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Dietary modulation of intestinal enzymes of the house sparrow (*Passer domesticus*): testing an adaptive hypothesis

Enrique Caviedes-Vidal [a], Daniel Afik [b,1], Carlos Martinez del Rio [c,2], William H. Karasov [b,*]

[a] *Departamento de Bioquímica y Ciencias Biológicas, Universidad Nacional de San Luis, 5700 San Luis, Argentina*
[b] *Department of Wildlife Ecology, University of Wisconsin, Madison, WI 53706, USA*
[c] *Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544-1003, USA*

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Abstract

Insectivorous/frugivorous passerine species studied so far lack the ability to modulate intestinal maltase activity, in contrast to galliformes. We tested for dietary modulation of small intestine (SI) enzymes including maltase in house sparrows to understand whether the difference between the galliformes on the one hand, and the passerines on the other, reflects a phylogenetic pattern (maltase modulated in galliformes but not passerines), a dietary pattern (maltase modulated in granivores but not insectivore/frugivores), some other pattern, or chance. We also tested the prediction that intestinal peptidase activity would be increased on a high protein (HP) diet. Birds were fed three diets high in starch, protein, or lipid for 10 days. For birds on the HP diet (60.3% protein) we observed the predicted upward modulation of aminopeptidase-N activity, as compared with the lower-protein, high starch (HS) (12.8% protein) diet. In contrast, birds eating the HS diet had similar maltase and sucrase activities, and only slightly higher isomaltase activity, compared with birds eating the high protein (HP), starch-free diet. Birds eating high lipid (HL) diet had low activities of both carbohydrases and peptidase. Considering that the statistical power of our tests was adequate, we conclude that house sparrows show little or no increase in carbohydrases in response to elevated dietary carbohydrate. We cannot reject the hypothesis that maltase lability among avian species has a phylogenetic component, or that high dietary fat has a depressing effect on both carbohydrase and peptidase activities.

Keywords: Birds; Digestion; Disaccharidases; Aminopeptidase-N; Maltase; Sucrase; Isomaltase

1. Introduction

Modulation of digestive enzymes is an important feature of digestive flexibility in animals, in addition to changes in nutrient absorption rate or digesta retention (time a meal spends in the gut) (Karasov and Hume, 1997). At the whole-animal level, such modulation is important in permitting or constraining diet switching or very high feeding rates. It has been argued that animals modulate, rather than maintain high constitutive levels of

* Corresponding author. Tel.: +1-608-263-9319; fax: +1-608-262-6099.
E-mail address: wkarasov@facstaff.wisc.edu (W.H. Karasov)
[1] Present address: Department of Biology, University of Haifa at Oranim, Oranim, Tivon 36003, Israel.
[2] Present address: Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA.

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specific enzymes, because the metabolic expense of synthesizing and maintaining large amounts of digestive enzymes would be wasted by animals feeding on diets with very low levels of the substrates for those enzymes. Thus, the a priori expectation for animals with biochemical lability is that, for dietary components such as carbohydrates, protein, and lipid, there will be a positive relationship between their level in the diet and the presence or amount of gut or pancreatic enzymes necessary for their breakdown.

Not all vertebrates modulate intestinal carbohydrase enzymes (Karasov and Hume, 1997). One suggested explanation is that the ability to modulate these has been selected for in omnivores that switch among diets with varying carbohydrate levels, but not in carnivores that always consume diets with little or no carbohydrates (Buddington et al., 1991; Afik et al., 1995). Studies with avian species, however, are not consistent with this simple hypothesis that links omnivory to modulation ability. Primarily granivorous chickens (Biviano et al., 1993) and turkeys (Sell et al., 1989) exhibit increased maltase activity when fed diets high in carbohydrate, whether starch, maltose, sucrose, or glucose. In contrast to these galliformes, the passerine birds European starlings (Martinez del Rio, 1990) and yellow-rumped warblers (Afik et al., 1995), which in the wild consume both insects, fruits and, in the case of starlings seeds, have no higher maltase activity when fed high carbohydrate diet than when fed low carbohydrate or carbohydrate-free diet. Thus, a question for birds is whether the difference between the galliformes on the one hand, and the passerines on the other, reflects a phylogenetic pattern (maltase modulated in galliformes but not passerines), a dietary pattern (maltase modulated in granivores but not insectivore/frugivores), or chance.

The present study of intestinal enzymes in house sparrows relates to this question, though it alone cannot serve as a definitive test because that will require a multispecies data set analyzed within a phylogenetic context (Harvey and Pagel, 1991). However, house sparrows are an important inclusion and a good study subject for several reasons. House sparrows are naturally omnivorous passerines, ingesting starchy seeds with large amounts of glucose, and also ingesting other nutrient mixes such as HP-moderate fat insects and high fat-moderate protein seeds (Martin et al., 1951). Also, their cosmopolitan distribution and ease of capture and laboratory maintenance make house sparrows good subjects for a variety of laboratory studies of avian physiology.

Our null hypothesis was that digestive carbohydrases would not differ significantly among house sparrow test populations eating varying levels of dietary carbohydrates. If this were the case, and given their documented dietary flexibility, this would be consistent with the hypothesis of a phylogenetic constraint. Alternatively, a finding that digestive carbohydrases increased in direct correlation with relative level of dietary carbohydrate would cast doubt on this phylogenetic hypothesis. We also predicted that peptidase activity would increase in direct correlation with dietary protein level, because this pattern of modulation has been documented in the passerine species studied so far (Martinez del Rio, 1990; Afik et al., 1995).

To examine our hypotheses, we assessed disaccharidase activity by measuring intestinal maltase, isomaltase, and sucrase activity. Maltose is the main by-product of the hydrolysis of complex polysaccharides such as starch, amylopectin, and glycogen (Alpers, 1987). Therefore, maltase activity resulting from the activity of two enzymes, maltase-glucoamylase and sucrase-isomaltase (Noren et al., 1986), is probably the single best estimator of the ability to assimilate complex soluble carbohydrates. Sucrase-isomaltase is a relatively unspecific enzyme that hydrolyzes sucrose, isomaltose and maltose (Hunziker et al., 1986). Within passeriforme birds, one taxonomic line, the Sturnidae–Muscicapidae line, entirely lacks sucrase-isomaltase (Martinez del Rio et al., 1995). The presence of sucrase-isomaltase within the Ploceidae, the family of house sparrows, is uncertain, and thus our tests for this enzyme constitute another new feature of our study.

Protein digestion is extremely complex due to the wide diversity of amino acids and possible peptides. Therefore, we have chosen to measure a representative dipeptidase, aminopeptidase-N (E.C. 3.4.11.2), also known as leucine-aminopeptidase and amino-oligopeptidase (Vonk and Western, 1984). This enzyme appears to account for almost all peptidase activity in the brush-border membrane (Maroux et al., 1973). It displays broad specificity in the cleavage of NH_2 terminal amino acid residues from nutrient oligopeptides to produce the final dipeptides and amino acids for absorption (Sjostrom et al., 1978).

2. Materials and methods

2.1. Animal care and housing

Twenty birds were captured with mist nets during early September at the University of Wisconsin, Madison, WI campus. They were housed alone in individual cages ($0.60 \times 0.45 \times 0.43$ m) under constant light cycle (12:12 h light–dark cycle), temperature (23°C) and relative humidity (45%). A branch was placed inside each cage for a perch. During the first 5 day adjustment period they were provided with seeds (Kaytee Products, Chilton, WI) and water (supplemented with vitamins) ad libitum.

Table 1
Composition of the semi-synthetic diets fed to house sparrows

Components (% w/w)	HS[a]	HP	HL
Casein[b]	10	57.52	13.48
Amino acid mixture[c]	2.77	2.77	3.74
Corn oil[d]	8	8	40.01
Corn starch[e]	61.52	14	18.88
Salt mixture[f]	5.5	5.5	7.42
H_3BO_3	9×10^{-4}	9×10^{-4}	1.2×10^{-3}
$Na_2MoO_4 \cdot 2H_2O$	9×10^{-4}	9.10^{-4}	1.2×10^{-3}
$CoSO_4 \cdot 2H_2O$	1×10^{-4}	1×10^{-4}	1.4×10^{-4}
Na_2SeO_3	2×10^{-5}	2×10^{-5}	2.7×10^{-5}
$NaHCO_3$	1	1	1.35
Choline chloride	0.2	0.2	0.27
Vitamin mix[g]	1	1	1.35
Cellulose[h]	2	2	2.7
Agar[i]	3	3	4.05
Ground silica sand	5	5	6.74
Totals	99.99	99.99	99.99
Gross energy content[j] ($kJ \times g^{-1}$)	17.66 ± 0.45	20.29 ± 0.04	24.03 ± 0.12
Water content (%w/w)	44.4 ± 0.82	47.05 ± 0.06	42.07 ± 0.94

[a] Murphy and King, 1982 basal-diet
[b] Casein (high nitrogen), Teklad Diets, Madison WI.
[c] Aminoacid mixture supplement Murphy and King, 1982, all the amino acids used were provided by Sigma Chemical Company, St. Louis, MO, as L-hydrochloride stereoisomers.
[d] Corn oil, Teklad Diets.
[e] Corn starch, Teklad Diets.
[f] Salt mixture Fox–Briggs (Spivey Fox and Briggs, 1960).
[g] Vitamin mixture AIN-76.
[h] Celufil-hydrolyzed, USB Corporation, Cleveland, OH.
[i] Agar bacteriological grade, USB Corporation.
[j] Values represent the mean of two measurements $\pm$ one S.E.M.

2.2. Diet acclimation

After the 5-day adjustment period the house sparrows were randomly divided into three groups of birds fed with different semi-synthetic diets (Table 1) prepared to resemble possible natural food types of the species. The high starch (HS) diet represents seeds with starch as the most important energetic substrate, the high lipid (HL) diet represents high fat seeds, and the HP diet, like insects, provided protein as the most abundant energetic source. All three diets were based on the semi-synthetic diet of Murphy and King (1982) and satisfy all the nutritional requirements (even during reproduction and feather synthesis periods). Food and water were offered ad libitum for 10 days, during which time food intake and body mass were monitored.

2.3. Sample collection

Birds were euthanized by decapitation. Immediately afterwards, the abdominal cavity was opened and the entire gastrointestinal tract (≈ 1 cm proximal to the stomach down to the cloaca) was removed and chilled in ice cold avian saline (Caviedes-Vidal and Karasov, 1996). Stomach and pancreas were removed, cleaned of extraneous tissue, weighed and stored for other studies. The complete small intestine (SI) with its content was measured for length and then divided into three equal-length parts, henceforth called proximal (P), medial (M) and, distal (D) SI. Under iced saline, each segment was slit open and the contents were removed. The intestinal pieces were then blotted, weighed, and stored in liquid N_2.

2.4. Sample preparation

Intestinal segments were thawed at 20–23°C and homogenized for 30 s using an Omni 5100 homogenizer (setting 6) in 350 mM mannitol in 1 mM Hepes/KOH (pH 7.5), using 8 ml g^{-1} tissue. We measured activity of membrane-bound enzymes in whole tissue homogenates rather than in mucosal samples or isolated brush-border membrane preparations to avoid underestimation of

activity as previously reported (Martinez del Rio, 1990).

2.5. Dissacharidases assays

We determined the activity of three intestinal dissacharidases, maltase (E.C. 3.2.1.20), sucrase (E.C. 3.2.1.48) and isomaltase (E.C. 3.2.1.10) in the intestinal homogenate. We used the colorimetric method developed by Dahlqvist (1984) and modified by Martinez del Rio (1990). Aliquotes of 100 μl of tissue homogenate, appropriately diluted, were incubated with 100 μl of 56 mM sugar (maltose, sucrose and isomaltose) solutions in 0.1 M maleate/NaOH, pH 7. After 10 min incubation at 40°C we arrested the reaction adding 3 ml stop/develop reagent. We prepared the stop/develop reagent dissolving one bottle of Glucose (Trinder) 315–500 reagent powder (Sigma Chemical, St. Louis, MO) in 250 ml 0.5 M phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7.0, plus 250 ml 1.0 M Tris/HCl). The arrested reactions were allowed to stand for 18 min and then the absorbances were measured at 505 nm at room temperature on a Beckman DU 64 spectrophotometer. Enzyme activity was determined using a glucose standard curve.

2.6. Aminopeptidase-N assay

We assayed aminopeptidase-N (E.C. 3.4.11.2) using L-alanine-*p*-nitroanilide as a substrate (Roncari and Zuber, 1969). We started the reaction adding aliquotes of 10 μl ($\approx$12 mg of protein/ml) of the tissue homogenate to 1 ml assay solution, made of 2.0 mM L-alanine-*p*-nitroanilide in 0.2 M phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7.0). The reaction was incubated during 10 min at 40°C and then arrested with 3 ml of chilled 2 M acetic acid. The absorbance was measured at 384 nm, and activity was determined using a *p*-nitroanilide standard curve.

2.7. Protein measurement

We estimated the concentration of protein in our samples using the commercial Bio-Rad Protein Assay (Bio-Rad, catalog number 500-0006). Absorbances were read at 595 nm and crystalline bovine serum albumin was used as standard.

2.8. Standardization of enzyme activities and calculation of summed hydrolysis activity

Enzymes activities for each intestinal region (P, M, D) were expressed as μmol min^{-1}, normalized to either measured tissue wet mass or measured nominal surface area. We provide conversion factors to normalize to tissue protein as well. The advantages of our normalization procedures are discussed by (Martinez del Rio, 1990). We calculated the summed hydrolisis activity of the entire SI, an index of the total hydrolysis capacity, by multiplying activity per gram tissue in each region by its respective mass, and summed over the three regions.

2.9. Determination of pH optima and kinetics

We determined the pH optima of maltase, sucrase and aminopeptidase-N in the medial portion of the SI in one bird chosen randomly from each diet treatment. The assays were performed using the homogenates and a 0.05 M maleate/NaOH buffer system with pHs ranging from 3.5 to 8.5 for the dissaccharidases and from 4.5 to 8.5 for aminopeptidase-N. We used the same homogenates for pH optima and for kinetics. We estimated the apparent binding constants (K_m^*), the concentration of substrate at which the rate of hydrolysis equals half the maximal hydrolysis rate (V_{max}) for maltase and sucrase. Enzyme activities were assayed at pH 7 and substrate concentration varying from 1 to 32 mM for dissaccharidases. To minimize individual variation, we calculated relative activity (i.e. activity at test pH or concentration normalized to activity at standard pH [7.0] or standard concentration in a sample from the same bird).

2.10. Data analysis

Results are given as means $\pm$1 S.E. (n = number of individuals per treatment). We used repeated measures analysis of variance (ANOVA) to examine the effect of diet and intestinal region on enzyme activities. The F-values of these and other analyses of variance are presented in the text with the relevant degrees of freedom as subscripts. The Tukey's Honest Significant Difference multiple comparison test was used to isolate diet differences in each region or in summed hydrolysis capacity. The significance level was set at

Table 2
Body mass and SI measures of house sparrows fed different diets 10 days[a]

Diet	*n*	Body mass (g)	SI mass (g)	SI length (cm)
HL	7	26.77 ± 0.95	1.20 ± 0.10	19.4 ± 0.5
HP	6	25.67 ± 0.75	1.49 ± 0.16	21.0 ± 0.47
HS	7	26.0 ± 0.82	1.44 ± 0.28	18.6 ± 0.9
P-value		>0.7	>0.7	0.07

[a] Values are means $\pm$ S.E. (n = number of sparrows). P-value for diet effect from ANOVA.

$P < 0.05$, and $0.05 < P < 0.1$ was taken to indicate a trend. Kinetic parameters were determined by fitting the kinetic data by nonlinear curve fitting (Gauss Newton routine, SYSTAT Wilkinson, 1992) to the equation relative activity $= (V_{max} \times \text{concentration})/(K^*_m + \text{concentration})$.

We used analysis of covariance (ANCOVA Wilkinson, 1992) to test whether the relationship between tissue mass and protein content differed by diet, and we tested the three intestinal regions separately because there is no straightforward way to do such an analysis by repeated measures. Interactions were not significant (i.e. slopes did not differ significantly) and are not reported. We used the same procedures to analyze the relationship between maltase and sucrase activity. Maltose is hydrolized by two independent enzymatic systems, the complex sucrase-isomaltase ('sucrase') and one or two maltase-glucoamylases (Semenza and Auricchio, 1989). Martinez del Rio (1990) found inter- and intraspecies linear correlation between the intestinal activities of maltase and sucrase. Theoretically, the slope of the regression of the activities of maltase on the sucrase provides an estimate of the contribution of the sucrase-isomaltase complex to the maltasic activity and the intercept provides an estimate of the independent activity of the maltase-glucoamylase complex. Therefore, we performed this regression analysis for each position, testing also for an effect of diet on the relation. Finally, we repeated these regression analyses using model II regression which gives unbiased estimates in cases where both X and Y variables are measured with error (Sokal and Rohlf, 1981). The residuals of each regression analysis were inspected (Wilkinson, 1992) and had constant variance, were independent, and were normally distributed.

Diet can have two types of effects on the expression of intestinal enzymes. It can lead to specific modulation, in which some substrates increase the activity of their corresponding enzymes, or it can show non-specific modulation in which ingesting a diet leads to correlated changes in all digestive enzymes (Karasov and Diamond, 1988; Sabat et al., 1998). To disentangle the effect of diet on specific and non-specific modulation, we conducted principal components analysis (PCA) on the summed activity of all enzymes. PCA can be a powerful technique to analyze the effect of diet on the activity of a suite of digestive enzymes because it reduces the number of variables (enzyme activities) to a smaller number of uncorrelated variables that can have simple physiological interpretations (see Sabat et al., 1998).

3. Results

3.1. Body mass and gut morphometrics

The sparrows acclimated to the laboratory with no apparent problems and ate similar amounts of the three diets daily (Caviedes-Vidal and Karasov, 1996). There were no significant differences by diet for final body mass or for SI mass (Table 2), but there was a trend for shorter SIs in sparrows fed the HS diet ($P = 0.07$) (Table 2). We previously reported shorter and lighter SIs in house sparrows fed the HS diet, compared with the other two diets (Caviedes-Vidal and Karasov, 1996).

3.2. Regional enzyme activities

Although we present enzyme activity normalized to tissue wet mass, our data can be compared with those of other studies that normalize activity to protein content (Fig. 1). Intestinal protein content was significantly correlated with intestinal mass in the proximal ($F_{1,17} = 138$, $P < 0.001$), mid ($F_{1,17} = 112$, $P < 0.001$), and distal ($F_{1,16} = 60$,

$P < 0.001$) intestinal regions. There were trends for diet to affect this relationship in the mid ($F_{2,17} = 3.0$, $P = 0.076$) and distal regions ($F_{2,16} = 3.2$, $P < 0.069$), though not the proximal region ($F_{2,17} = 1.9$, $P = 0.18$), which is one reason we chose not to normalize enzyme activities to tissue protein content. After excluding diet as a factor in the analyses, because it was not statistically significant, the intercepts were not significantly different from zero in either the proximal (intercept $= 4.3 \pm 3.5$ mg protein, $P = 0.2$), mid (1.3 ± 2.5, $P = 0.6$) or distal positions (-0.2 ± 2.5, $P = 0.9$), and the three intestinal positions all had very similar slopes (96 ± 7, 88 ± 7, and 89 ± 7, respectively, all $P < 0.001$). Therefore, each gram intestine contained about 90 mg protein.

The activities of sucrase and maltase decreased distally along the intestine (Fig. 2, Table 3). In contrast, isomaltase activity did not differ with intestinal position whereas aminopeptidase-N activity increased along the intestine.

Diet had a significant effect on regional activities of all enzymes (Fig. 2, Table 3). In the case of aminopeptidase-N, house sparrows fed HP diet had higher activity, as predicted. Tukey multiple comparison tests of aminopeptidase-N activity at each position showed significant differences among all three diets in the proximal region (HP > HL > HS) and one significantly different diet in both the medial region (HP > HL, HS) and distal region (HP, HL > HS).

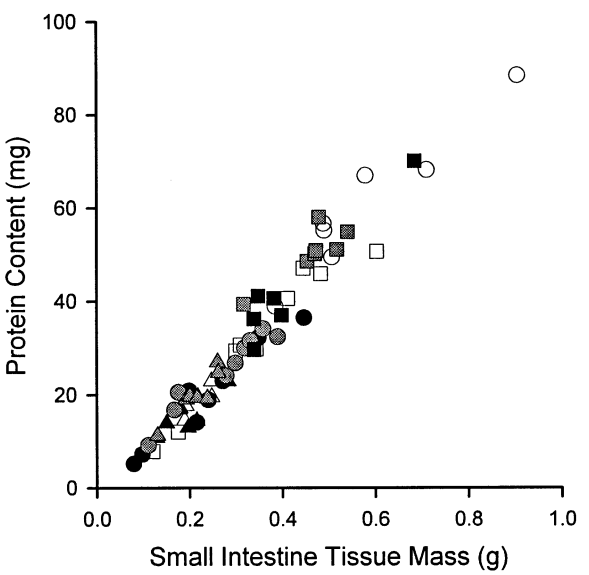

Fig. 1. The relationship between intestinal protein content and intestinal mass. Values are coded according to the house sparrow's diet (black, HS; unfilled, HP; grey, HL) and the intestinal position the tissue was collected from (square, proximal; circle, medial; triangle, distal). The data indicate that each gram intestine contains about 90 mg protein (see text).

In the case of the carbohydrases, the diet effects were not as statistically significant as for the peptidase (i.e. higher P-values; Table 3). Indeed, Tukey multiple comparison tests at each position were nonsignificant ($P \geq 0.05$) for both maltase and sucrase, though for both enzymes there were trends ($0.05 < P < 0.1$) at every position for lower activity in the HL diet group. Comparisons of isomaltase indicated a trend for a diet effect in the proximal region (HS > HL, $P = 0.06$; HP not different from either), a significant diet effect in the medial region (HS > HL, $P = 0.03$; HP not different from either) and a significant diet effect in the distal region (HS, HP > HL). Looking at all the carbohydrases together (Fig. 2), the main diet effect seems to have been lower carbohydrase activity in house sparrows fed the HL diet, a pattern clarified in the univariate and PCA of summed hydrolysis capacity.

3.3. Summed hydrolysis capacity

Diet had a significant effect on the summed hydrolysis rates for maltase ($F_{2,17} = 8.7$, $P = 0.002$), sucrase ($F_{2,17} = 13.6$, $P < 0.001$), isomaltase ($F_{2,17} = 10.6$, $P = 0.001$), and aminopeptidase-N ($F_{2,17} = 5.3$, $P = 0.017$) (Fig. 3), but for each carbohydrase the difference was due to lower summed activity in the HL group (post-hoc Tukey comparisons in Fig. 3A–C). For summed hydrolysis capacity of aminopeptidase-N, birds fed HP diet had significantly higher values than those fed HL or HS (Fig. 3D).

PCA reduced the summed hydrolysis capacities for these four enzyme activities to two PCA axes that accounted for 86% of the variation (Table 4). The first component axis (PCA axis 1) was positively correlated with the activity of all dissaccharidases but was uncorrelated with the activity of aminopeptidase-N (Table 4). In contrast, the second component axis (PCA axis 2) was weakly correlated with sucrase and maltase activities, weakly negatively correlated with isomaltase activity, and highly correlated with the activity of aminopeptidase-N. Thus, we interpret PCA axis 1 as a dissacharidase expression axis, and PCA axis 2 as an aminopeptidase-N expression axis. As Fig.

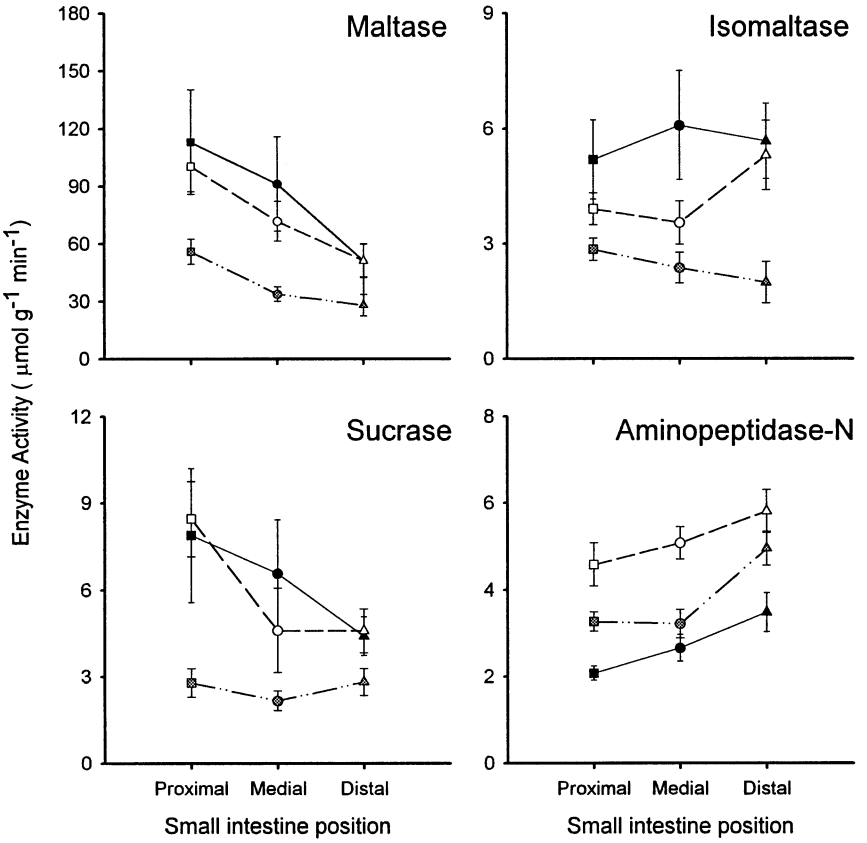

Fig. 2. Intestinal brush border enzyme activity in house sparrows. Values are coded according to the house sparrow's diet (black solid line, HS; unfilled dashed line, HP; grey, dashed and dotted line, HL) and the intestinal position the tissue was collected from (square, proximal, circle, medial, triangle, distal). Statistical comparisons are in Table 3.

4 indicates, diet had a different effect on these two axes: the HL diet led to significantly depressed disaccharidase activities (ANOVA on PCA axis 1, $F_{2,15} = 7.0$, $P < 0.005$) relative to the activities found in birds fed on HP and high sugar diets. Multiple comparisons (Tukey's HSD test) revealed no significant difference in PCA axis 1 between the HP and the HS treatments ($P > 0.1$), but significant differences between these two treatments and the HL treatment ($P < 0.05$). Diet also had a significant effect on PCA axis 2 (ANOVA, $F_{2,15} = 5.6$, $P < 0.05$). The HS diet had significantly lower values in this axis than the HP diet. The HL diet did not differ significantly from

Table 3
Repeated measures ANOVA table showing effects of diet habituation, position along the intestine, and their interaction on house sparrow intestinal brush border enzyme activity per milligramme intestine

Treatments	df[a]	Enzyme activity							
		Maltase		Sucrase		Isomaltase		Aminopeptidase-N	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Diet	2,17	3.65	0.048	4.45	0.028	7.21	0.005	13.0	<0.001
Position	2,34	20.3	<0.001	7.1	0.003	0.25	0.8	45.0	<0.001
Diet × position	4,34	1.33	0.28	1.6	0.2	1.3	0.3	2.1	0.10

[a] df, degrees of freedom in the ANOVA.

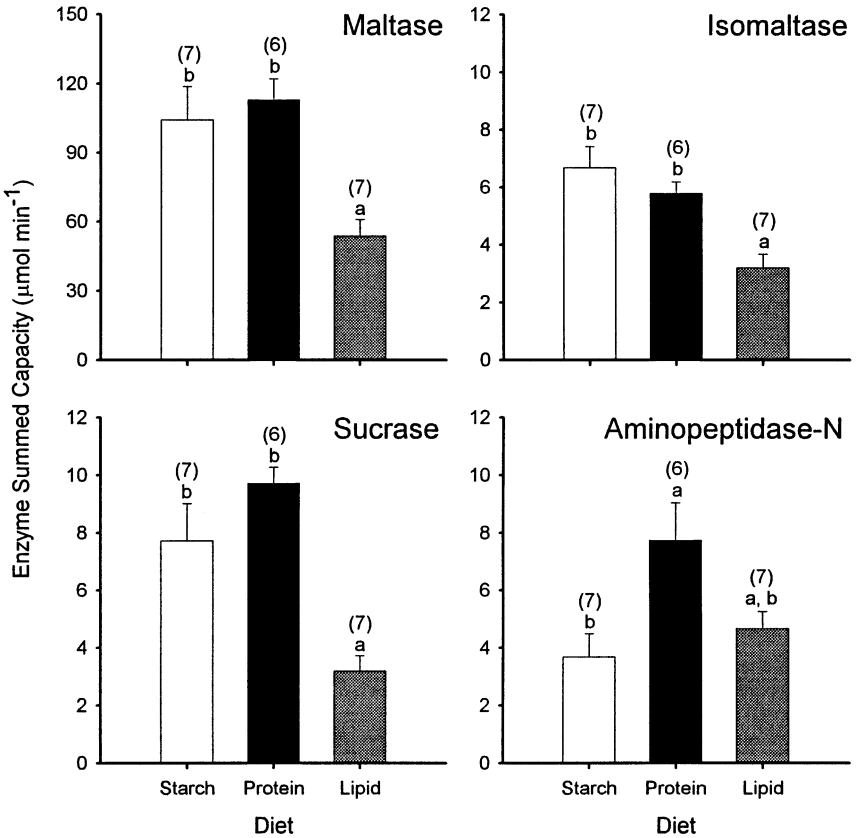

Fig. 3. Intestinal summed hydrolysis capacity in house sparrows. Bars are means ± S.E. (n = number of birds). Columns that share a letter reflect means that are not significantly different.

either the HP or the HS diet in this axis.

In summary, PCA supports the conclusions reached by the univariate analyses (Fig. 3). The HL diet appeared to have a depressing effect on carbohydrases but no significant effect on the expression of aminopeptidase-N. The expression of aminopeptidase-N, as measured by PCA on axis 2, was influenced by diet and was higher in the protein diet than in the carbohydrate diet, but the HL did not differ significantly from either the HP or the HS diets.

3.4. Relationship between maltase and sucrase

We regressed the activity per gramme of maltase against sucrase, as a partial test for sucrase-independent maltase-glucoamylase activity (Fig. 5). Intestinal maltasic activity was significantly correlated with intestinal sucrasic activity in the proximal ($F_{1,15} = 22.4$, $P < 0.001$), mid ($F_{1,15} = 6.3$, $P = 0.02$), and distal ($F_{1,14} = 225$, $P < 0.001$) intestinal regions. In no case did diet have a significant effect on this relationship (all $P > 0.18$). After removing diet as a factor, because it was not statistically significant, we computed the regression for each region by both Models I and II linear regression (Table 5). In the proximal region the intercepts were positive and statistically

Table 4
PCA axes derived from analysis of the activities of four intestinal enzyme activities in house sparrows

Principal component	1	2
Factor loadings		
Sucrase	0.98	0.53
Maltase	0.89	−0.01
Isomaltase	0.76	−0.37
Aminopeptidase-N	0.25	0.944
Eigenvalue	2.40	0.60
% Variance explained	0.60	0.26
% Cummulative variance explained	0.60	0.86

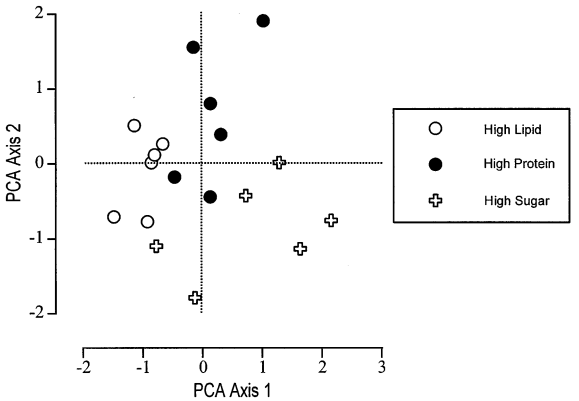

Fig. 4. Results of PCA. PCA axis 1 can be interpreted as a carbohydrase axis whereas PCA axis 2 can be interpreted as an aminopeptidase-N axis. Note that PCA separated diets according to treatments.

significant by both analyses (Table 5). For more distal intestinal regions, however, the intercepts approached the origin. Slopes were generally similar by both analyses in all three intestinal regions. The contribution of sucrase to maltasic activity in the proximal region was estimated by multiplying the slope of the maltase vs. sucrase relationship (11.1 Table 5) by the mean sucrase activity (6.1 ± 1.0 μmol min^{-1} g^{-1}), and maltase thus apparently accounted for 77.7% of the mean maltasic activity (87.3 ± 11.1 μmol min^{-1} g^{-1}). In other words, in the proximal region there was evidence for sucrase-independent maltase-glucoamylase activity that accounted for 22.3% (100–77.7) of all maltasic activity. Based on the regression statistics in the medial and distal intestinal regions (Table 5), this activity diminishes in the proximal to distal direction.

3.5. pH and kinetics

Measured pH optima was 6 for maltase, 5.5 for sucrase and 7.5 for aminopeptidase-N (Fig. 6A). Thus our use of pH 7 for measuring enzyme activity should provide estimates for 75–79% (average, 76%), 32–48% (average, 42%) and, 80–100% (average, 93%) of the maximal enzyme activity of maltase, sucrase and aminopeptidase-N, respectively. Consequently, our measurements can be corrected upward, but we do not expect the difference to affect our conclusions regarding positional and dietary effects on enzyme activities. In the range of concentration used in our study, maltase, sucrase and aminopeptidase-N exhibited

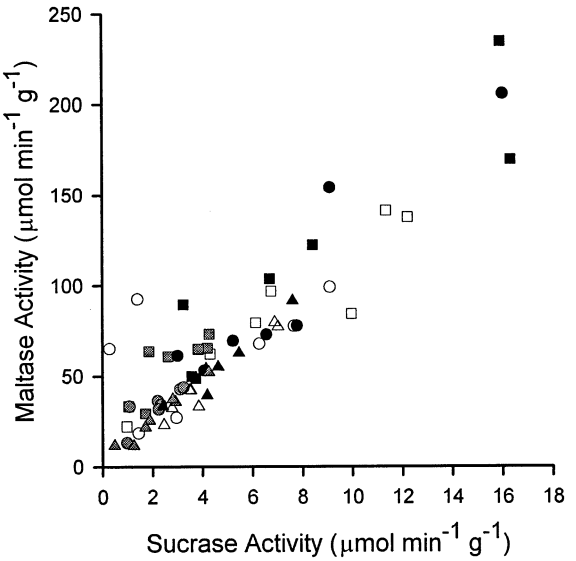

Fig. 5. The relationship between intestinal maltase activity and sucrase activity. Values are coded according to the house sparrow's diet (black, HS; unfilled, HP; grey, HL) and the intestinal position the tissue was collected from (square, proximal; circle, medial; triangle, distal). Statistical analyses of these data are in Table 5.

saturable kinetics that were adequately described by the equation relative activity $= (V_{max} \times \text{concentration})/(K_m^* + \text{concentration})$ (Fig. 6B). The correlation coefficients (r^2) for the individual birds tested ranged from 0.97 to 1. The values of K_m^* reflecting apparent affinity between enzyme and substrate, were 6.7 ± 2.57 and 3.0 ± 0.2 mM sucrase and maltase respectively ($n = 3$ for each enzyme).

4. Discussion

4.1. Pattern and magnitude of dietary modulation of intestinal enzymes

In this study, freshly captured house sparrows were habituated for 10 days to synthetic diets that varied in protein, carbohydrate and fat (Table 1). For birds on the HP diet (60.3% protein) we observed the predicted upward modulation of aminopeptidase-N activity, as compared with the lower-protein HS (12.8% protein) and HL (17.2% protein) diets (Figs. 2 and 3). The approximate doubling in aminopeptidase-N activity in house

Table 5
Regression coefficients and statistics for the relationship in Fig. 5 between maltasic and sucrasic activity in the three intestinal regions

Intestinal region	Model I regression				Model II regression			
	Intercept value	*t*-Statistic	Slope value	*t*-Statistic	Intercept value	95% C.I.[a]	Slope value	*t*-Statistic
Proximal	24.0 ± 7.2	3.35**	10.3 ± 0.9	10.9***	19.2 ± 7.3	3.9–34.4*	11.1 ± 1.0	11.6***
Mid	18.5 ± 8.2	2.26*	10.3 ± 1.4	7.43***	11.0 ± 8.5	−7–29	12.0 ± 1.4	8.3***
Distal	2.1 ± 2.4	0.9	11.1 ± 0.6	19.3***	1.1 ± 2.4	−4–6.2	11.4 ± 0.6	19.7***

* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.
[a] 95% Confidence interval.

sparrows was similar or greater in magnitude to induction of this enzyme in yellow-rumped warblers (Afik et al., 1995) and European starlings (Martinez del Rio et al., 1995) switched from low protein foods to HP foods, and to the typical increases seen in other intestinal brush border

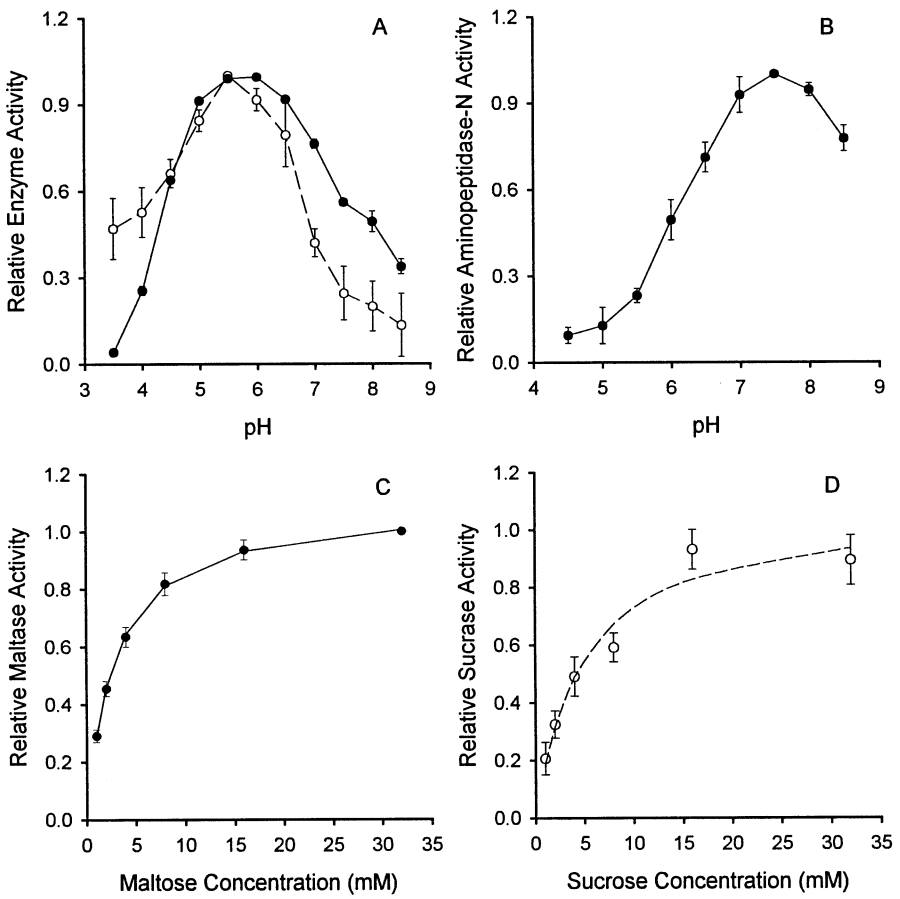

Fig. 6. The effects of pH and substrate concentration on intestinal brush border enzyme activity in house sparrows. (A) The relationship with solution pH for maltase activity (solid circles, solid line) and sucrase activity (unfilled circles, dashed line), and (B) for aminopeptidase-N activity. (C) The relationship with substrate concentration for maltase activity and (D) for sucrase activity. In all figures, each point is the mean ± S.E. for three birds, each from a different diet group. In the case of pH relationships, the lines are fitted through the points. The lines in the kinetic figures are the fits by nonlinear curve fitting to the equation relative activity $=(V_{max} \times \text{concentration})/(K_m^* \times \text{concentration})$.

digestive enzymes in mammals and birds (Karasov and Hume, 1997). Thus, these results for house sparrows seem consistent with our a priori prediction that peptidase activity would increase in direct correlation with dietary protein level.

The responses of house sparrow carbohydrases to dietary carbohydrate manipulation were not uniform. Birds eating the high carbohydrate diet (HS, 61.5% carbohydrate) had significantly higher activities for sucrase and maltase than birds eating one of the lower carbohydrate diets (HL, 18.9% carbohydrate) but not the other (HP, 14% carbohydrate). For isomaltase, the high carbohydrate birds had significantly higher activities than both other diet groups, but only by a small amount in the case of the HS vs. HP comparison (Fig. 3). It does not seem likely that our 10-day habituation period was not long enough for carbohydrase modulation, because this occurs within 1 day in rodents (Cezard et al., 1983; Goda et al., 1983) and within 4 days in chicken (Biviano et al., 1993).

Many species more than double carbohydrase activity on high carbohydrate diets (Karasov and Hume, 1997). House sparrows do not, however, based on a power analysis (Zar, 1984) of our data. Despite our relatively small sample sizes and large variances, and using an α value of 0.05, we had high probabilities of detecting a doubling in sparrow maltase activity had it actually existed (e.g. for summed maltase $P = 1 - \beta > 0.99$, for maltase in the proximal region $1 - \beta = 0.8$). Thus, our measurements lend us a high degree of certainty that house sparrows do not substantially increase carbohydrase activity when eating high carbohydrate diet. Interestingly, this was also the conclusion of our survey of pancreatic enzymes (Caviedes-Vidal and Karasov, 1995).

Because all enzyme activities were low in birds on the HL diet, we cannot rule out the possibility that high dietary fat simply has a depressing effect on the intestinal enzymes that we measured. Interestingly, the earlier finding in starlings (Martinez del Rio et al., 1995) of lower enzyme activities in those fed insects (high fat, HP, low carbohydrate) compared with those fed synthetic carbohydrate free diet (low fat, HP, no carbohydrate) are also consistent with the idea that dietary fat has a depressing effect on brush-border enzyme activities. This possibility merits further study.

4.2. Dietary, physiological, and phylogenetic correlates of intestinal enzyme levels and intestinal lability

We have concluded that house sparrows do not show the expected positive correlation between intestinal carbohydrase activity and dietary carbohydrate. None of the passerine species studied so far show the pattern (Table 6). Future studies might test whether results differ under more natural, energy-stressful conditions. It might be argued that the passerine species studied to date make poor test cases for testing the hypothetical correlation between omnivory and digestive lability. For example, there is no doubt that house sparrows do eat arthropods as well as seeds in the wild, but seasonal analysis of wild house sparrows in North America show them to be overwhelmingly granivorous year-round (Martin et al., 1951). Arguably they should be considered specialized granivores and be expected to maintain consistently high levels of intestinal carbohydrases. In a similar vein, both starlings and yellow-rumped warblers might best be considered specialized insectivore/frugivores that only consume grains in the wild during crucial periods of food shortage (Martinez del Rio et al., 1995), in which case they might be expected to maintain consistently low levels of intestinal carbohydrases. Tests for modulation in a passerine that shows clearer temporal reliance in the wild on seeds vs. insects would be most instructive. However, even the chingolo (*Zonotrichia capensis*), the granivore whose consumption of insect in the wild varies seasonally from 11 to 37% of diet, did not exhibit higher carbohydrase activity when eating higher carbohydrate diet in both field and laboratory (Sabat et al., 1998). Thus, the studies so far with passerine granivores cast doubt on the hypothetical association between digestive lability and omnivory. Surely, the application of this idea to wild species based on studies in two domesticated species (chicken and turkey) is premature. The enigmatic finding that passerine dissaccharidases exhibit relative constancy, but aminopeptidase-N shows lability, further underscores this cautionary note. Sabat et al. (1998) suggest that the difference might relate to different time courses for enzymatic regulation.

Table 6
Summary of patterns of regulation of intestinal digestive enzymes in birds[a]

Species/GI tract	Diet	Body mass (g)	Response to higher dietary protein — increase peptidase activity?	Response to higher dietary carbohydrate — increase maltase activity?	Source
Passerine/lack cecum					
Yellow-rumped warbler	Fruit, insects	12	Yes	No	Afik et al., 1995
Pine warbler	Insects	12	Yes	No	Caviedes-Vidal et al., 1994
House sparrow	Seeds, insects	26	Yes	No	This study
Chingolo	Seeds, insects	22	Yes	No	Sabat et al., 1998
Common diuca	Seeds, insects	33	Yes	No	Sabat et al., 1998
European starling	Insects, fruit	76	Yes	No	Martinez del Rio et al., 1995
Non-passerine/ cecal digesters					
Chicken	Seeds, insects	3000		Yes	Biviano et al., 1993
Turkey	Seeds, insects	1000		Yes	Sell et al., 1989
Snow goose	Plants, insects	2000	No	Yes	Ciminari et al., 1999
Canada goose	Plants, insects	3500	No	Yes	Ciminari et al., 1998

[a] Empty cells indicate no data available.

What about the aforementioned expected correlation between intestinal carbohydrase levels and ability to rely on high carbohydrate seeds? Here, again, we do not see emerging the expected pattern in the variation among species that a certain level of maltase activity is permissive for eating and maintaining energy balance on foods high in starch (Martinez del Rio et al., 1995). Of the five passerine species discussed above, for example, the house sparrow had higher maltase capacity normalized to metabolic live mass (9.1 μmol min^{-1} $g^{-3/4}$) than either European starling (2.08; Martinez del Rio et al., 1995), yellow-rumped warbler (5.6; Afik et al., 1995), chingolo (2.25; Sabat et al., 1998) or common diuca (*Diuca diuca*) (2.74; Sabat et al., 1998). The house sparrow, chingolo, and common diuca can thrive on a HS diet whereas the warbler cannot (Afik et al., 1995), and the starling seems to digest grain inefficiently (Thompson and Grant, 1968; Coleman, 1974) and can fail to meet energy demands when feeding on it (Feare and McGinnity, 1986). Thus, there is no correspondence between ability to rely on HS seeds and intestinal maltasic capacity. We do not think that this comparison is confounded by species differences in pH sensitivity or maltase kinetics. All the assays were performed similarly at a pH (Caviedes-Vidal and Karasov, 1996) slightly above the optima for all the species (all the species studied so far have pH optima for maltase in the same range, 5.5–6; Martinez del Rio, 1990; Afik et al., 1995; Sabat et al., 1998, this study) and at a maltose concentration (56 mM) that is somewhat saturating relative to the maltase K_m^* of all the species (2–21 mM). Possibly, intestinal carbohydrases exist in considerable excess of need, and the limiting step in starch utilization lies elsewhere.

It is interesting that the carbohydrase capacity of the SI is so much larger ($10\times$) than the peptidase capacity (Fig. 3), even though the differences in dietary substrate level are not this high. This pattern is apparent in other species as well (Afik et al., 1995; Sabat et al., 1998). Perhaps for intestinal and pancreatic peptidases there is a severe cost of excess production of enzyme-rapid degradation of other digestive enzymes. A thorough analysis of the relation between enzyme

capacities and nutrient loads, including whether low enzyme activity limits reliance on starchy foods, may require additional consideration of the interaction of pancreatic and intestinal enzyme activities with digesta retention (time a meal spends in the gut) and nutrient absorption.

One other pattern also underscores how an integrative framework may be necessary to understand patterns of digestive regulation. Though none of the passerines show the expected positive correlation between intestinal carboydrases and dietary carbohydrate, they all show the expected positive correlation between peptidase and dietary protein (Table 6). In notable contrast to these passerine species, all the nonpasserine species studied to date do exhibit the expected correlation for the intestinal carbohydrases, but not for peptidase (Table 6). Thus, we cannot reject the hypothesis that the pattern of digestive lability among avian species has a phylogenetic component, or perhaps the patterns relate functionally to features correlated with the phylogenetic difference such as body size or the presence/absence of a functional cecum. For example, assuming that natural selection has favored a microbial fermentation in the cecum because of certain functional advantages (and many are proposed Vispo and Karasov, 1997), perhaps it also favored correlated small intestinal features that somehow are significant for supporting that microbial fermentation.

One such feature might be permitting small intestinal escape of amino nitrogen as peptides to the cecum, to support microbial growth. In contrast, the SI of the passerine has perhaps been selected to extract the maximum available amino nitrogen rather than excreting it as waste. Final nutrient extraction in birds with a functional cecum may occur in that organ and, indeed, cecal active sugar and amino acid transport have been described (Obst and Diamond, 1989). We might also predict the presence of peptidase activity in this organ as well, though this remains to be tested.

What about possible correlated responses between intestinal hydrolysis and transport? Interestingly, in house sparrows and yellow-rumped warblers high dietary protein induced intestinal amino acid active transport in parallel with the induction of the peptidase activity. Possible coordination in the regulation of intestinal hydrolysis and absorption has not been tested for in nonpasserines. In the only nonpasserine tested so far, the northern bobwhite, amino acid transport was not enhanced on a HP diet (Karasov et al., 1996). It would be interesting if the apparent differences between the groups in Table 6 in patterns of regulation of intestinal hydrolase activity were mirrored by differences in the pattern of regulation of transport activity. Thus, we especially look forward to additional studies of regulation of digestive biochemistry, both hydrolysis and absorption, in nonpasserine species.

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