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ORIGINAL PAPER

Modulation of digestive enzyme activities in the avian digestive tract in relation to diet composition and quality

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Abstract In nature, birds are faced with variable food sources that may differ in composition (protein vs. carbohydrates) and quality (highly digestible material vs. indigestible fiber). Studies in passerine birds and some commercial poultry demonstrate that the gastrointestinal tract can respond to varying diet composition and quality by changing morphology and/or activities of digestive enzymes. However, studies in additional avian species are warranted to understand generalities of these trends. We first fed juvenile mallards (*Anas platyrhynchos*), chickens (*Gallus gallus*), and quails (*Coturnix coturnix*) on either high-carbohydrate or high-protein diets. For the most part, birds fed the high-carbohydrate diet had higher small intestinal and cecal disaccharidase activities (maltase and sucrase). However, only mallards exhibited higher small intestinal aminopeptidase-N (APN) activities when fed the high-protein diet. These results differ from passerine birds, which largely modulate small intestinal proteases, but not disaccharidases. In another trial, we fed Canada geese (*Branta canadensis*) diets that varied in both their protein and fiber concentrations for approximately 3.5 months. Birds fed the high-fiber diets had significantly longer small intestines and caeca compared to those fed low-fiber diets. Additionally, geese fed the high-fiber diets exhibited lower mass-specific activities of small intestinal sucrase, and higher activities of APN when summed across the small intestine and ceca. Similar to the avian species above, geese fed the high-protein diets did not exhibit flexibility in their small intestinal APN activities. Overall, these experiments demonstrate that responsiveness of the avian digestive tract to diet composition may have phylogenetic or ecological constraints. Studies on other avian taxa are needed to understand these patterns.

Keywords Birds · Digestion · Digestive physiology · Fiber · Maltase

Introduction

The avian gastrointestinal tract is a dynamic and multifunctional organ (McWhorter et al. 2009). One important function of the digestion and absorption of ingested food is to provide the nutrients and energy required for birds to survive and reproduce. In nature, birds experience

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considerable variation in the composition and quality of food that they consume (McWhorter et al. 2009). For example, changes in the abundance of arthropods and fruits may impact the relative amounts of dietary protein, fat, and carbohydrates ingested by birds over time (Parrish 2000) and the digestion and absorption of these macronutrients (Karasov 1990). Additionally, plant material can vary widely in fiber content, presenting nutritional challenges to herbivorous birds (Richman et al. 2015). Given the likelihood of variable diets, it has been hypothesized that the avian gastrointestinal tract should be responsive to changes in diet composition and quality such that optimal digestion is maintained (McWhorter et al. 2009).

Modulation of digestive enzyme activities is one mechanism by which the gastrointestinal tract can respond to changes in food composition and quality. Specifically, the adaptive modulation hypothesis suggests that activities of digestive enzymes should be matched to substrate levels in an animal's diet so as to fully digest available nutrients while avoiding synthesis and membrane space costs of unneeded enzymes (Diamond and Hammond 1992; Diamond 1993). To date, most studies investigating the effects of diet composition on small intestinal digestive enzyme activities in birds have focused on passerines (Afik et al. 1995; Brzęk et al. 2010b; Caviedes-Vidal et al. 2000; Levey et al. 1999; Maldonado et al. 2011; Martínez del Rio et al. 1995; Sabat et al. 1998). Collectively, these studies demonstrate that many adult passerines are able to modulate small intestinal proteases, but not carbohydrases (though see Levey et al. 1999). An additional study conducted in pigeons seemed to match the trend observed in passerines (Ciminari et al. 2005). Conversely, limited work in commercial poultry species demonstrates that chickens and turkeys are able to modulate small intestinal carbohydrase activities (Sell et al. 1989; Biviano et al. 1993; Siddons 1972), though studies in more species are warranted. Additionally, these previous works have only focused on regulation of digestive enzymes in the small intestine. However, some birds absorb substantial amounts of nutrients in their caeca (Obst and Diamond 1989; McWilliams 1999), and thus enzymatic digestion and modulation may be occurring here as well. Studies of additional avian species and other parts of the digestive tract are needed to better understand the possible ecological or evolutionary trends underlying the capacity for modulation of digestive enzyme activities.

Variation in diet quality has also been demonstrated to change the structure and function of the gastrointestinal tract. Addition of fiber to diets largely results in enlargement of digestive tissues, presumably to increase retention time and digestion of the diet (Savory and Gentle 1976; Williamson et al. 2014; Kehoe et al. 1988). Investigations on the effects of dietary quality on digestive enzymes in birds are scarce and are limited to studies using mannan oligosaccharides in commercial poultry, where these fibers induce intestinal protease and carbohydrase activities (Yang et al. 2007; Iji et al. 2001). These results are consistent with some previous studies in mammals (Chinery et al. 1992; Hedemann et al. 2006), though other studies have demonstrated that addition of fiber to the diet lowers digestive enzyme activities (Khokhar 1994; Thomsen and Tasman-Jones 1982). These conflicting results could be due to the fact that different fiber components (e.g., pectin vs. cellulose) can have differential effects on enzyme activities (Thomsen and Tasman-Jones 1982). Therefore, to better understand the effects of fiber on avian digestive enzymes, studies should be conducted in herbivorous species using complex fiber sources (whole plant material rather than isolated components) to better replicate the diets these animals face in nature.

Here, we present a series of feeding trials maintaining several bird species on diets of varying composition and quality. To study the effect of diet composition, we maintained young mallard ducks (*Anas platyrhynchos*), domestic chickens (*Gallus gallus*), and Common quail (*Coturnix coturnix*) on diets either high in starch or high in protein. In accordance with the adaptive modulation hypothesis, we predicted that carbohydrase activities would be higher in birds fed the high-starch diet, while peptidase activities would be higher in birds fed the high-protein diet. To study the effects of diet quality, we conducted a feeding trial on young Canada geese (*Branta canadensis*), which are largely herbivorous (McLandress and Raveling 1981). Here, we varied both diet composition (high protein vs. low protein) and fiber (high vs. low) to mimic variation in these components that can occur in natural forage (Richman et al. 2015). Consistent with the predictions above, we expected birds fed the high-protein diets to have higher peptidase activities. Additionally, we expected birds fed the high-fiber diets to have higher enzyme activities (both carbohydrase and peptidase), as has been demonstrated in domestic chickens and some mammals (Chinery et al. 1992; Hedemann et al. 2006; Iji et al. 2001).

Materials and methods

We investigated the effect of diet composition and quality on small intestinal and cecal digestive enzyme activities of birds by conducting several feeding trials at the University of San Luis, Argentina and University of Wisconsin, Madison, USA. In general, birds in all trials were maintained under laboratory conditions and fed synthetic diets of different composition. In the first series of experiments, we varied the percentage of carbohydrates (starch) and protein by at least four times (>400 %). In a second series of experiments, we also varied fiber content by about 50 %.

Diet compositions for all trials are given in Tables 1 and 2. Appropriate animal ethics committees approved all procedures used in the described trials. All goose eggs were collected under Scientific Permit from Environment Canada and followed animal care and use guidelines of the University of Wisconsin-Madison. Animal maintenance, trial protocols and sample collection protocols were approved by the Animal Care Committee of the Facultad de Química, Bioquímica y Farmacia of the Universidad Nacional de San Luis. At the time of these experiments, this committee was not issuing protocol numbers.

Table 1 Composition of the synthetic diets fed to chickens, quails and ducks

Components (% w/w)	High starch	High protein
Milk protein concentrate[a]	12.3	60.3
Corn oil[b]	8	8
Corn starch[c]	62	14
Salt mixture	5.5	5.5
Sodium bicarbonate	1	1
Choline chloride	0.2	0.2
Vitamin mixture	1	1
Cellulose	5	5
Ground silica sand	5	5

Composition is based on percent dry matter

[a] Milk protein concentrate (Milkaut S.A., Argentina)

[b] Aceite Mazola (Aceitera General Deheza S.A., Argentina)

[c] Maizena Cornstarch (Unilever S.A., Argentina)

Trial 1: effects of protein and starch on enzyme activities

Young (1–3 days old) mallards, broiler chickens, and quails were purchased from commercial shops (Forrajera San Luis, San Luis, Argentina). Birds were housed in individual cages (0.60 × 0.45 × 0.43 m) in our animal facility under constant environmental conditions (room temperature 25.2 ± 0.3 °C, relative humidity 50 ± 9 %, photoperiod 14:10 h light–dark) with ad libitum water and food. Before the start of our experiment (described below), birds were raised on commercial diet (Ganave® Alimentos Pilar S.A., Argentina; composition: protein 20 %, fat 4 %, crude fiber 3.9 %).

Beginning at ages 80 days (mallards), 42 days (chickens), and 45 days (quail), birds were fed one of two diets (see Table 1) designated High Starch (HS) or High Protein (HP). No samples of these diets remain for chemical analysis, but based on the chemical composition reported by the manufacturers of each component, the HS diet can be estimated to have contained approximately 10.5 % protein and 52.8 % carbohydrate (mainly starch), and the HP diet contained approximately 49.7 % protein (mainly unhydrolyzed casein) and 11.9 % carbohydrate. The mallards and chickens were fed these diets for 15 days, and the quail for 30 days. All three species were juveniles during our

Table 2 Composition (% dry mass) of four synthetic diets fed to geese that were a factorial combination of two levels of dietary protein and two levels of fiber

	High protein		Low protein	
	High fiber (HP–HF)	Low fiber (HP–LF)	High fiber (LP–HF)	Low fiber (LP–LF)
Component composition (% dry mass)				
Beet sugar	1	9.2	19.4	32.7
Soybean meal	35.2	25.8	13.6	1.3
Grass hay	15	21	15	29
Wheat middlings	1	35	1	35
Oat hulls	46.8	8	50	1
Nutritional composition (% dry mass)				
Protein	18.1 ± 0.6[a]	18.3 ± 0.7[a]	9.5 ± 0.5[b]	10.9 ± 1.4[c]
Neutral detergent fiber	46.3 ± 3.6[a]	32.1 ± 2.0[b]	44.3 ± 2.5[a]	30.7 ± 2.8[b]
Acid detergent fiber	25.2 ± 2.1[a]	16.1 ± 1.1[b]	23.3 ± 1.8[a]	15.7 ± 1.1[b]
Lignin	2.8 ± 0.2	2.5 ± 0.3	2.7 ± 0.2	2.1 ± 0.3
Lipids	1.5 ± 0.2	2.8 ± 0.2	1.3 ± 0.1	2.2 ± 0.2
Soluble carbohydrates	34.1	46.9	44.9	56.2

See text for description of methods used to determine nutritional composition. All diets contained 1 % vitamin mix. Soluble carbohydrate content was estimated by subtraction (% soluble carbohydrates = 100 % − % protein − % fiber/lignin − % lipid). It should be noted that this method does not account for variation in percent ash content of diets. Measurements of nutritional composition with different superscripts are significantly different ($P < 0.05$ by Fischer's multiple comparison test) experiment. The starting points for the experiments were at or near time of slaughter for these species in the poultry industry (Baeza et al. 2012; Camci et al. 2002). While the length of the experiment varied slightly across species (15 days for mallards and chickens and 30 days for quail), it is unlikely that these differential lengths influenced our interspecies comparisons of the capacity to modulate intestinal enzymes, given that enzyme levels in turkey poults respond within 3 days of diet shifts (Sell et al. 1989). Moreover, we were not particularly interested in differential adjustments across species. Our data are compared statistically within a species, but only qualitatively across species (e.g., is modulation exhibited or not?).

Sample sizes were (HS and HP diets, respectively): 7 and 7 for mallards; 7 and 6 for chickens; 8 and 7 for quails. Cornstarch and milk protein were used as sources of carbohydrate and protein, respectively. These synthetic diets were formulated according to previous experiments (Caviedes-Vidal et al. 2000; Murphy and King 1982). Data from the chicken samples have been published previously (Ciminari et al. 2014); however, we present the data again here for a holistic comparison of several avian species.

Trial 2: effects of protein and fiber on enzymes activities

Canada goose eggs were collected in May of 1995, on Akimiski Island (53°10′N, 81°40′W), James Bay, Canada (see Richman et al. 2015 for details about egg collection and gosling rearing). All eggs were collected under Canadian Wildlife Service Scientific Permit. Eggs were transferred to the laboratory in Madison, Wisconsin where they were incubated until hatching at 36 ± 1 °C, with 60 % relative humidity, under a controlled light cycle (12:12 h light–dark cycle). Hatchling Canada geese were assigned to one of four groups that were then fed diets composed of either high or low protein and high or low fiber (Table 2). As described in more detail in Richman et al. (2015), goslings were reared communally by diet group for the first week post-hatch in round cardboard enclosures (46 cm tall, 2.5 m diameter) and thereafter were housed individually in cages (46 × 74 × 71 cm, 2.5 cm^2 mesh, 20 cages per rack). Enclosures and cage racks were housed indoors and maintained at ambient temperature (22 °C) with a 12:12 h light–dark cycle. Raising of goslings followed Animal Care and Use guidelines of the University of Wisconsin-Madison.

The food and water, and oyster shell and sand for grit were offered ad libitum. Fiber content was altered by varying the amount of different grass components in the diet (orchard grass, oat hulls, and wheat middlings), thus using complex fiber sources that are similar to natural forage consumed by young geese in arctic and subarctic breeding habitat (Richman et al. 2015). The birds used here were part of a larger experiment investigating the effects of fiber and protein on growth and survival of Canada geese (Richman et al. 2015). In the full experiment, some birds were switched between diets to consolidate groups. Animals that underwent diet switches in the larger experiment were not included in the present study so as to avoid potential lasting effects of previous diets. Thus, the sample sizes for the current study are: High Protein–High Fiber (HP–HF): 4; High Protein–Low Fiber (HP–LF): 4; Low Protein–High Fiber (LP–HF): 7; Low Protein–Low Fiber (LP–LF): 7. At the time of dissection, animals were 96.6 ± 0.7 days old, with no significant differences in age across groups.

Table 3 Summary of statistics from ANOVAs of mass-specific intestinal activities in various bird species

	Mallard		Chicken		Quail	
	F	*P*	*F*	*P*	*F*	*P*
Maltase						
Diet	3.19	0.09	5.26	**0.045**	31.07	**0.0001**
Region	57.99	**<0.0001**	55.65*	**<0.0001***	2.73	0.11
Diet × region	1.55	0.26	0.59*	0.52*	2.98	0.051
Sucrase						
Diet	15.86	**0.002**	2.60	0.14	5.18	**0.042**
Region	31.18	**<0.0001**	89.88	**<0.0001**	13.38	**0.001**
Diet × region	0.12	0.86	0.73	0.51	0.01	0.98
Aminopeptidase-N						
Diet	31.42	**0.0001**	0.34	0.57	0.40	0.54
Region	316.82*	**<0.0001***	22.06	**0.0003**	1.89	0.19
Diet × region	40.81*	**<0.0001***	0.82	0.47	3.09	0.08

Statistics with asterisks did not meet the assumption of sphericity, and thus a Huynh–Feldt correction was applied to the statistics

Significant values are in bold

Diet samples were dried, homogenized, and then nutrient composition was directly measured using standard methods (see Richman et al. 2015 for further details). Total nitrogen was measured using the Kjeldahl method (Lang 1958) at the University of Wisconsin Soil and Plant Analysis Lab. Kjeldahl nitrogen content was multiplied by 6.25 to estimate total protein concentration (Karasov and Martinez del Rio 2007). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin contents were measured using established methods (Goering and Van Soest 1970; Undersander et al. 1993). Lipids were extracted using petroleum ether solvent refluxed for 6 h in a Goldfisch extraction apparatus (Dobush et al. 1985). Last, soluble carbohydrate content was estimated by subtraction (% soluble carbohydrates = 100 % − % protein − % fiber/lignin − % lipid). It should be noted that this method does not account for variation in percent ash content of diets.

Sample collection

Briefly, birds were killed by cervical dislocation, and the entire gastrointestinal tract was immediately removed and chilled in ice-cold saline solution (350 mmol). The small intestine was separated from the rest of the gastrointestinal tract and extraneous tissues were removed. The contents were flushed out with cold saline solution, and the small intestine was measured for length and then blotted dry and weighed.

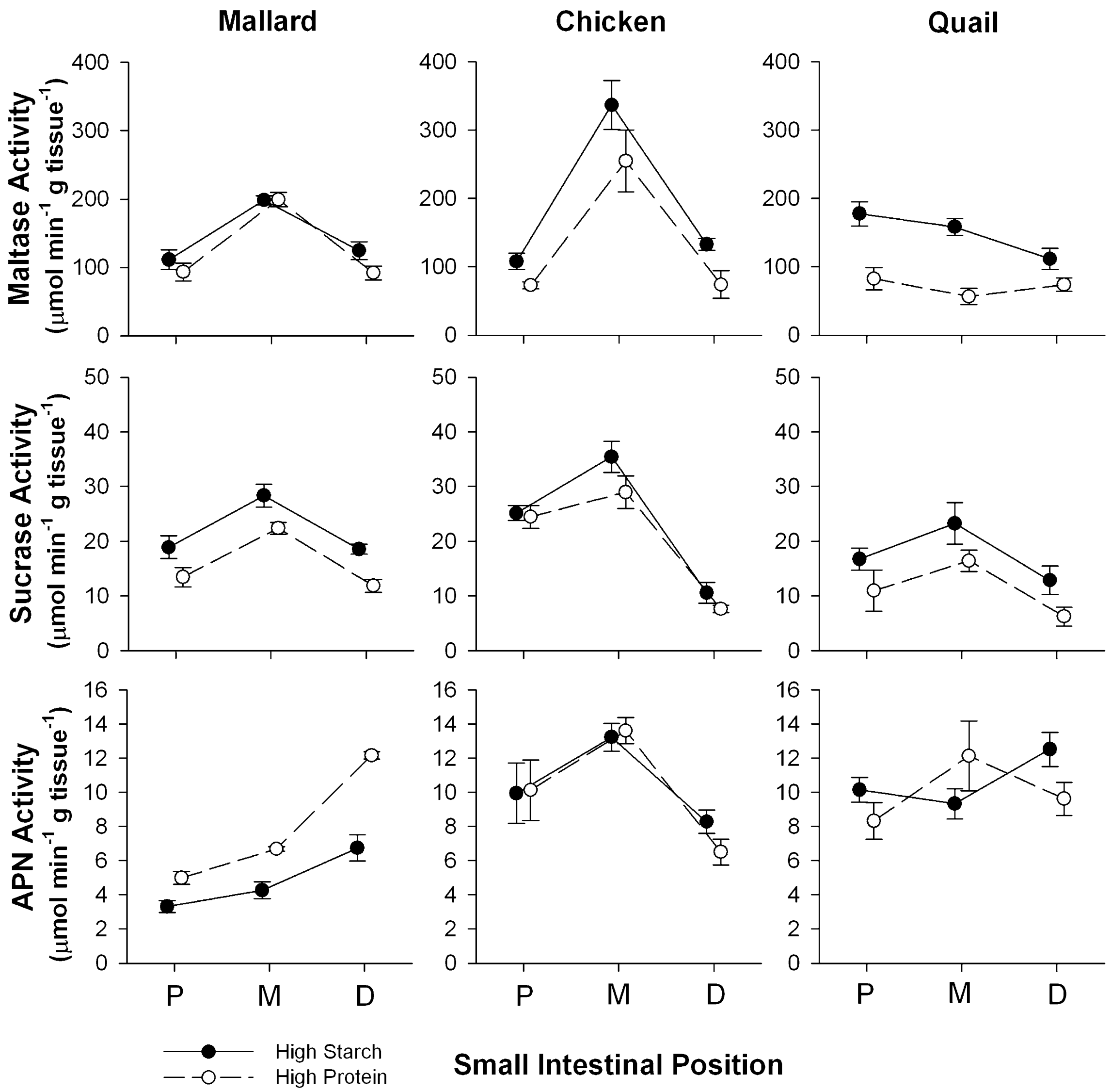

Fig. 1 Mean (±SEM) mass-specific enzyme activities from intestinal tissues of various bird species. *P* proximal small intestine, *M* middle small intestine, *D* distal small intestine. Sample sizes are as follows: mallards (high starch, 7; high protein, 7), chickens (high starch, 7; high protein, 7), quail (high starch, 8; high protein, 6). Data from the chicken samples have been published previously (Ciminari et al. 2014)

Immediately thereafter, 10-cm-long segments from the proximal, medial and distal parts (relative to the pylorus) of the small intestine were collected. Pieces were weighed, rapidly frozen, and stored at −80 °C. The two caeca were processed exactly as the small intestines and were divided into three portions: the neck section (closest to the small intestine; C1), the middle section (C2) and the end section (the fundus; C3).

Enzyme assays

Small intestinal and caeca segments were thawed at 4 °C and homogenized for 30 s using a Fisher Scientific homogenizer in 350 mM mannitol in 1 mM Hepes/KOH (pH 7), using 10 ml per gram tissue. The activity of membrane-bound enzymes was measured in whole tissue homogenates (Martínez del Rio 1990).

Disaccharidase activities (maltase and sucrase) were determined using a colorimetric method (Dahlqvist 1984; Martínez del Rio 1990). Briefly, aliquots of 40 µl of appropriately diluted tissue homogenate were incubated with 40 µl of 56 mM sugar (maltose or sucrose) solutions in 0.1 M maleate/NaOH, pH 6.5. After a 10-min incubation at 40 °C, we added 1 ml of Glicemia Enzimática work reagent (glucose oxidase 1000 U/ml, peroxidase 120 U/ml, 26 mM/l 4-aminophenazone, 55 M/l phenol, 0.92 M/l Tris buffer, pH 7.4; Wiener® Laboratorios, Argentina). The mix was allowed to stand at room temperature and after 20 min the absorbance was read at 505 nm in a Spectronic 21D spectrophotometer. Enzyme activity was determined using a glucose standard curve.

Aminopeptidase-N activity was assayed using L-alanine-*p*-nitroanilide as a substrate (Maroux et al. 1973). Aliquots of 10 µl of the tissue homogenate were added to 1 ml of 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). The reaction was incubated for 10 min at 40 °C and then stopped with 3 ml of chilled 2 M acetic acid. Absorbance was measured at 384 nm, and activity was determined using a *p*-nitroanilide standard curve. All enzyme assays for each sample were run with technical duplicates, and activity was averaged for each sample. Additionally, we always corrected against a blank tube where substrates were not added until the completion of the assay, to prevent the reaction from occurring.

We measured pH optima of maltase, sucrase, and aminopeptidase-N for all birds from Trial 1, using both small intestinal and cecal samples. The above assays were performed using a 0.05 M maleate:NaOH buffer system with increasing pH in increments of 0.5, ranging from 5 to 7.5 for the disaccharidases, and a 0.1 M sodium phosphate buffer with pH ranging from 5.5 to 8.5 for aminopeptidase-N. The pH optima were determined as the pH with the highest measured enzyme activities.

Mass-specific enzyme activities were calculated as activity per unit small intestinal (or caecal) wet mass (μmol min^{-1} g wet tissue^{-1}). The summed hydrolysis activity of the entire small intestine and caeca was calculated by multiplying activity per gram tissue in each region by 1/3 of the small intestine and caeca total mass, and summed over the three regions.

Data analysis

Within each species, body mass was compared across treatments using *t* tests (Trial 1) or ANOVAs (Trial 2). Organ

Table 4 Summary of statistics from ANOVAs of mass-specific cecal activities in various bird species

	Mallard		Chicken		Quail	
	F	*P*	*F*	*P*	*F*	*P*
Maltase						
Diet	4.49	**0.05**	0.49	0.50	3.24	0.09
Region	1.98	0.18	5.25*	**0.03***	1.87	0.20
Diet × region	1.08	0.37	0.26*	0.68*	0.13	0.87
Sucrase						
Diet	6.17	**0.03**	0.63	0.44	92.81	**<0.0001**
Region	0.43	0.66	4.56*	**0.04***	2.96	0.09
Diet × region	0.46	0.64	0.06*	0.88*	1.19	0.34
Aminopeptidase-N						
Diet	0.86	0.37	0.47	0.50	0.88	0.37
Region	27.02	**<0.0001**	6.60	**0.01**	4.89	**0.03**
Diet × region	2.16	0.16	0.83	0.46	0.91	0.43

Statistics with asterisks did not meet the assumption of sphericity, and thus a Huynh–Feldt correction was applied to the statistics. Data from the chicken samples have been published previously (Ciminari et al. 2014)

Significant values are in bold

masses and lengths were compared using ANCOVA, with body mass as a covariate. Repeated measures ANOVA was used to examine the effect of the diets and gut region on mass-specific enzyme activities. Here, gut region was used as repeated measure and diet was included as an experimental variable. Data were tested for sphericity, and if the data failed the assumption of sphericity, a Huynh–Feldt correction was applied to the statistics (Roberts and Russo 1999). Summed activities were compared using ANCOVA incorporating body mass as a covariate. All statistical analyses were conducted in JMP 12.0 with $\alpha = 0.05$. We present values as mean ± SEM.

Results

Trial 1: effect of starch and protein on enzyme activities

Body mass and gut morphology

Diet did not affect body masses of mallard (2517.7 ± 68.2 g; diet effect: $P = 0.83$) or quail (174.5 ± 7.1 g; diet effect: $P = 0.32$). Chickens fed the HS diet were roughly 27.5 % smaller than chickens fed the HP diet (HP 1771.5 ± 84.9 g; HS 1283.1 ± 67.9; diet effect: $P = 0.001$).

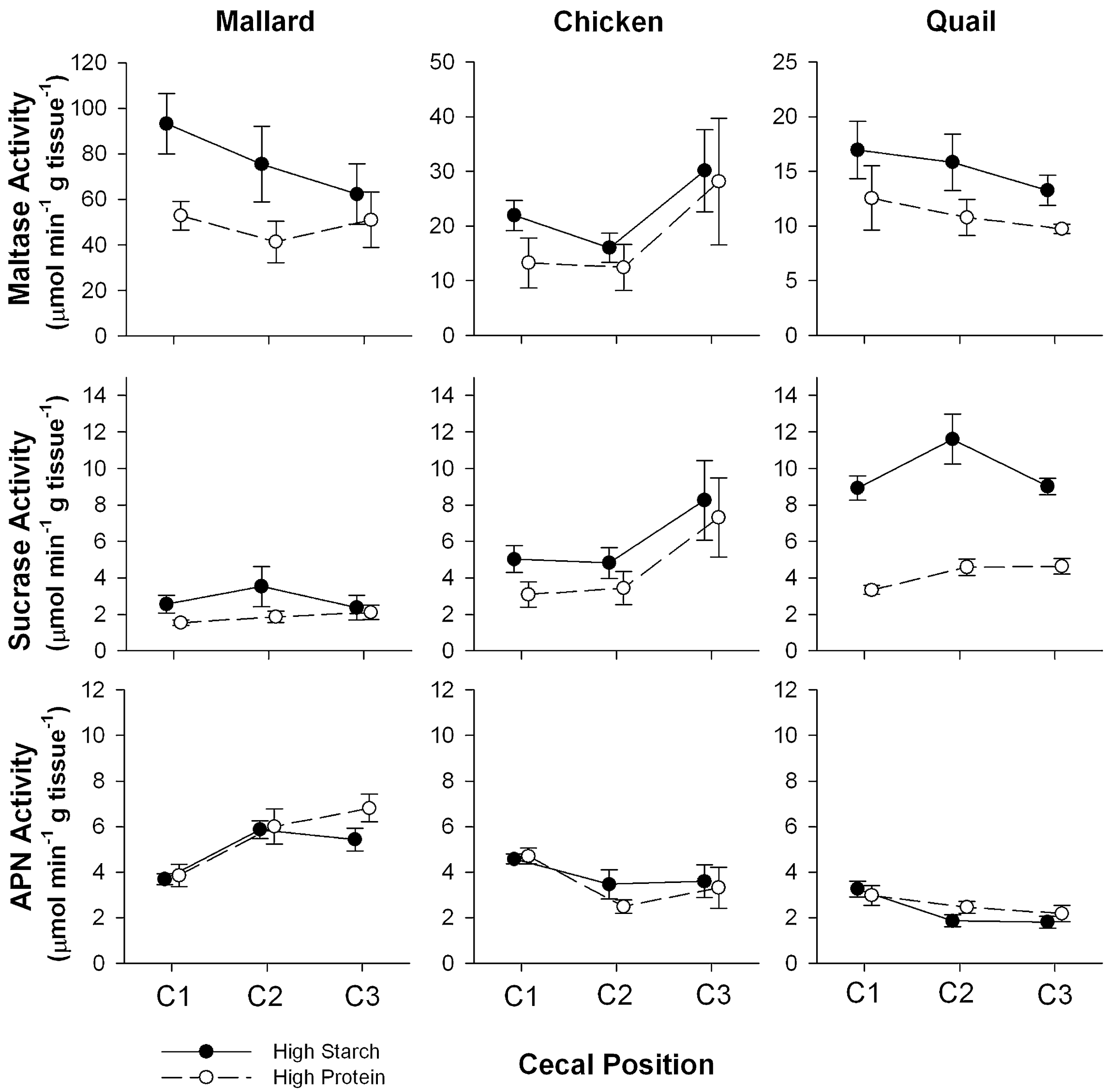

Fig. 2 Mean (±SEM) mass-specific enzyme activities from cecal tissues of various bird species. *C1* section closest to the small intestine, *C2* middle cecum, *C3* the end section, or the fundus. Sample sizes are as follows: mallards (high starch, 7; high protein, 7), chickens (high starch, 7; high protein, 7), quail (high starch, 8; high protein, 6). Data from the chicken samples have been published previously (Ciminari et al. 2014)

Diet did not impact small intestine mass or length in any species when including body mass as a covariate (ANCOVA—diet effect: $P > 0.05$ for all species). Similarly, diet did not affect the mass or length of caeca in chickens or quails. Mallards fed the HS diet had significantly larger caeca in terms of mass (ANCOVA with body mass as a covariate—diet effect: $P = 0.038$), but not length ($P = 0.26$).

Tissue mass-specific enzyme activities

The responses of mass-specific enzyme activities to diet composition varied across enzymes, avian species, and gut regions. Mass-specific small intestinal maltase activity was significantly higher in chickens and quails fed the HS diet, with no difference in mallards (Table 3; Fig. 1). Small intestinal maltase activity varied significantly across regions in mallards and chickens, but not quail (Table 3; Fig. 1). There were no significant diet × region effects on small intestinal maltase activity (Table 3). Mass-specific cecal activities of maltase were significantly higher in HS-fed mallards (Table 4; Fig. 2). There was no difference in cecal maltase activity in quail or chickens (Table 4; Fig. 2). Maltase activities differed significantly across cecal regions in chickens, but not other bird species, and no diet × region effects were detected for cecal maltase in any species (Table 4). The pH optimum of maltase for all three bird species, both diets, and both gut regions (small intestinal and cecal) was 6.5.

Mass-specific small intestinal sucrase activity was significantly higher in mallards and quails fed the HS diet, though no difference between diets was observed in chickens (Table 3; Fig. 1). Small intestinal sucrase activities varied significantly across regions in all three bird species, but no diet × region effects were detected in any species (Table 3). Similarly, mass-specific cecal sucrase activity was significantly higher in mallards and quails fed the HS diet, while there was no difference in chickens fed different diets (Table 4; Fig. 2). Cecal sucrase activities varied significantly across regions in chickens, but not mallards or quails (Table 4). The pH optimum of sucrase for most samples (both small intestinal and cecal samples from all three bird species on both diets) was 6.5. Only the cecal samples from the chicken differed, and exhibited a pH optimum of 5.5 (with no effect of diet).

Mass-specific small intestinal aminopeptidase-N (APN) activity was significantly higher in mallards fed the HP diet, while there were no differences in small intestinal APN activity in chickens or quails fed different diets (Table 3; Fig. 1). Small intestinal APN activities varied significantly across regions in mallards and chickens (Table 3). There was a significant diet × region effect for small intestinal APN activities in mallards, such that differences between diet groups were larger in the distal small intestine (Table 3; Fig. 1). Mass-specific cecal APN activity exhibited no diet-induced differences in any species. (Table 4; Fig. 2). The pH optimum of APN for all three bird species, both diets, and both gut regions (small intestinal and cecal) was 7.0.

Summed activities

Summed enzymatic activities for each bird were calculated by multiplying mass-specific activities by tissue weight and summing over the small intestinal and cecal masses. Body mass was a significant covariate for several cases (Table 5;

Table 5 Summary of statistics from ANCOVAs of summed digestive enzyme activities in various bird species

	Mallard		Chicken		Quail	
	F	*P*	*F*	*P*	*F*	*P*
Maltase						
Diet	13.68	**0.004**	0.53	0.48	7.12	**0.02**
Body mass	11.45	**0.007**	0.01	0.93	0.70	0.42
Diet × body mass	3.43	0.09	3.38	0.09	0.26	0.62
Sucrase						
Diet	30.33	**0.0003**	0.08	0.79	2.44	0.15
Body mass	11.70	**0.007**	2.06	0.18	0.31	0.59
Diet × body mass	4.16	0.07	0.28	0.61	0.29	0.60
Aminopeptidase-N						
Diet	7.18	**0.02**	0.06	0.81	0.31	0.59
Body mass	3.93	0.08	8.75	**0.02**	0.76	0.40
Diet × body mass	0.69	0.43	0.07	0.79	0.03	0.86

Graphs depicting data can be found in Supplementary Fig. 1. Data from the chicken samples have been published previously (Ciminari et al. 2014)

Significant values are in bold

Suppl. Figure 1) and was included in all statistical models. Summed maltase activity was significantly higher in mallards and quails fed the HS diet, though there was no difference between diets in chickens (Table 5; Suppl. Figure 1). Summed sucrase activity was significantly higher in HS-fed mallards, while summed APN activity was higher in HP-fed mallards. Diet did not significantly affect summed activities of sucrase or APN in chickens or quails (Table 5; Suppl. Figure 1).

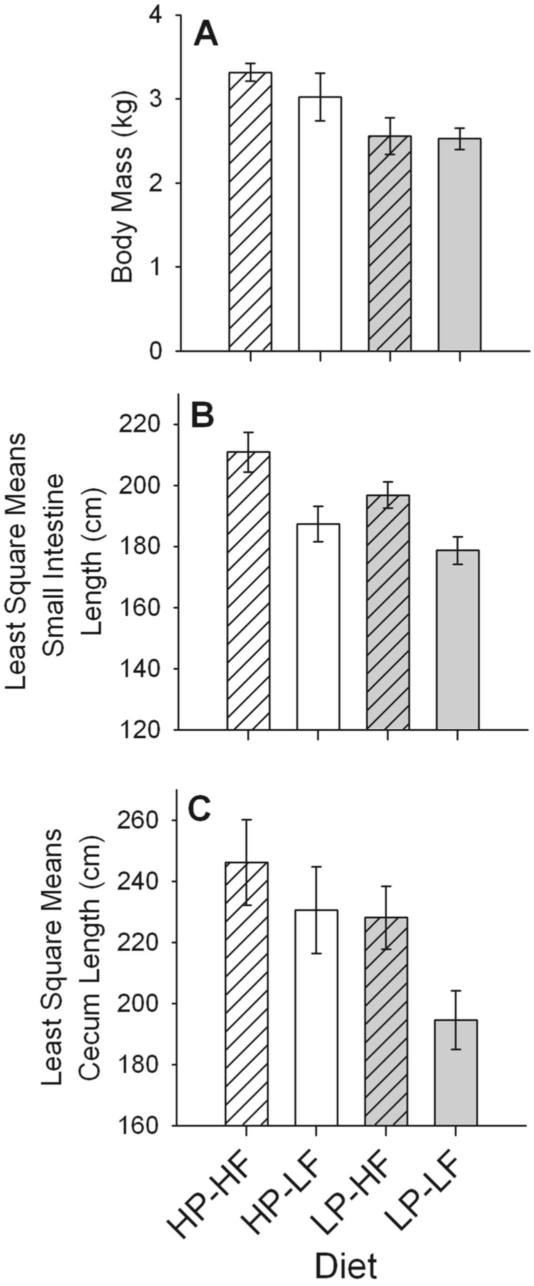

Fig. 3 **a** Mean (±SEM) body mass of geese ~96 days old fed one of four diets (see Table 2). **b** Least square means of small intestine length and **c** least square means of cecum length of geese fed one of four diets. Lengths of paired caeca were averaged within an individual. Sample sizes are as follows: HP–HF 4, HP–LF 4, LP–HF 7, LP–LF 7

Trial 2: effect of fiber and protein on digestive enzyme activities

In trial 2, there was a significant effect of dietary protein on body masses of geese ($P = 0.006$; Fig. 3a), though there was no effect of fiber ($P = 0.44$) and no significant interaction ($P = 0.52$). For a given body mass, geese fed the high-fiber diets had significantly longer small intestines (Fig. 3b; ANCOVA—protein: $P = 0.08$; fiber: $P = 0.0007$; protein × fiber: $P = 0.60$; body mass: $P = 0.001$). Geese fed the high-fiber diets also had significantly longer caeca (Fig. 3c; ANCOVA: protein: $P = 0.06$; fiber: $P = 0.05$; protein × fiber: $P = 0.45$; body mass: $P = 0.92$).

Diet quality significantly altered digestive enzyme activities. For example, small intestinal maltase activities exhibited a significant protein × fiber interaction, such that maltase activity was higher in the LP–LF group when compared to the LP–HF group, while there we no differences between the HP–HF and HP–LF groups (Fig. 4; Table 6). No differences were observed in cecal or summed activities. Birds fed the high-fiber diets had significantly depressed mass-specific small intestinal sucrase activities when compared to birds fed low-fiber diets (Fig. 4; Table 6), though there were no differences in cecal activities. Last, birds fed diets containing high levels of protein exhibited higher cecal APN activity, especially in the C1 section (Fig. 4; Table 6). A number of enzymes and tissues also exhibited differences that bordered statistical significance (Fig. 4; Table 6). When investigating summed activities across the whole digestive tract, the groups fed high-fiber diets exhibited higher summed APN activities, while there were no significant differences in summed maltase or sucrase activities (Fig. 4; Table 6).

Discussion

Here, we present a series of experiments testing the effects of diet composition and quality on digestive enzyme activities in non-passerine birds. First, we predicted, in accordance with the adaptive modulation hypothesis, that activities of carbohydrases and peptidases would match substrate levels in animals' diets. Overall, we found support for this hypothesis in carbohydrases, but less so for the measured peptidase in the species studied. Second, we predicted that high-fiber diets would increase enzyme activities, as has been demonstrated in other species. We found that fiber increased summed APN activity across the small intestine and ceca, but decreased small intestinal sucrase activity. We

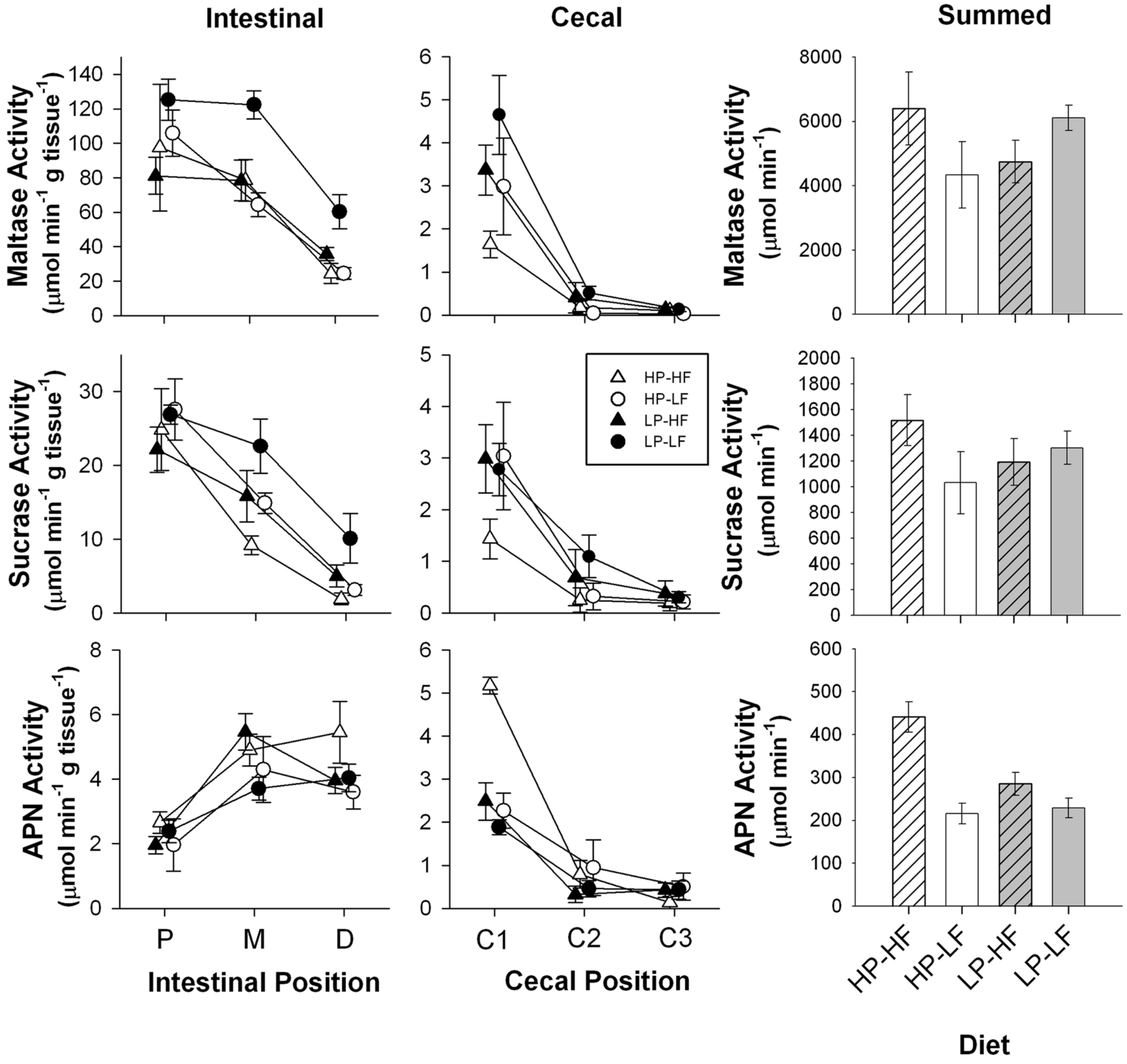

Fig. 4 Mean (±SEM) mass-specific enzyme activities from small intestinal and cecal tissues of geese. In addition, summed enzymatic activities across the small intestinal and cecal regions: *P* proximal small intestine, *M* middle small intestine, and *D* distal small intestine. *C1* section of the cecum closest to the small intestine, *C2* middle cecum, and *C3* the end of the cecum, or the fundus. Sample sizes are as follows: HP–HF 4, HP–LF 4, LP–HF 7, LP–LF 7

discuss these findings below in relation to what has been observed in other avian species.

Birds fed the high-starch diet largely had higher mass-specific activities of small intestinal carbohydrases (four of six cases tested, Table 3), in agreement with the adaptive modulation hypothesis. This upregulation of carbohydrases likely enhances the digestion and absorption of starch material in the diet. However, modulation in small intestinal peptidases by dietary protein content was less common, and was only observed in juvenile mallards (this study) and domestic turkeys (Foye and Black 2006). Our findings are in agreement with work conducted in other commercial poultry species, which modulate small intestinal carbohydrases, but not peptidases (Sell et al. 1989; Biviano et al. 1993; Siddons 1972). However, the results presented here are contrary to what has been observed in adult passerine birds, which modulate peptidase activities, but not carbohydrases (Afik et al. 1995; Brzęk et al. 2010b; Caviedes-Vidal et al. 2000; Levey et al. 1999; Maldonado et al. 2011; Martínez del Rio et al. 1995; Sabat et al. 1998; Brzęk et al. 2010a). Thus, it seems possible that there is a phylogenetic basis for the ability of adult birds to modulate carbohydrase activities (McWhorter et al. 2009).

It has been proposed that differences in the capacity for regulation of peptidase activities could have a functional basis (McWhorter et al. 2009). The avian species that modulate peptidase activities (passerines and pigeons) do not have cecal chambers, whereas most of the species that

Table 6 Summary of statistics from ANOVAs and ANCOVAs of mass-specific and summed enzyme activities in geese fed contrasting diets

	Intestinal		Cecal		Summed	
	F	*P*	*F*	*P*	*F*	*P*
Maltase						
Protein	4.10	0.058	3.98	0.063	0.07	0.79
Fiber	4.01	0.061	1.35	0.26	0.12	0.74
Protein × fiber	4.92	**0.039**	0.06	0.80	4.17	0.058
Body mass	–	–	–	–	0.15	0.70
Sucrase						
Protein	3.84	0.065	1.36	0.26	0.11	0.74
Fiber	5.98	**0.025**	0.58	0.45	0.57	0.46
Protein × fiber	0.42	0.52	0.43	0.52	1.81	0.20
Body mass	–	–	–	–	0.67	0.42
Aminopeptidase-N						
Protein	0.23	0.63	7.11	**0.017**	2.02	0.17
Fiber	3.99	0.061	3.89	0.066	19.18	**0.0005**
Protein × fiber	0.55	0.46	1.71	0.21	6.60	**0.02**
Body mass	–	–	–	–	1.18	0.29

Region effect was significant for all intestinal and cecal enzymes ($P < 0.0001$ for all). Body mass was not included in the models for mass-specific intestinal or cecal enzyme activities

Significant values are in bold

do not modulate peptidase activities in relation to dietary protein content (chickens, quail, geese) maintain caeca. The simple guts of passerines may be specialized to digest and absorb all ingested protein, whereas other avian species may allow some undigested protein to pass through the small intestine to support microbial growth in the caeca (McWhorter et al. 2009), thereby allowing additional digestion and absorption of protein in the caeca (McWilliams 1999; Sedinger 1997). This hypothesis may be difficult to test, given that no passerine birds maintain cecal chambers. However, there is diversity in the presence, size, and structure of caeca across avian taxa (Clench and Mathias 1995; McLelland 1989), and thus further studies could examine flexibility in peptidase activity in relation to the presence, size and structure of caeca.

Fiber decreased mass-specific activities of small intestinal sucrase in geese. This is in contrast to what has been demonstrated in chickens fed mannan oligosaccharides, which increased disaccharidase activity (Iji et al. 2001). These differential results could be due to the fact that different fiber components (e.g., pectin vs. cellulose) can have differential effects on enzyme activities (Thomsen and Tasman-Jones 1982). Summed enzyme activities of APN were higher in geese fed the high-fiber diets, which is in agreement with previous studies conducted in chickens (Yang et al. 2007; Iji et al. 2001). The mechanistic basis and adaptive function of modulation of digestive enzymes in response to diet quality are poorly understood, but seem unrelated to changes in epithelial structure (Hedemann et al. 2006; Montagne et al. 2003). Our study is the first, to our knowledge, to study the effects of ecologically relevant fiber levels (Richman et al. 2015) on digestive enzyme activities in an herbivorous bird, and thus further studies are needed to better understand these results.

Very few studies have measured the activities of endogenous digestive enzymes in avian cecal tissues. Protease activities have been detected in the caeca of domestic poultry, with the suggestion that these enzymes aid in the digestion of microbial protein (Lepkovsky et al. 1964). In our study, all species expressed significant activities of carbohydrases and peptidases in the caeca. Additionally, activities of several enzymes responded to diet in accordance with the adaptive modulation hypothesis, suggesting that these enzymes may digest dietary substrates, and not only microbial products. However, it could also be argued that cecal enzymes are subject to pleiotropic effects from the regulation of small intestinal enzymes. The activity of cecal enzymes only contributed 2–3 % of the total maltase activity when summed across the small intestine and ceca, while they contributed 4–12 % of total APN activity. Avian species are known to exhibit significant interspecific variation in nutrient uptake rates in the caeca (Obst and Diamond 1989). For example, the cecal contribution to total uptake capacity (estimated across the entire gut) for specific amino acids was higher for geese (6–19 % depending on amino acid) compared to chickens (ca. 4 %), but was modest compared to grouse (ca. 25–50 %; Obst and Diamond 1989). These findings are consistent with the hypothesis that Galliformes rely more on cecal digestion than do geese and other waterfowl (Sedinger 1997; McWilliams 1999). It would be interesting to measure the digestive enzyme activities in cecal tissues of other avian species to see if this tissue accounts for a higher proportion of digestive capacity. For example, do grouse, which have a higher capacity for cecal absorption, also have a higher capacity for cecal hydrolysis?

Overall, modulation of digestive enzymes in response to dietary composition and quality is hypothesized to optimize digestion while avoiding excess energetic costs. It is interesting that support for the adaptive modulation hypothesis varies across avian groups (Galloanserae vs. passerines) and across enzyme types (carbohydrases vs. peptidases). Additionally, the adaptive nature of changes in enzyme activities in response to fiber is poorly understood. We encourage further studies on additional bird groups with varying gut anatomy and ecology to better understand the mechanisms and limitations for flexibility in the avian digestive tract.

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