups had a lower mass than control birds at the end of the study period, and birds provided with an average of 80 g of food had a higher mass than the corresponding 60 g individuals (Table 2).

Final spleen mass and size-corrected spleen mass did not differ among treatment groups (two-way ANOVAs of spleen mass: predictability  $F_{1,1,43} = 0.1$ , P = ns, abundance  $F_{1,1,43} = 0.22$ , P = ns; size-corrected spleen mass: predictability  $F_{1,1,43} = 0.1$ , P = ns, abundance  $F_{1,1,43} = 0.19$ , P = ns).

The PHA index and haematocrit values are not available for the 60 g groups. The 80 g unpredictable group and its 80 g control differed in final haematocrit value ( $F_{1,26} = 5.14$ , P < 0.04) and PHA index ( $F_{1,26} = 5.37$ , P < 0.03); the low haematocrit value and small reaction to PHA indicated a worsening of health condition in the 80 g unpredictable group (Fig. 1).

## 4. Discussion

#### 4.1. Food abundance

The different quantities of food delivered for 15 d led to relevant variations in magpie mass. The lower food supply (50 g/d, 279 kJ) caused a mass decrease, while the groups receiving an intermediate amount of food (60 g: 335 kJ, and

Table 2



Dependent variable variation	Mean ± S.D.	MANOVA independent variables										
		Variability		Quantity		Initial ES rate		Initial heterophils		Initial lymphocytes		
		F <sub>1,38</sub>	Р	$F_{1,38}$	Р	F <sub>1,38</sub>	Р	F <sub>1,38</sub>	Р	$F_{1,38}$	Р	
ES rate	$+0.036 \pm 0.013$	3.43	ns	3.50	ns	0.43	ns	3.40	ns	1.21	ns	
Heterophils	$+ 3.7 \pm 27.2$	2.55	ns	0.05	ns	1.28	ns	3.28	ns	0.17	ns	
Lymphocytes	$+46.4 \pm 79.5$	0.19	ns	1.31	ns	3.33	ns	1.89	ns	2.39	ns	
Change in mass												
60 g predictable	$-3.1 \pm 8.4$	6.98	< 0.01	5.15	< 0.02	0.63	ns	0.16	ns	0.84	ns	
60 g unpredictable	$-21.5 \pm 5.7$											
80 g predictable	$+2.5\pm6.6$											
80 g unpredictable	$-10.3 \pm 2.34$											





Fig. 1. Comparison (mean  $\pm$  S.D.) of haematocrit value and PHA index in two groups fed with a mean of 80 g/d and with different schedules (predictable and unpredictable).

70 g: 391 kJ) or a daily administration of 80 g (446 kJ) roughly maintained the initial body mass. This reflects the metabolic needs of magpies. According to the equation of Aschoff and Pohl (1970), the basal metabolic rate is 428 kJ/d, while in free conditions these birds need about 630 kJ/d for their basal metabolism plus flying and daily activities (Hogstedt, 1981; Harper et al., 2001). The daily food shortage did not cause changes in health parameters (ES rate and leukocytes), contrary to our previous experiment on the hooded crow, where we observed an increase of ES rate with the lowest food supplement (Acquarone et al., in press). Other studies of fasting in raptors, gulls and serins also found an effect of food scarcity on blood metabolites or leukocytes or immune response (Garcia-Rodriguez et al., 1987; Ferrer and Dobado-Berrios, 1998; Romero-Pujante et al., 1999; Totzke et al., 1999; Alonso-Alvarez and Tella, 2001).

#### 4.2. Food unpredictability

Magpies in the variable groups responded to the unpredictable food supply by decreasing their mass more than controls. The pattern of mass loss is similar to that reported for the hooded crow (Acquarone et al., in press) and for chicks of the Japanese quail Coturnix coturnix (Boon et al., 1999). Our results do not fit the classical model of an adaptive fattening response in unpredictable situations (Witter et al., 1995). However, they should be discussed in relation to the following points. (1) The study was conducted in spring, when climatic conditions are less extreme than in winter. As it has been found that the adaptive response could change in different seasons (Witter et al., 1995), it is possible that the appearance of adaptive fattening responses is caused only by an interaction of food unpredictability with harsher conditions (e.g. in winter). (2) Fattening strategies might be more useful in small birds because of their relatively high metabolic requirements and high risk of starvation (Witter et al., 1995), while larger birds have a greater margin for survival in environmental extremes. Magpies are intermediate in weight (200 g) between small passerines (less than 40 g) and crows (400 g). However, according to the Aschoff and Pohl (1970) equation, their relative metabolic requirement is more similar to that of bigger crows than that of passerines. (3) Magpies are opportunistic resident birds that live in agro-ecosystems, which are generally food-rich habitats. Human-generated resources are probably more constant and predictable than natural ones. Moreover, since magpies can occasionally hoard food and use these external reserves when food is not available (Birkhead, 1991), then a fattening strategy, because of its costs (Lima, 1986; McNamara and Houston, 1990) may not be advantageous.

Our results, and those of other studies (Boon et al., 1999; Acquarone et al., in press), suggest that there is a need of more data for a more reliable theory of mass variation or, more widely, of energy allocation in birds in unpredictable situations.

As Gustafsson et al. (1994) pointed out, animals must allocate resources for body reserves, reproduction and maintenance of the immune system. In theory, there are trade-offs between investments in life-history components and immune defence (Norris and Evans, 2000), and it can be hypothesized that a shortage of food can cause a worsening of health condition (Klasing, 1988; Gustafsson et al., 1994). In this study, the individuals subjected to a variable schedule showed a lower haematocrit and a weaker reaction to PHA than controls, suggesting that their higher mass loss was accompanied by a negative effect on health condition.

In conclusion, our study of magpies shows that food unpredictability can lead to a response which results in poorer health state and body mass decrease. Future longterm studies on the effects of environmental variability will help to clarify the trade-offs between the costs and benefits associated with body mass regulation and physiological and immunological conditions.

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