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Low activities of digestive enzymes in the guts of herbivorous grouse (Aves: Tetraoninae)

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Abstract

Avian herbivores face the exceptional challenge of digesting recalcitrant plant material while under the selective pressure to reduce gut mass as an adaptation for flight. One mechanism by which avian herbivores may overcome this challenge is to maintain high activities of intestinal enzymes that facilitate the digestion and absorption of nutrients. However, previous studies in herbivorous animals provide equivocal evidence as to how activities of digestive enzymes may be adapted to herbivorous diets. For example, "rate-maximizing" herbivores generally exhibit rapid digesta transit times, and high activities of digestive enzymes. Conversely, "yield-maximizing" herbivores utilize long gut retention times and express lower activities of digestive enzymes. Here, we investigated the activities of digestive enzymes (maltase, sucrase, aminopeptidase-N) in the guts of herbivorous grouse (Aves: Tetraoninae), and compared them to activities measured in several other avian species. We found that several grouse species exhibit activities of enzymes that are dramatically lower than those measured in other birds. We propose that grouse may use a "yield-maximizing" strategy of digestion, which is characterized by relatively long gut retention times and generally lower enzyme activities. These low activities of intestinal digestive enzyme could have ecological and evolutionary consequences, as grouse regularly consume plants with compounds known to inhibit digestive enzymes. However, more comprehensive studies on passage rates, digestibility, and microbial contributions will be necessary to understand the full process of digestion in herbivorous birds.

Keywords: aminopeptidase-N, digestive physiology, maltase, sucrase

Introduction

The process of digestion provides animals with the energy and essential nutrients required for survival and reproduction. Due to the importance of these processes, animals exhibit a number of adaptations for optimizing energy acquisition, such as modulating gut size, motility, or expression of digestive enzymes to increase digestive efficiency (Foley and Cork 1992; Karasov and Douglas 2013). Avian herbivores experience substantial digestive challenges due to competing demands associated with flying. Birds must maintain a relatively low body mass to decrease the amount of energy needed to work against gravity while flying. Birds have adapted to this demand with several mass-reducing traits such as the loss of teeth and development of hollow bones (Gill and Coe 1990). Similarly, birds have decreased the size of their digestive systems compared to non-flying mammals to decrease their overall body mass (Price et al. 2015; Sedinger 1997). However, birds have higher basal and field metabolic rates than mammals, and thus require higher daily food consumption to meet their increased energy demands (Nagy 1987; Nagy 2001). These challenges are more difficult for avian herbivores, as plants are depleted in nutrients such as protein, the existing proteins provide unbalanced ratios of amino acids, and the process of digesting complex plant material may require larger digestive tracts (Karasov and Martínez del Rio 2007; Sedinger 1984; Sedinger 1997). Therefore, avian herbivores experience a tradeoff in that they must have a small enough gut as to not compromise flight, but also maintain digestive efficiency at an appropriate level to obtain the necessary nutrients from their diets.

One potential mechanism of physiological adaptation to a herbivorous diet is differential production of digestive enzymes that target protein, carbohydrates, lipids, or fiber, as the concentrations of these nutrients can vary across available and selected food types. The "adaptive modulation hypothesis" proposes that digestive enzyme activities will be correlated to the substrate levels in an animal’s diet, allowing for full digestion of available nutrients while avoiding the energy and space costs of unneeded enzymes (Kohl et al. 2017b). For example, birds in the superorder Galloanserae (orders Galliformes and Anseriformes) upregulate carbohydrase enzymes in the intestine, such as maltase and sucrase, when feeding on high-starch diets (Kohl et al. 2017b). The logic and predictions of the adaptive modulation hypothesis can also be extended to interspecies comparisons. For example, passerine birds are unable to flexibly modulate activities of starch-digesting enzymes in response to diet, and instead there is variation across species that exhibits evolutionary matching between activities of starch-digesting enzymes such as maltase and sucrase and the levels of starch in their natural diets (Kohl et al. 2011; Ramirez-Otarola et al. 2011). Conversely, the “nutrient balancing hypothesis” hypothesizes that herbivores may maintain high activities of digestive enzymes to digest and absorb limiting nutrients, especially protein (Clissold et al. 2010; German et al. 2010). Overall, regulation of digestive enzymes offers a mechanism by which avian herbivores could maintain optimal digestion of plant material.

Studies investigating digestive enzyme activities in herbivores are limited, even outside of avian herbivores. A study examining the Rufous-tailed Plantcutter (*Phytotoma rara*), a small herbivorous bird, found exceptionally high maltase activities, that matched the bird’s high carbohydrate diet, and lower aminopeptidase-N (APN) activity, again correlating with the bird’s low protein ingestion (Meynard et al. 1999). Conversely, herbivorous geese exhibit carbohydrase enzyme activities that are lower than omnivorous species, and regulate these activities differentially based on the fiber and protein content of their diets (Kohl et al. 2017b). Similarly, a herbivorous rodent the common degu (*Octodon degus*) exhibits lower carbohydrase and APN enzyme activities compared to an omnivorous species, the Darwin’s leaf-eared mouse (*Phyllotis darwini*; Sabat et al. 1999). Yet, herbivorous mudskipper fish (*Boleophthalmus pectinirostris*) exhibit higher maltase and sucrase activities compared to *Bostrichthys sinensis* a closely-related carnivorous fish consuming food with relatively high protein content (Wu et al. 2009). Overall, there is still much that remains unknown concerning the adaptations of digestive enzyme activities associated with herbivorous diets, as previous studies provide equivocal evidence as to how activities of digestive enzymes may be adapted to herbivorous diets.

Here, we directly measured activities of two disaccharidases (maltase and sucrase) and aminopeptidase-N enzymes in the guts of herbivorous grouse (Tetraoninae) species and domestic chickens (*Gallus gallus domesticus*). A comparison between these birds is relevant as during winter, herbivorous grouse generally survive on a diet low in crude protein (<10%), high in fiber (generally <20%), and variable in carbohydrates (50-80%) (Ellison 1976; Gurchinoff and Robinson 1972; Treichler et al. 1946). Conversely, both domestic chickens and their wild counterparts are omnivorous and consume diets that contain relatively higher concentrations of crude protein (~25%) and similar amounts of carbohydrates (~70%) compared to grouse (Arshad et al. 2000; Malheiros et al. 2003). Additionally, we compare our activities to values measured in previous studies of numerous avian species (an additional dataset from domestic chickens, and also Mallard [*Anas platyrhynchos*] and Japanese Quail [*Coturnix japonica*]), all of which are considered omnivorous (Cheng et al. 2010; Combs and Fredrickson 1996; Dabbert and Martin 2000; Kawahara 1973), and Canada Geese (*Branta canadensis*), which are herbivorous, but are considered grazers and consume less woody material compared to grouse (Sedinger 1997). Our prediction for disaccharidase activities was that there would be similar levels of activity between grouse, geese and omnivorous birds, as their diets contain relatively similar concentrations of simple carbohydrates. For aminopeptidase-N, we predicted that if APN activity follows the 'adaptive modulation hypothesis' in herbivores, there will be lower APN activities in grouse and geese, as the protein content of plant material is relatively low compared to that of other avian species. However, if herbivores act to digest and absorb the scarce amount of protein in their diets, in accordance with the ‘nutrient balancing hypothesis’, we may see similar or higher activities of APN in grouse and Canada geese compared to omnivores.

Materials and Methods

Animal Collection

Licensed hunters generously collected grouse in the field between 26 February and 07 April 2016, during the boreal winter when birds were feeding predominantly on plant material (Moss 1974; Pulliainen and Tunkkari 1983). Collection was approved by regional wildlife authorities: Jordbruksverket (the Swedish Board of Agriculture) for Black Grouse (*Tetrao tetrix*, n=5) and Capercaillie (*Tetrao urogallus*, n=1) in Sweden; Icelandic Institute of Natural History for Rock Ptarmigan (*Lagopus muta*, n=5) in Iceland; Swedish Environmental Protection Agency for Willow Ptarmigan (*Lagopus lagopus*, n=4) in Sweden; and by the Boise State University Institutional Animal Use and Care Committee (006-AC15-012). Black Grouse and Capercaillie were collected in forests dominated by Scots Pine (*Pinus sylvestris*, 59° 41.261'N, 15° 26.155'E). Rock Ptarmigan were collected in alpine areas dominated by *Betula pubescens* shrubs (65° 37.460'N, 17° 2.621'W). Willow Ptarmigan were collected in birch forests (*Betula pubescens*, 61° 55.585'N, 13° 19.888'E). Tissues from domestic chickens (n = 6) were collected in November 2016 from the University of Wisconsin – Madison. Adult Cobb Cornish Rock hens were fed a standard commercial diet (Purina Start and Grow SunFresh Poultry feed) and housed under standard poultry production procedures. Domestic chickens were covered under IACUC Protocol #A005392.

Immediately after death, a midline incision was made on the left lateral surface of the bird to expose the abdominal cavity. All animals were dissected within 10 minutes of death. A small section of approximately 5 mm^3 was cut from the proximal section of small intestine (duodenum) and from the midpoint of a single ceca respectively for each animal, using sterile scalpels and forceps. In the field, all samples were immediately placed in a cryogenic dry shipper charged with liquid nitrogen (validated to maintain temperatures at -150°C or colder) and transported in the cryoshipper to the laboratory where they were stored at -80°C until enzyme assays were conducted.

Contents of crops of collected grouse were qualitatively evaluated to confirm that species were primarily consuming plant material. Inspection of crop contents indicated that Black Grouse were consuming cones and needles of Scots pine and berries of lingonberry (*Vaccinium vitis-idaea*) and the Capercaillie was consuming only Scots pine. The crop contents of Rock Ptarmigan were all dominated by twigs and buds of *Betula* spp. and *Salix* spp., which is consistent with previous dietary reports for this species. The only Willow Ptarmigan with crop contents was consuming terminal leaves and stems of crowberry *Empetrum nigrum*, stems of *Vaccinium* spp., twigs of *Betula* spp., and berries of lingonberry. All of these food items are consistent with previous dietary reports for these species (Summers et al. 2004; Thomas 1984; Wegge and Kastdalen 2008).

Intestinal Enzyme Assays

We evaluated the activity of membrane-bound enzymes in whole-tissue homogenates of samples from small intestinal and cecal tissues. We assayed disaccharidase (maltase, sucrase) activity using an adaption of the colorimetric method developed by Dahlqvist (1984). Assays are described in detail elsewhere (Martinez del Rio 1990; Fassbinder-Orth and Karasov 2006). In brief, tissues were thawed at 4°C, rinsed thoroughly in physiological saline to remove gut contents, and homogenized in 350 mM mannitol in 1 mM N-2-hydroxyethylpiperazine-N -2-ethanosulfonic acid

(Hepes)-KOH, pH 7.0. Intestinal homogenates (30 μL) diluted with 350 mM mannitol in 1 mM Hepes-KOH were incubated with 30 μL of 56 mM maltose or 56 mM sucrose in 0.1 M maleate and NaOH buffer, pH 6.5, at 40ºC for 20 min. Next, 400 μL of a stop-develop reagent (GAGO-20 glucose assay kit; Sigma Aldrich, St. Louis) was added to each tube, vortexed, and incubated at 40°C for 30 min. Last, 400 μL of 12 N H_2SO_4 was added to each tube, and the absorbance was read at 540 nm.

We used l-alanine-p-nitroanilide as a substrate for aminopeptidase-N. To start the reaction, we added 10 μL of the homogenate to 1 mL of assay mix (2.0 mM l-alanine-p-nitroanilide in 1 part of 0.2 M NaH_2PO_4/Na_2HPO_4 buffer, pH 7, and 1 part of deionized H_2O) previously warmed to 40°C. The reaction solution was incubated for 20 min at 40°C and then terminated with 3 mL of ice-cold 2 N acetic acid, and absorbance was read at 384 nm.

Statistical Analyses

We combined our new enzyme activity measurements with those measured from other avian species using the same enzymatic assays. We collected these data from the literature for the following species: Mallard, Japanese Quail, domestic chickens, and Canada Goose (Kohl et al. 2017b) and the Greater Sage-Grouse (*Centrocercus urophasianus*; Kohl et al. 2015). Enzyme activities were compared using nested Analysis of Variance (ANOVA) with the main effects of "Group" and Species nested within "Group". Our Groups were as follows: Omnivores (Mallard, Quail, Chicken), Grouse (Sage-Grouse, Black Grouse, Capercaillie, Rock Ptarmigan, Willow Ptarmigan), and Canada Goose (the only species in this group). While we present the data from the domestic chicken separately for published and new data in the figures, both published and new measurements were coded as "Species: Chicken" for analysis. We then conducted post-hoc

Tukey's HSD Test on the Least-Squares means to test for significant differences in enzyme activities across Groups of birds. Finally, we ran an ANOVA and conducted post-hoc Tukey's HSD Test with only data from Grouse species to test for significant differences in enzyme activities across species within the Grouse Group. All analyses were conducted in JMP 14.0, with an α threshold of 0.05.

Results

Small intestinal enzyme activities

In the small intestine, "Groups" of birds exhibited significantly different activities of maltase (Fig. 1; $F_{2,78} = 24.80$, $P < 0.0001$) and sucrase (Fig 1; $F_{2,78} = 64.39$, $P < 0.0001$). Specifically, Omnivores and Canada Geese exhibited mass-specific activities of maltase and sucrase that were roughly 10x higher than Grouse. When only comparing within Grouse, there were still significant differences across species in mass-specific maltase (Fig. 1; $F_{4,14} = 9.89$, $P = 0.0005$) and sucrase activities (Fig 1; $F_{4,14} = 4.00$, $P = 0.023$). Specifically, Black Grouse exhibited significantly higher activities than Rock Ptarmigan and Willow Ptarmigan (Tukey's HSD Test).

Intestinal APN activities varied significantly by Group (Fig 1; $F_{2,78} = 58.06$, $P < 0.0001$). Grouse and Canada Geese exhibited similar APN activities, while Omnivores exhibited activities that were ~3.2x higher. When only comparing Grouse species, APN activities varied significantly across species (Fig 1; $F_{4,14} = 3.28$, $P = 0.043$). Specifically, Rock Ptarmigan exhibited activities of intestinal APN that were 4.7x higher and significantly different from activities measured in Sage-Grouse.

Cecal enzyme activities

Overall, mass-specific maltase and sucrase activities were much lower in cecal tissue compared to small intestinal tissues (Fig. 2). Activities of both cecal maltase (Fig. 2; $F_{2,79}$ = 27.01, P <0.0001) and sucrase (Fig 2; $F_{2,79}$ = 27.72, P <0.0001) varied significantly across Groups of birds. Here, Grouse and Canada Geese all exhibited low activities, while Omnivores show activities of cecal maltase and sucrase that were ~31.5x and ~10.8x higher, respectively. When only comparing Grouse species, there were subtle differences in mass-specific maltase activities (Fig. 2A; $F_{4,14}$ = 2.89, P = 0.062), such that the Black Grouse exhibited ~2x higher activities compared to other species (though this result was not significant based on Tukey's HSD test). There were no significant differences in mass-specific cecal sucrase activities across Grouse species (Fig 2B; $F_{4,14}$ = 0.49, P = 0.74).

Cecal APN activities were also substantially lower than those measured in the small intestine. Activities of cecal APN varied across Groups of birds ($F_{2,78}$ = 67.55, P <0.0001), such that activities in Omnivores were ~2.5x higher than Grouse and Canada Geese. When comparing only amongst Grouse species, there was a significant difference in mass-specific cecal APN activities (Fig 2C; $F_{4,14}$ =4.45, P = 0.016), such that the Sage-Grouse exhibited ~3.2x higher activities compared to Black Grouse (Tukey's HSD Test).

Discussion

We found that herbivorous grouse exhibited lower activities of disaccharidases, compared with omnivorous chickens and other omnivorous species. These differences based on feeding strategies are consistent with previous results in herbivorous rodents and geese (Kohl et al. 2017b; Sabat et al. 1999). In support of the 'adaptive modulation hypothesis', grouse had considerably lower APN activities in both small intestinal and cecal tissues compared to chicken and other omnivorous species, which may be related to the relatively low protein content of their herbivorous diets.

Herbivorous grouse exhibited distinct enzyme activities from other omnivorous species. For example, mass-specific maltase and sucrase activities in the small intestine were roughly 10-fold higher in omnivore than in grouse. Omnivores also showed 3x higher activities of the APN enzyme in the small intestine, matching their typically high-protein diet. These differences are substantially larger than differences observed across gut regions (Kohl et al. 2017b), and so the fact that we only measured activities in the duodenum should not affect our overall conclusions. It should be noted that chickens were being fed a commercial diet rich in metabolizable carbohydrates and protein, and as production animals they may have digestive enzymes that have high activity due to artificial selection for growth rates (Al-Marzooqi et al. 2019). Our results are consistent with a study in rodents, where an omnivorous rodent species had higher enzyme activities than a herbivorous species (Sabat et al. 1999).

There were several examples where we observed interspecific differences in enzyme activities across grouse species. For example, the Black Grouse exhibited higher activities of maltase and sucrase in the small intestine than some other grouse species. Also, the Sage-Grouse exhibited higher mass-specific cecal APN activities compared to the other grouse species. While all grouse species are considered herbivorous, the contributions of berries with higher sugar content (e.g., *Vaccinium*) and invertebrates or buds with higher protein content varies geographically, seasonally, and developmentally due to different foraging strategies and other environmental factors. (Bocca et al. 2014; Garcia-Gonzalez et al. 2016; Starling-Westerberg 2001). While we did not have a sufficient number of crops with food content to determine contribution of berries compared to stems and buds, differences in enzyme activities may correlate with the average concentrations of substrates in their diets prior to collection, as has been observed in song birds (Kohl et al. 2011).

Overall, the small intestine had much higher enzyme activities compared to the cecum. These findings are consistent with studies on other avian species (Kohl et al. 2017b). While these results indicate a relatively low contribution of host digestive capacity in the ceca of grouse, they do not necessarily suggest low absorption capacity in the ceca. Previous studies in grouse show that mass-specific rates of amino acid uptake are higher in the ceca than in the small intestine, and cecal chambers of some grouse species can be responsible for nearly half of the integrated glucose uptake capacity (Obst and Diamond 1989). Thus, the cecal chambers of grouse may be important for maintaining nitrogen balance. It is also possible that the function of the gut microbiome could compensate for low enzyme activity of hosts. Like mammals, grouse harbor diverse microbial communities in their hindguts that produce considerable amounts of short chain fatty acids as a product of fermentation which may contribute up to 18% of the energetic costs associated with physiological maintenance of the host (McBee and West 1969; Moss and Parkinson 1972). The hindgut microbiota is often nitrogen limited, especially in herbivorous hosts (Reese et al. 2018). Thus, forgoing digestion by the host may increase the availability of nutrients to the hindgut microbial community (Kohl et al. 2017a; McWhorter et al. 2009), allowing them to carry out important functions that assist the host in maintaining nutritional balance. Furthermore, the collective metagenome of the gut microbial community of grouse is enriched in genes associated with the biosynthesis of essential amino acids (Kohl et al. 2016) and degradation of endohemicellulose and starch (Salgado-Flores et al. 2019) which may minimize the host's reliance on their own digestive enzymes.

Different mechanisms of digestion in herbivores (focusing on digesting cell contents versus cell walls) may also be influencing these results. Two digestive strategies, rate-maximizing and yield-maximizing have been shown to contribute to differences in digestive physiology. Rate-maximizers tended to assimilate only the most digestible components of their food while passing the rest through feces. To compensate for the rapid digesta transit time and high rates of food intake, ‘rate maximizing’ species generally exhibit higher digestive enzyme activities (Crossman et al. 2014; German 2009). For example, an herbivorous fish species that uses rate-maximizing (*Xiphister mucosus*) exhibits significantly higher activities of amylase throughout the intestine when compared to a related herbivorous species that exhibits yield-maximizing (*Cebidichthys violaceus*; (German et al. 2015). It is possible that herbivorous Rufous-tailed Plantcutters, which exhibit exceptionally high activities of digestive enzymes, are using this rate-maximizing digestive strategy (Meynard et al. 1999). Conversely, yield-maximizing animals generally have slower digesta transit, lower food intake, and higher overall digestibility (Choat et al. 2004; Clements and Rees 1998). However, enzyme activities tend to be lower in yield-maximizing species. For example, *C. violaceus* showed lower levels of amylase activity across the intestine compared with the rate-maximizing species (German et al. 2015). Herbivorous grouse may be yield-maximizers during winter, which would explain their low levels of digestive enzyme activities. Dry matter digestibility values from grouse tend to be lower than 45%, which is on par with capacity for rate-maximizing Rufous-tailed Plantcutters to digest low-quality diets (48%), though lower than the yield-maximizing Hoatzin, which digests roughly 70% of ingested food material (Graial 1995; Lopez-Calleja and Bozinovic 1999; Moss 1983). Moreover, the passage of food material in herbivorous grouse is complex, and is rather rapid unless the material enters the cecal chambers (Gasaway et al. 1975). For example, intestine length increases from autumn to winter as the composition of woody plants increases in the diets of Rock Ptarmigan (Moss 1974) and Willow Ptarmigan (Pulliainen and Tunkkari 1983). The link between diet composition and gut length thus suggests that grouse may rely on rate-maximizing strategies when berries are available and yield-maximizing strategies when foods higher in carbohydrates and protein are not available.

The dynamics and processes of digestion are poorly understood in herbivorous birds and require studies the directly connect diet composition and quality with morphology and physiological function of intestines. Our study adds data to a body of work comparing the digestive strategies of herbivorous grouse and herbivorous geese with that of other birds. In general, grouse and geese have similar intestinal sizes, though grouse maintain significantly larger cecal chambers (Sedinger 1997). As a result, grouse retain food material within their gut for a longer residence time when compared to geese (Clemens et al. 1975; Gasaway et al. 1975; Prop and Vulink 1992), which may increase digestive efficiency and explain the relatively low nitrogen requirements observed in grouse when compared to geese (Sedinger 1997). The fast-throughput by geese supports the hypothesis that geese are typical “rate-maximizers”. Contrary to the “rate-maximizer” hypothesis, geese still have relatively low digestive enzyme activities when compared to other birds (Kohl et al. 2017b), but in support of the hypothesis, geese have relatively higher intestinal maltase and sucrase activities compared to grouse (this study). Our functional enzyme assay offers one path towards collecting comparable data that could overcome the limitations of existing measurements of digestive physiology in herbivorous birds (food intake, retention time, digestibility) that are scattered across studies using different techniques and avian taxa (Sedinger 1997). In addition, enzyme activity can be integrated into chemical reactor theory and kinetic models as a promising avenue to model the dynamics of digestion to better understand the processes that optimize digestion in herbivorous birds (Penry and Jumars 198; Penry and Jumars 1986).

The low enzyme activities of herbivorous grouse could have potential ecological and evolutionary consequences, given that digestive function is important for animal fitness (Brittas 1988). Herbivorous grouse species specialize on a number of plants that are relatively high in fiber and rich in plant secondary metabolites (PSMs), and so might be faced with limited digestibility associated with both fiber and toxins. We have previously shown that some PSMs, such as phenolics and monoterpenes can inhibit digestive enzymes (Kohl and Dearing 2011; Kohl et al. 2015). Though, the enzymes of grouse can be more resistant to inhibition by PSMs when compared to domestic chicken (Kohl et al. 2015). Global climate change is expected to increase the concentrations of PSMs in plant tissues and decrease concentrations of nitrogen (Ayres 1993; Forbey et al. 2013). Thus, grouse may face challenges in the future in digesting and acquiring adequate energy and protein given their exceptionally low activities of digestive enzymes.

Overall, our results suggest that digestive enzyme activities of herbivorous grouse differ considerably from omnivores. Our results add to the body of knowledge regarding digestion in herbivorous birds, which is important given that these organisms face the contrasting pressures of digesting difficult foods which may require larger intestines (Moss 1974; Moss 1983), yet needing to reduce body mass to optimize flight. As stated above, more comprehensive studies on passage rates, digestibility, and microbial contributions will be necessary to understand the full process of digestion in herbivorous birds.

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Figure Legends

Figure 1. Mass-specific activities (umol/min/g tissue) of maltase, sucrase, and aminopeptidase-N from intestinal tissues. Bars represent mean ± standard error enzyme activities from the proximal section of the small intestines of all species with newly generated data from this study (those bars with double hatch marks on the bottom x-axis) and species with activities gathered from published literature (those without double hatch marks). Samples sizes for samples newly analyzed in this paper are as follows: Chicken (new): 6; Black Grouse: 5; Capercaillie: 1; Rock Ptarmigan: 5; Willow Ptarmigan: 4. Uppercase letters represent results from Tukey's HSD test on the Least-Square means of Groups (Omnivores, Grouse, Canada Goose). Groups not sharing the same upper-case letter are statistically significant from one another. We then conducted a post-hoc Tukey's test only among species within the Grouse group, and bars not sharing the same lower-case letter are statistically significant from one another.

Figure 2. Mass-specific activities (umol/min/g tissue) of maltase, sucrase, and aminopeptidase-N from cecal tissues. Bars represent mean ± standard error enzyme activities from the medial section of the cecal tissues of all species with newly generated data from this study (those bars with double hatch marks on the bottom x-axis) and species with activities gathered from published literature (those without double hatch marks). Samples sizes for samples newly analyzed in this paper are as follows: Chicken (new): 6; Black Grouse: 5; Capercaillie: 1; Rock Ptarmigan: 5; Willow Ptarmigan: 4. Uppercase letters represent results from Tukey's HSD test on the Least-Square means of Groups (Omnivores, Grouse, Canada Goose). Groups not sharing the same upper-case letter are statistically significant from one another. We then conducted a post-hoc Tukey's test only among species within the Grouse group, and bars not sharing the same lower-case letter are statistically significant from one another.