Diet quality affects postnuptial molting and feather quality of the house sparrow (*Passer domesticus*): interaction with humoral immune function?

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Abstract: We investigated the effects of nutritional limitation, humoral immune activation, and their interaction on postnuptial molting of aviary-kept house sparrows (*Passer domesticus* (L., 1758)). In a 2 × 2 experimental design, we measured the progress of molting and the quality of feathers produced during molting by house sparrows exposed to different diet qualities (high and low) and humoral immune activation with sheep red blood cells (SRBC). Food quality, but not the activation of humoral immunity, affected significantly the body mass and the process of molting. Sparrows feeding on low-quality food had decreased body mass and longer molts than the high-quality group. Low-quality food, but not the activation of humoral immunity, reduced significantly the length and mass (i.e., the quality) of primaries grown during molting. Birds responded significantly to injection with SRBC compared with the control group, but the immune response was similar between nutritional groups. The absence of a negative effect of humoral immunity on molting in house sparrows might be related to the low energy and nutritional requirements of mounting and maintaining a humoral immune response.

Résumé : Nous étudions les effets des restrictions alimentaires, de l'activation immunitaire humorale et de leur interaction sur la mue post-nuptiale chez des moineaux domestiques (*Passer domesticus* (L., 1758)) gardés en volière. Dans un plan d'expérience 2 × 2, nous avons mesuré le progrès de la mue et la qualité des plumes produites par des moineaux exposés à des régimes alimentaires de qualité variable (haute et faible) et à une activation immunitaire humorale à l'aide d'érythrocytes de mouton (SRBC). La qualité de la nourriture, mais non l'activation immunitaire humorale, affecte significativement la masse corporelle et le processus de mue. Les moineaux nourris d'un régime de faible qualité ont une masse corporelle réduite et une mue prolongée par rapport aux moineaux bien nourris. La faible qualité de l'alimentation, mais non l'activation de l'immunité humorale, réduit significativement la longueur et la masse (c'est-à-dire la qualité) des pennes primaires qui poussent pendant la mue. Les oiseaux réagissent significativement à l'injection de SRBC par comparaison au groupe témoin, mais la réponse immunitaire est semblable dans les deux groupes alimentaires. L'absence d'effet négatif de l'immunité humorale sur la mue des moineaux domestiques s'explique peut-être par les exigences énergétiques et alimentaires basses requises pour l'établissement et le maintien d'une réponse immunitaire humorale.

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Introduction

One of the most energy- and nutrition-demanding periods in a bird's life is the period of increased somatic cell growth during the regular replacement of body and flight feathers (postnuptial molting; Lindström et al. 1993; Jenni and Winkler 1994; Klaassen 1995). Given the high caloric content of feathers (Murphy and King 1982) and the very low efficiency of their production (compared with other animal tissues; Murphy and King 1984; Reeds 1991 cited in Klaassen 1995), the energetic cost of feather production is comparable with that of reproduction (Lindström et al. 1993; Klaassen 1995; Kuenzel 2003). Besides the increased energy requirement for molting, the need for high-quality food is also essential (Murphy and King 1982, 1986; Cherel et al. 1994). Another important energy- and nutrition-dependent

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life-history trait of a bird is the immune system. Immune activity elevates energy expenditure (Svensson et al. 1998; Ots et al. 2001; Martin et al. 2003; Eraud et al. 2005), and hence probably diverts energy from other costly functions such as growth, reproduction, and molting (e.g., Ilmonen et al. 2000; Hanssen et al. 2004; Sanz et al. 2004; Martin 2005; Mauck et al. 2005). The trade-off between the immune system and other costly activities was demonstrated by experiments where the energy expenditure has been increased. For instance, experimentally increased parental duties resulted in reduced immunocompetence (reviewed in Lochmiller and Deerenberg 2000). In addition, the effectiveness of the defense system depends on the amount and the quality of the food ingested (Lochmiller et al. 1993; Saino et al. 1997; Klasing 1998; Gonzalez et al. 1999; Alonso-Alvarez and Tella 2001; Smith et al. 2007). Birds experiencing feeding deficiency, or more specifically feeding on low-quality food, usually have a reduced immune capacity (Lochmiller et al. 1993; Saino et al. 1997; Gonzalez et al. 1999; Råberg et al. 2003; Hangalapura et al. 2005; Smith et al. 2007), which may reduce their ability to keep infections within bounds. The effects of the quality of food on the humoral immune system in wild birds are, however, still waiting to be tested. On the other hand, the energetic costs of mounting a humoral immune response is generally low (8%–13% of the basal metabolic rate; Svensson et al. 1998; Ots et al. 2001; Eraud et al. 2005), suggesting that the trade-off between the humoral immune system and the other resource-demanding functions (e.g., reproduction, molting) might be less accentuated.

Considering the energetic and nutritional costs of molting along with those related to the deployment of immune defenses, it seems reasonable to assume the existence of resource-based trade-offs between these two traits. This trade-off may be manifested as a decrease in molting speed during an immune challenge (Martin 2005), or inversely, the depression of immune response during intense feather replacement (Martin et al. 2006). In critical estates, e.g., during the molting–breeding overlap, when the energetic demand is probably at a ceiling, an induced immune response may result in delaying the onset of postnuptial molting (Sanz et al. 2004).

In the present study, we test the hypothesis that molting is traded off with food quality and humoral immunity. The hypothesis is based on the assumption that food quality and the activated humoral immunity affect condition of the birds. We addressed these questions by using a 2 × 2 experimental design, where within the nutritional groups half of the birds were challenged with sheep red blood cells (SRBCs), while the rest of them served as a control receiving the same dose of phosphate-buffered saline. We first explore the effects of nutritional limitation on molting and feather quality of house sparrows (*Passer domesticus* (L., 1758)) feeding under low- and high-quality foods. Second, we study the effect of humoral immune response on molting of birds from different nutritional groups.

Materials and methods

Studied species, capture, and aviary conditions

We captured 56 adult male house sparrows from a farm situated near Cluj Napoca (46°46′N, 23°33′E), Transylvania, central Romania, during two netting sessions on the 16th and 21st of July 2006. The birds were then transported into aviaries situated at the campus of the Babeş-Bolyai University, Cluj Napoca. At capture, none of the birds had started molting. Following capture, birds were randomly distributed among four aviaries (5 m long × 2 m wide × 2.5 m high).

Group feeding is a characteristic of the house sparrow social system (Anderson 2006). For this reason, birds were not housed in individual cages, but in groups to stimulate group feeding. However, the drawback of group living is the dominance interaction between individuals, which can influence the effect of manipulation, resulting in various responses of individuals with different social statuses. The food was provided in two separate dishes in each aviary to reduce dominance interactions between individuals (see McGraw and Hill 2000). Observations made during the experiment confirmed the reduced competition between birds, since the aggressive interactions between feeding individuals were almost lacking. There were no differences between the four groups in wing length ($F_{[3,52]} = 1.2$, $P = 0.33$), tarsus length ($F_{[3,52]} = 2.5$, $P = 0.07$), wing-bar area ($F_{[3,52]} = 1.13$, $P = 0.94$), and bib size ($F_{[3,52]} = 0.37$, $P = 0.77$) at capture (for measurement details see Pap 2002; P.L. Pap and C.I. Vágási, unpublished data). After 2 days of accommodation, we started the nutritional experiment. Birds in two randomly chosen aviaries (14 birds each) received low-quality food during the whole period of molting, while birds in the other two aviaries received high-quality food. In the wild, house sparrows feed on insects and seeds, with a high proportion of protein-rich insects during the molting period (Cramp 1994), but with considerable variation between populations and years. We designed the diet treatments to simulate the natural range of diet quality. The low-quality diet treatment consisted entirely of seeds, simulating the harsh conditions faced when insects are scarce and protein content of the diet is low. Groups with low-quality diet were fed ad libitum a mixture of seeds containing ground corn, sunflower, millet, oat, and barley; they also received twice weekly fresh dandelions. The composition of the dry matter of the seed mixture comprised 2.1% ash, 15.9% protein, 4.2% fat, and 77.8% carbohydrate. Every 2nd day their water was supplemented with vitamins and minerals recommended for canaries (Vita-Plus; Promedivet Ltd., Sovata, Romania). Groups with high-quality diet received the same grain mixture in addition to being supplemented with one grated boiled egg / group on every even day (comprising 3.7% ash, 51.3% protein, 40.4% fat, and 4.6% carbohydrate) and two mealworms/bird on odd days throughout molting (comprising 2.8% ash, 79.2% protein, 14.6% fat, and 3.4% carbohydrate). To suppress coccidian infection in the birds, we administered a coccidiostatic cure developed for veterinary use by adding sulfachinoxalin (Coccistops, Pasteur Ltd., Bucharest, Romania) to the drinking water of all groups during the experiment. To increase the comfort of the birds, we provided shelter (small bushes), perches, and nest boxes inside of each aviary. Sand and water were available ad libitum throughout the experiment. At capture and before release, we measured the wing and tarsus lengths, and the condition of the birds was assessed by measuring their body mass (with a Pesola spring balance

with an accuracy of 0.1 g) at weekly intervals throughout molting.

Experimental protocol

We used a 2 × 2 experimental design with two replicates of the nutritional groups (2 low-quality and 2 high-quality groups, respectively). Within each aviary, half of the birds were challenged with an antigen (see below), while the rest of them served as controls receiving the same dose of phosphate-buffered saline (PBS). Thus within each aviary we had 7 immunized and 7 control birds, totaling 14 house sparrows in each of the following groups: low-quality × SRBC, low-quality × PBS, high-quality × SRBC, and high-quality × PBS. One bird from the low-quality group was injured and another one from the high-quality group escaped during the second half of the molting period, hence the data for these birds on the molting process and feather quality were omitted from the analyses.

Seventy-seven percentage of the birds survived until the end of the study (the duration of the experiment was 125 days). The survival rate is comparable with those of other studies on aviary-kept house sparrows (63% during 3 months in Gonzalez et al. 1999; 71% during 4 months in Poston et al. 2005), and the death of the birds may be related to the stress associated with nutritional manipulation, repeated handling, or other factors. The survival rate of house sparrows were slightly higher than in the studies mentioned above, which is expected given the higher survival rates of adults relative to juveniles (Anderson 2006). After the birds had finished molting and feathers had been collected at the end of November, all birds were fed high-quality (protein-rich) food. After 2 weeks of intensive feeding, they were released in good condition at the farm where they had been captured. The body mass of the house sparrows at capture and before their release were similar between the nutritional groups ($F_{[1,42]} = 2.83$, $P = 0.10$); the body mass was significantly higher after 2 weeks of intensive feeding compared with the body mass at capture (Tukey's post hoc test from repeated-measures ANOVA; high-quality group: $P < 0.001$; low-quality group: $P < 0.001$).

Measurements: molting, feather quality, and biometry

Based on our observation and literature data (Jenni and Winkler 1994), the molting of primary feathers characterizes the complete postnuptial molting in birds; thus the use of the primaries gives a good general picture about the molting progress of house sparrows. Birds started molting shortly after they were introduced into the aviaries; on average, 13.5 ± 4.7 days following the start of the nutritional manipulation experiment. There was no difference between the four aviaries in the days elapsed between the start of nutritional manipulation and the start of molting (Kruskal–Wallis test: $\chi^2_{[3]} = 5.3$, $P = 0.15$). Following the settlement of birds in the aviaries, birds were captured once a week to assess the stage of molting (measure sessions hereafter). We categorized the molting stage of individual primaries following the scheme of Newton (1966): the dropped feathers were scored as 1; a quarter-, half-, or three-quarter-regrown feathers were scored as 2, 3, and 4, respectively; and fully regrown feathers were scored as 5. Old feathers received a score of 0. The molting pattern of the house sparrows is similar to that of most passerines: primary (P) flight feathers are shed from the wing in sequential order (Jenni and Winkler 1994), from innermost P1 to outermost P9 (P10 is rudimentary in sparrows). For each measure session, the molting stage of birds was assessed by summing the scores obtained for each individual primary feather, which is called the molting score. The molting score ranges between 0 (before the beginning of molting: all primaries old) and 45 (finished molting: all primaries completely replaced). To determine the speed of growth of individual feathers, we also measured the distance between the tip and the base of the untied part of the feather with a caliper (the length of the unbounded distal part). For the analyses, we only used the measurements of those feathers that were growing at least at two consecutive measure sessions and were assigned codes between 2 and 4. We considered that molting had started if at least one primary was dropped, while molting had ended when the outermost primary was fully grown.

At the end of molting (when all primary feathers were replaced), we plucked out the P2 and P7 feathers (for the rationale of choosing these particular feathers see below) on both wings to determine the effect of experimental manipulation on the quality of feathers grown. We characterized the quality of feathers through three parameters: feather length, rachis diameter, and feather mass. The length of the feathers was measured with an accuracy of 0.5 mm by extending them on a ruler. The rachis diameter was measured with a digital caliper (with an acccuracy of 0.01 mm) at 1.5 cm from the base of the shaft. To get a more exact measure of dry mass, we dried the feathers for 24 h at 40 °C in a desiccator to evaporate the absorbed moisture, after which the feathers were weighed on an electronic balance with an accuracy of 10^{-4} g. The repeatability of all feather-quality traits estimated by remeasuring 14 feathers was significantly high ($r > 0.8$, $P < 0.0001$). To increase the accuracy of the data, we used the mean values of the feathers from the left and right wings.

Immunization protocol and assessment of humoral immunity

Humoral immune system activation against SRBC was measured using a standard haemagglutination test (e.g., Eraud et al. 2005). We assessed the primary humoral immune response of house sparrows following injection with 100 μL of 20% fresh SRBC suspension into the pectoralis muscles (Ots et al. 2001). The dose calculated for unit of body mass (71 mL × concentration of SRBC/body mass) falls within the range used in avian studies, is generally higher than the dose used in ecological immunology studies, and falls well below the maximum concentration used (P.L. Pap, C.I. Vágási, and G.Á. Czirják, unpublished data). Birds were injected when the start of their primary molt was first observed. Therefore, the effects of activating humoral immunity on the development of the next-to-be-molted primaries (P2) and their quality can be studied. At the same measuring session when SRBC or PBS was administered, 75 μL of blood was drawn from the brachial vein to determine the pre-exposure levels of antibodies. Seven days later (see Roulin et al. 2000), during the next measuring session, a second blood sample was collected to determine the post-immunization primary antibody titre in plasma. Thirty-five

days after the first injection, an identical second injection was given and 7 days later a blood sample was taken to test for the presence of antibodies of the secondary response. During the period of the second immunization, most of the birds were shortly before dropping or just started growing the P7, which permitted us to study the effect of immune activation on molting and quality of this primary. Following blood collection in heparinized capillaries, the plasma was separated by centrifugation at 10 000 rev/min (16 060*g*) for 10 min and preserved at –20 °C until further analysis. Antibody titres were measured using a base-2 serial dilution haemagglutination test conducted with 15 μL of heat inactivated plasma (30 min on 56 °C) on U-shaped 96-well microtitre plates. Samples were serially diluted starting with 15 μL of PBS and to each well 15 μL of a 1% suspension of SRBC in PBS was added. Plates were incubated at 37 °C for 1 h. Titres are given as the $\log_2$ of the reciprocal of the highest dilution of plasma showing positive haemagglutination.

We quantified the total immunoglobulins concentration, part of the constitutive humoral immune system, in the blood samples collected before and 1 week after the two injections. The concentration of immunoglobulins in plasma was quantified using a spectrophotometrical method. Concentrations as low as 24 mg/L of heavy metal salts precipitate the immunoglobulins, since the electric charge and colloidal stability of gamma globulins are lower than those of serum albumins at pH 7.4. We mixed a volume of 3.3 μL of plasma with 197 μL of a 0.024% barbital buffer zinc sulphate solution and allowed immunoglobulins to precipitate for 30 min at room temperature (22–23 °C). Immunoglobulin concentration expressed in optical density units was read spectrophotometrically (λ = 475 nm, d = 0.5 cm; multichannel spectrophotometer SUMAL PE2; Karl Zeiss, Jena, Germany) (Khokhlova et al. 2004). The repeatability of immunoglobulins measurements was highly significant (r = 0.82, n = 14, $P < 0.001$).

Statistical analyses

The effect of nutritional and immunological manipulations on body mass and the growth rate of individual feathers was analyzed with general linear models using two-way repeated-measures ANOVAs, where body mass and growth rate were introduced as dependent variables. Primary molting increases in a nonlinear S-shaped function, which is very similar with the growth pattern of body mass of the developing birds. To describe the molting pattern, we fitted to each bird separately a logistic growth curve (Ricklefs 1973), which is a model used to study avian growth. The logistic growth curve has the form $y = a/\{1 + e^{[-K(t - I)]}\}$, where y denotes the molting score of a bird at time t, a is the final score or asymptote (which has a fixed value of 45 in our study), K is the growth constant, I is the inflection point on the time axis in which molting changes from accelerating to decelerating, and e is the base of natural logarithm. Following the calculation of molting parameters, we tested the effect of nutritional and immunological treatments on molting speed (K) and inflection point (I) using a two-way ANOVA. The molting speed can be characterized by the number of simultaneously growing feathers and the growth rate of individual feathers. Because for most of the measuring sessions the number of growing feathers did not follow a normal distribution and had nonhomogeneous variance, we calculated the mean number of growing feathers at each measuring session obtained during the whole molting period, which was then used in the following analyses. The effect of nutritional manipulation and immunization on the mean number of growing feathers was analyzed by two-way ANOVAs. The daily growth rate of individual feathers was calculated as the difference between the length of the unbounded distal part of feathers measured at two consecutive sessions (1 week apart) divided by the number of days elapsed between the two measures. Since in 25 of 43 birds the unbounded distal part of at least 1 of the 9 primary feathers could not be measured in one of the two consecutive measure sessions because the feather was unbounded (score 1) or reached the final length (score 5), we had a reduced sample size in measuring the effect of manipulation on the growth rate of primary feathers. The effect of nutritional manipulation on the probability of producing antibody titre against SRBC was tested with a generalized linear (binomial) model using a logit link function, while the difference in immune response of immunized birds between nutritional treatments was tested with a two-tailed t test. Values reported in the text are means ± SD unless otherwise stated.

Molting house sparrows under the same nutritional conditions but located in different aviaries did not differ significantly in the starting date of molting (Mann–Whitney U test; low-quality diet: z = 1.6, n_1 = 14, n_2 = 14, P = 0.12; high-quality diet: z = 0.6, n_1 = 14, n_2 = 14, P = 0.57), molting process (low-quality diet: $F_{[1,13]}$ = 0.9, P = 0.37; high-quality diet: $F_{[1,21]}$ = 0.4, P = 0.52), change in body mass (low-quality diet: $F_{[1,13]}$ = 2.8, P = 0.12; high-quality diet: $F_{[1,21]}$ = 0.01, P = 0.90), and the quality of feathers grown ($P > 0.5$ in all analyses). None of the interactions between variables and experimental groups were significant, except the change in body mass between birds fed the high-quality diet in the two aviaries during molting ($F_{[14,294]}$ = 5.6, $P <$ 0.0001). However, the difference in change in body mass between birds fed the high-quality diet molting in different aviaries was minor compared with the change in body mass between low- and high-quality groups (see below), thus pooling the data of house sparrows from aviaries with high-quality diet had an insignificant effect on the results. We found no significant difference between aviaries of the same nutritional groups in humoral immunity and survival of birds ($P > 0.05$ in all cases). Therefore, in the subsequent analyses, we pooled the data of birds from the same experimental group that were kept in separate aviaries.

Results

The relationship between diet quality, humoral immunity, condition, and survival

Forty-three out of 56 house sparrows (77%) survived until the end of the study. Survival rate differed between nutrition groups (low-quality diet: 61% survival, n = 28; high-quality diet: 96% survival, n = 28; generalized (binomial) linear model with a logit link function, $\chi^2_{[1]}$ = 11.6, P = 0.001), but not among immunization treatments ($\chi^2_{[1]}$ = 0.6, P = 0.46); the interaction between nutrition type and immunization was not significant ($\chi^2_{[1]}$ = 1.1, P = 0.29).

The body mass of house sparrows in the low-quality

Table 1. The effect of nutritional and immunological manipulations on body mass and individual feather growth rate of house sparrows (*Passer domesticus*).

Source of variation	df	Sums of squares	*F*	*P*
Body mass				
Nutritional treatment	1	215.7	6.3	0.02
Immunological treatment	1	0.7	0.02	0.89
Subject within groups	38	1306.9	—	—
Repeated measures	14	374.0	60.3	<0.0001
Nutritional treatment × immunological treatment	1	20.0	0.6	0.45
Nutritional treatment × repeated measures	14	45.4	7.3	<0.0001
Immunological treatment × repeated measures	14	1.6	0.3	1.00
Repeated measures × subjects within groups	532	235.8	—	—
Growing rate of individual feathers				
Nutritional treatment	1	0.1	0.3	0.57
Immunological treatment	1	0.4	1.4	0.25
Subject within groups	14	4.2	—	—
Repeated measures	8	8.4	6.6	<0.0001
Nutritional treatment × immunological treatment	1	0.4	1.4	0.26
Nutritional treatment × repeated measures	8	1.2	1.0	0.46
Immunological treatment × repeated measures	8	1.3	1.1	0.41
Repeated measures × subjects within groups	112	17.8	—	—

Note: None of the nutritional treatment × immunological treatment × repeated-measures interaction is significant.

group was significantly lower than in the high-quality group (Table 1). None of the preinjected house sparrows had detectable SRBC antibodies, and following injection with PBS of control birds, only 1 out of 28 birds produced a reaction against SRBC. In contrast, SRBC treatment significantly elevated antibody titre compared with controls in both nutritional groups, as 93% of birds (25 of 27) produced haemagglutination against SRBC ($\chi^2_{[1]} = 56.8$, $P < 0.0001$; nutritional treatment × immune response: $\chi^2_{[1]} = 0.0$, $P = 1.00$). Nutritional manipulation had no significant effect on the primary antibody response in birds immunized with SRBCs ($F_{[1]} = 0.4$, $P = 0.56$), and including the two "negative" SRBC-treated birds (individuals that produced no antibody titre against the antigen), the mean antibody titre between low- and high-quality groups was similar (4.2 ± 2.4 vs. 4.6 ± 1.2; $t = 0.6$, $n_1 = 14$, $n_2 = 13$, $P = 0.57$). Secondary injection with SRBC produced detectable antibody titre in all but 1 bird (24/25), and the magnitude of the immune response was similar between primary and secondary immune responses (paired sample *t* test; low-quality group: $t = 0.66$, $n = 11$, $P = 0.53$; high-quality group: $t = 0.59$, $n = 13$, $P = 0.57$). Again, the nutritional treatment had no effect on the probability of producing antibody titre ($\chi^2_{[1]} = 1.4$, $P = 0.25$) or on the mean antibody titre (low-quality vs. high-quality groups: 4.6 ± 2.1 vs. 4.2 ± 1.9; $t = 0.5$, $n_1 = 12$, $n_2 = 13$, $P = 0.59$). The correlation between primary and secondary antibody titres of SRBC-injected birds was significant ($r = 0.44$, $n = 24$, $P = 0.03$). Preimmunization immunoglobulin concentrations were not significantly affected by nutritional condition ($t = 1.7$, $n_1 = 26$, $n_2 = 25$, $P = 0.09$), and postimmunization immunoglobulin concentrations did not differ between nutritional groups ($F_{[1,44]} = 0.9$, $P = 0.36$). Immunoglobulin concentrations at the time of secondary SRBC-injection was similar between nutritional groups ($t = 0.7$, $n_1 = 23$, $n_2 = 25$, $P = 0.52$); however, after 1 week following the immune challenge, their concentration dropped significantly in the low-quality groups compared with the high-quality groups ($F_{[1,48]} = 6.4$, $P = 0.02$).

Immunization with SRBC produced no significant effect on body mass (Table 1). Postimmunization immunoglobulin concentrations did not differ between SRBC and PBS-injection groups ($F_{[1,44]} = 0.8$, $P = 0.37$). SRBC treatment did not affect immunoglobulin concentrations following secondary injection ($F_{[1,48]} = 0.02$, $P = 0.88$). In none of the cases, the interactions between experimental groups were significant.

Effect of nutritional manipulation and immune activation on molting and feather quality

The start of molting in house sparrows held under low- and high-quality nutritional conditions did not differ significantly ($z = 0.9$, $n_1 = 28$, $n_2 = 28$, $P = 0.39$; Fig. 1). However, the nutritional condition had a significant effect on the process of molting, as reflected in the significant increase in the *K* value and decrease in *I* value for birds feeding on high-quality food compared with birds feeding on low-quality food (*K* value: $F_{[1,37]} = 9.9$, $P < 0.01$; *I* value: $F_{[1,37]} = 8.6$, $P < 0.01$). Immunization with SRBC caused no significant change in molting parameters (*K* value: $F_{[1,37]} = 0.1$, $P = 0.74$; *I* value: $F_{[1,37]} = 0.8$, $P = 0.39$). The absence of the significant interaction between immunization and nutritional manipulation (*K* value: $F_{[1,37]} = 0.0$, $P = 0.92$; *I* value: $F_{[1,37]} = 0.0$, $P = 0.94$) indicated that the effect of nutritional manipulation was similar in groups injected with either SRBC or PBS. The duration of molting (calculated as the time elapsed between the first and the last measuring sessions, namely when a dropped innermost primary was observed first and when all primary feathers were fully grown) in birds fed a high-quality diet was 82.6 ± 9.9 days ($n = 25$) compared with 96.7 ± 10.6 days ($n = 16$) for birds

Fig. 1. Mean (SE) changes in molting score of aviary-kept house sparrows (*Passer domesticus*) held under low-quality (○) and high-quality (●) nutritional conditions during postnuptial molting between July and November 2006. The sample size for low- and high-quality groups are 17 and 25, respectively.

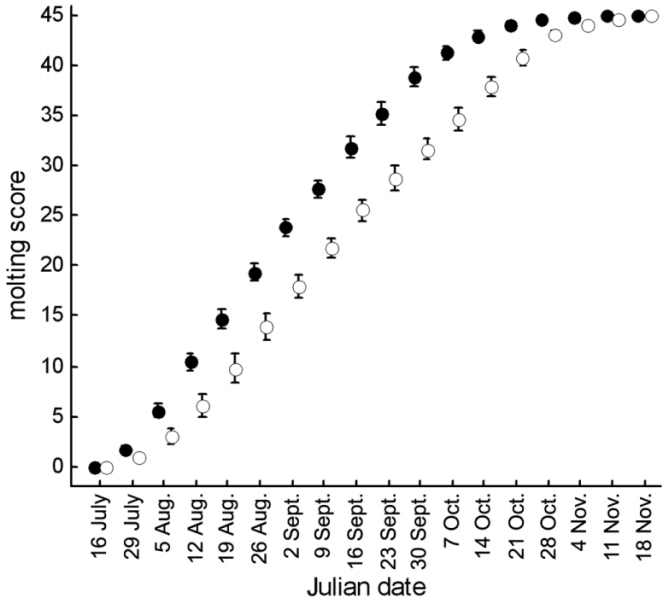
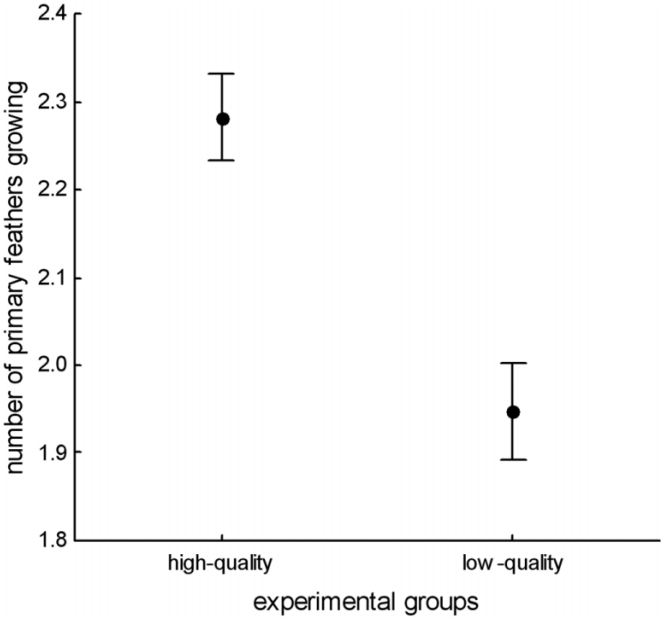


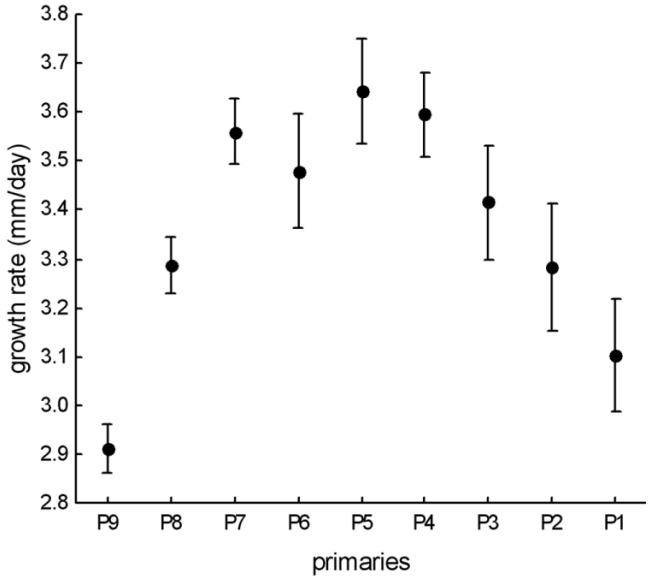
Fig. 2. Mean (SE) numbers of growing primary feathers of the left wing of aviary-kept house sparrows (*Passer domesticus*) molting under low- and high-quality nutritional conditions.



fed a low-quality diet ($F_{[1,37]}$ = 17.7, P = 0.0001). SRBC injections and the interaction between nutritional and immunization manipulations had no significant effect on the duration of molting (SRBC injections: $F_{[1,37]}$ = 1.3, P = 0.27; nutrition × SRBC injections: F = 0.1, P = 0.75).

Molting speed has two components: the number of simultaneously growing feathers and the growth rate of individual feather lengths. Nutritional condition had a significant effect on the mean number of growing feathers through molting,

Fig. 3. Mean (SE) daily growth rates of primary feathers of the aviary-kept house sparrows (*Passer domesticus*) (n = 18).



namely birds feeding on low-quality diet grew less primaries simultaneously than did birds feeding on the high-quality diet ($F_{[1,37]}$ = 18.7, P = 0.0001; Fig. 2). In contrast, SRBC injections had no significant effect on the number of growing feathers ($F_{[1,37]}$ = 0.6, P = 0.44). The nonsignificant interaction between nutritional and immunization manipulations ($F_{[1,37]}$ = 0.1, P = 0.74) indicates that the effect of nutritional manipulation is similar in SRBC and saline-injected groups. There was no significant difference in the daily growth rate of individual feathers between nutritional and immunization groups (Table 1), which indicates that the difference between nutritional groups in molting was due to the increased number of growing primaries in the high-quality group compared with the low-quality group. The highly significant repeatability of growth rate of primary feathers shows the similarity of the growing rate of primaries within individual birds; however, the daily increase in length varied between feathers in different positions (Table 1, Fig. 3). The growth rate of primaries increased from the inner to the middle positions (Fisher's LSD post hoc test of the repeated-measures ANOVA; e.g., P1 vs. P5, P < 0.0001), and then decreased from the middle to the distal positions (e.g., P5 vs. P9, P < 0.0001).

Feather quality, measured through the feather length, rachis width, and feather mass differed significantly between birds feeding on low- and high-quality foods (Table 2). House sparrows feeding on high-quality food grew longer and heavier feathers and tended to have thicker rachis of proximal primaries compared with birds feeding on low-quality food, while none of the variables differed between immunized and control birds (P > 0.5 for all tests).

Discussion

Molting is recognized as one of the most energy-demanding activities of birds. The metabolic rate during molting increases by more than 100% of its premolting value (Lindström et al. 1993). In addition, the increase in metabolic

Table 2. The quality of proximal (P2) and distal (P7) primary feathers grown by house sparrows (*Passer domesticus*) molting under low- and high-quality nutritional conditions.

	Group (mean ± SE)			
	Low-quality	High-quality	*F*	*P*
Feather length (mm)				
Primary 2	57.2±0.3 (24)	58.2±0.3 (25)	5.62	<0.05
Primary 7	67.5±0.4 (19)	68.6±0.3 (25)	5.01	<0.05
Rachis width (mm)				
Primary 2	0.83±0.01 (24)	0.85±0.01 (25)	3.34	0.07
Primary 7	0.99±0.01 (19)	1.00±0.01 (25)	0.23	0.64
Feather mass (1×10^{-4} g)				
Primary 2	97.3±1.2 (24)	101.6±1.2 (25)	6.01	<0.05
Primary 7	144.9±2.1 (18)	154.5±1.8 (25)	12.48	<0.01

Note: Sample sizes are in parentheses.

rate during molting is size-dependent (Lindström et al. 1993), i.e., the cost of molting is higher in species with reduced size. Therefore, it is reasonable to expect that molting can be energetically limited and as a result is negatively affected by the immune activation that consumes the same energy resources. However, contrary to expectations, our results indicated that molting and feather quality seem uncompromised by humoral immunity, as immune response to SRBC antigen had no effect on either feather growth and quality or on condition of the birds. Hence, our results do not confirm the previous findings regarding the trade-off between molting and immune response (Sanz et al. 2004; Martin 2005). Interestingly, a recent study on the annual variation of health status and immune function in the great tit (*Parus major* L., 1758) showed that immunoglobulin concentrations are highest during molting (P.L. Pap, C.I. Vágási, J. Tökölyi, G.Á. Czirják, and Z. Barta, unpublished data), questioning further the existence of a strong trade-off between humoral immune system and molting. However, we did not exclude the possibility that during critical stages of the annual cycle of the birds, such as at the molting–breeding overlap, the cost of mounting a humoral immune response may have a significant deleterious effect on fitness (Sanz et al. 2004).

Molting is a nutrition-demanding activity for birds because the feathers that are synthesized compose about 30% of the total protein mass of the birds (Murphy et al. 1988). Therefore, it is reasonable to expect that molting can be limited by nutrition. In line with this assumption, we found that house sparrows feeding on low-quality food molted slower because of the reduced number of simultaneously growing feathers and grew proximal and distal primaries of reduced quality compared with house sparrows feeding on high-quality food. The slower feather replacement of birds feeding on low-quality food resulted in a 14-day delay in the final molt (high-quality food vs. low-quality food: 82.6 vs. 96.7 days). Moreover, house sparrows feeding on low-quality food could not compensate for the nutrition deficiency by expanding the molting period as shown by the production of the low-quality feathers. These results confirm the findings of the few previous studies regarding the nutritional limitation of feather growth and quality (e.g., Murphy et al. 1988; Murphy and King 1991). Our study also demonstrated that house sparrows could not fulfill their nutritional needs during molting with seeds alone (but for other species see Allen and Hume 2001), which is supported by the observation of house sparrows selectively feeding on insects during this period of the annual cycle (Cramp 1994).

Nutritional limitation had no effect on the individual growth rate of feathers. Contrary to a previous study involving European starlings (*Sturnus vulgaris* L., 1758) (Dawson 2003), we have shown that the growth rate of primaries varied relative to their position, namely the more proximal and distal feathers had a reduced daily growth rate compared with the primaries in the middle of the wing. In line with Dawson (2003), we found the growth rate of the outermost feather to be the lowest, which may be related to the increased mass of this primary compared with the proximals (Dawson 2005) and (or) the cost of feather replacement of secondaries that began to molt during the growth of the distal primaries.

Our experimental study showed that the humoral immunity, assessed using primary and secondary responses against SRBCs, was not affected by the food quality in molting house sparrows. Furthermore, the amount of anti-SRBC antibodies produced by house sparrows in the current study was comparable with the amount produced by other wild-living avian species (e.g., Lochmiller et al. 1993; Roulin et al. 2000; Ots et al. 2001; Hanssen et al. 2004; Eraud et al. 2005). In contrast, immunoglobulin concentrations decreased following the second immunization of birds feeding on low-quality food, which supports the nutritional limitation of constitutive humoral immunity. Following injection, the antigen-specific antibodies increase in the peripheral blood (e.g., Hanssen et al. 2004), and total immunoglobulin concentrations include both the constitutive and the acquired humoral immunities. However, the haemagglutination assay showed that the SRBC antigen-specific immune response was not affected by the nutritional manipulation, leading to the conclusion that the decreased immunoglobulin concentrations in the low-quality nutritional group compared with the high-quality nutritional group could be the result of suppression of nonspecific immunoglobulins. The absence of nutritional limitation on acquired humoral immunity and the reduction of immunoglobulin concentrations only following

prolonged malnutrition appear surprising, since both specific and nonspecific antibodies are nutrition-dependent, hence their production depends on the quality of food ingested. However, during nutritional limitations birds may use nutrition reserves deposited in muscles to supply the nutrition need for physiologically important functions, such as the immune system (Cherel et al. 1994), as is the case for migratory birds following long flights (Landys et al. 2005). In fact, birds feeding on low-quality food reduced significantly their body mass during molting. However, the duration of nutritional manipulation was probably long enough to deplete the nutritional reserves of the birds well before the termination of molting, which is supported also by the increased death rate of birds feeding on low-quality food compared with those feeding on high-quality food (see also Birkhead et al. 1999), as well as the reduced molting rate of birds feeding on low-quality food. Our results are in accordance with previous studies regarding the low energetical and nutritional costs of humoral immunity (Lochmiller et al. 1993; Gonzalez et al. 1999; Cichoń 2000; Ilmonen et al. 2002; Grindstaff et al. 2005; Poston et al. 2005), but contradicts the two recent studies that showed this branch of the immune system to be nutrition-limited (Gasparini et al. 2006; Smith et al. 2007). House sparrows on a low-quality diet mounted increased antibody responses (Buchanan et al. 2003), while sparrows on a protein-rich diet decreased antibody responses (Gonzalez et al. 1999). In addition, our study demonstrated that even during intensive protein use, such as during molting, humoral immune system is less limited by nutrition.

In conclusion, house sparrows feeding on low-quality food experienced prolonged molting and produced low-quality feathers, which may seriously reduce their future reproductive value because of increased cost of thermoregulation, higher abrasion rate of feathers, and reduced aerodynamical capacity of the flight feathers. Furthermore, we have shown that the food quality had no effect on the induced humoral immune response of the house sparrows during the energy- and nutrition-demanding periods of the molting process. Finally, the activation of humoral immunity had no effect on molting, which supports the absence of a trade-off between humoral immunity and molting in house sparrows. We propose further studies that would examine the role of energetically and nutritionally costly branches of the immune system (e.g., cellular immunity) in shaping the molting of birds.

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