Phenotypic flexibility of digestion in white-browed sparrow-weavers (*Plocepasser mahali*): limits to digestive flexibility and dietary enzyme modulation

by

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DECLARATION

I **Sekgwari Mpho Malematja** declare that the dissertation/thesis, which I hereby submit for the degree **MSc (Zoology)** at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

ETHICS STATEMENT

The author, whose name appears on the title page of this dissertation/thesis, has obtained, for the research described in this work, the applicable research ethics approval.

The author declares that she has observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research.

Signature: 

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“I come as one, but I stand as ten thousand”- Dr Maya Angelou.

This dissertation is dedicated to my ten thousand, those who have passed on and those who are living, they have carved the path for me to reach this point, it was not smooth sailing, but it was possible because you walked this journey before me and guided me till the end. To my supervisors, Prof Andrew McKechnie and Prof William Karasov, and my advisor, Prof Enrique Caviedes- Vidal, thank you for; setting the bar high and always encouraging me to be better, for investing your valuable time and effort into ensuring my Masters is a success. Watching the three of you hard at work, has taught me what it really means to be passionate about science, I feel blessed to have been mentored by the best. Thank you to the following research assistants who were involved in making this project a success; Matthew Noakes, Shannon Conradie, Nevanya Lubbe, Jones Matladi, Mokgadi Malematja and Alicia Fick.

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RESEARCH OUTPUTS

Conference presentations

Oral presentations

SANBI-NZG AGM 2018- Pretoria, South Africa

Hot birds research project meeting 2017– Prince Albert, South Africa

University of Pretoria, Department of Zoology and entomology AGM 2017/ 2018- Pretoria, South Africa

Poster presentations

International ornithological congress 2018-Canada, Vancouver

SUMMARY

Phenotypic flexibility of digestion in white-browed sparrow-weavers (*Plocepasser mahali*): limits to digestive flexibility and dietary enzyme modulation

By

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Degree: MSc (Zoology)

Many aspects of animal digestive form and function vary with ecological factors including diet composition and food availability. I examined phenotypic flexibility of digestive traits in white-browed sparrow-weavers (*Plocepasser mahali*), a widespread southern African passerine in which the ratio of insects to plant matter consumed varies seasonally. I predicted that digestive traits of *P. mahali* are modulated in response to diet composition. For experiment one, I caught forty-five sparrow-weavers from the Kalahari Desert population in South Africa, transported the birds to experimental facilities and acclimated each bird to one of three experimental diets [100 % seed, 100 % insect or control (70% seed, 30% insects), n = 15 per diet] while monitoring food intake and body mass (M_b). All three groups initially received the control diet, and thereafter gradually acclimated to the respective experimental

diets. Once experiment one was concluded, I randomly selected 24 individuals from experiment one, and maintained the birds on the control diet for two weeks. I euthanized the birds after an additional 8 days of acclimation to either a 100 % seed or 100 % insect diet (n=12), and thereafter determined liver mass, pancreas mass, gizzard mass, intestine mass, intestine length, and also quantified intestinal digestive enzyme activity of aminopeptidase-N (APN), maltase and sucrase. Diet emerged as a significant predictor of M_b. Birds acclimated to the seed diet lost ~0.07 % of M_b per day, whereas birds acclimated to the insect diet and the control diet gained ~0.15 % and ~0.08% of M_b per day respectively. Of all the digestive organs assessed, the sizes of only the liver and gizzard varied significantly with diet. Analysis of digestive enzyme activity indicated modulation correlated with diet composition. For example, in birds acclimated to the higher protein insect diet compared to the lower protein seed diet, APN activity summed over the entire intestinal length was 2.6-fold higher. For maltase and sucrase, activity in the proximal and medial section of the small intestine was statistically indistinguishable between the two diet groups, however, for birds acclimated to the insect diet, activity in the distal section was 78 % higher for maltase and 85 % for sucrase. Maltase activity summed over the entire intestinal length was 1.4-fold higher in birds fed the insect diet whereas sucrase activity remained similar between the two diet groups. These results indicate that digestive system features of *P. mahali* are modulated relative to diet composition, thus providing the capacity to maintain energy and nutrient balance despite variable diet compositions observed in different populations.

CHAPTER 1 INTRODUCTION

Digestive systems of animals directly link nutrients, energy and water in their environments to survival, growth and reproduction. For this reason, the field of digestive physiology is vital for understanding animal ecology and evolution (Karasov, 1990, Karasov *et al,*. 2011). Many aspects of digestive system structure vary with ecological factors, such as diet chemistry and food availability, resulting in considerable physiological diversity among digestive systems (Stevens and Hume, 1995, Karasov *et al.,* 2011).

Birds are particularly interesting within the context of digestive physiology. Birds have comparatively high basal metabolic rates, combined with a relatively low gut surface area and retention time of meals in the gut (Nagy, 2001, McWhorter *et al.,* 2009). These characteristics present physiological challenges to the digestive performance of the avian gut that act in addition to various ecological constraints related to food availability and quality (Karasov, 1990). In order to avoid mismatches between energy supply and demand, birds require higher food intake rate relative to similarly sized mammals. But, with smaller intestines they may have limited spare digestive capacity, which is the extra biochemical or mechanical capacity to process food relative to that required for the routine intake load (McWilliams and Karasov, 2014). A compensatory response to this situation is phenotypic flexibility in gut structure and function, which may be the norm in many avian species (McWilliams and Karasov, 2001, Battley and Piersma, 2005).

Phenotypic flexibility of digestion is associated with reversible changes in the digestive system morphology and physiology, as a response to an alteration of specific environmental variables (Stearns, 1989, Piersma and Lindstrom, 1997, Piersma and Drent, 2003, McWilliams and Karasov, 2014). In addition to the adjustment of body mass, the main mechanisms involved in phenotypic flexibility of avian digestion are modulation of

digestive organ structure, dietary enzyme activity, retention time, and absorption rate. (Karasov, 1996). My thesis focuses on modulation of the first two features.

Previous studies have revealed the capacity for up- or down-regulation of gastrointestinal organs of birds relative to diet, specifically changes in the mass and length of the intestine, gizzard, pancreas, and liver following acclimation to either a diet of varying nutritional content or periods of hyperphagia and hypophagia (Fenna and Boag, 1974, Al Jaborae, 1979, Savory, 1986, Starck, 1999, Lee *et al.*, 2002, McWilliams and Karasov, 2014).

The gizzard is an important organ to consider in this context. The principal function of the avian gizzard in the context of this study is to grind food in order to increase the rate of digestive breakdown (Piersma and Lindstrom, 1997, Starck, 1999). The up-regulation of the gizzard is largely due to either an increased digestive load caused by a low nutritional value diet, high meal coarseness, or a constant large meal consumption (Starck, 1999, Dekinga *et al.*, 2001, Starck and Rahman, 2003). The changes are often reversible, as per the conditions of phenotypic flexibility (Starck, 1999, McWilliams and Karasov, 2001). Japanese quails (*Coturnix japonica*), were switched from a diet of low fibre content to a higher fibre content diet. As a result, the size of the gizzard doubled within 7 days. The authors performed a reverse experiment, and noted a significant down-regulation of the gizzard (Starck, 1999).

Up-regulation of liver size is largely associated with increased nutrient intake (Battley and Piersma, 2005). For example, yellow-rumped warblers (*Dendroica coronata*) were kept on a restricted diet (hypophagia) and as a result, displayed relatively smaller liver size compared to birds that were fed *ad libitum* (Lee *et al.*, 2002). Chaffinches (*Fringilla coelebs)* displayed up-regulation of the liver size during hyperphagia (Donlik and

Blyumental, 1967). It was suggested that the observed decrease in the liver size of chaffinches, occurred due to a combination of, a reduction of the glycogen reserves and an increase in the lipogenesis rate in the liver, corresponding to an alteration of carbohydrate: fat ratios in the diet (Donlik and Blyumental, 1967).

Although there is limited data available on digestive modulation of the pancreas, smaller pancreas sizes have been observed in birds that were kept on a restricted diet (hypophagia) compared to birds that were fed ad libitum (Lee *et al.,* 2002). Furthermore, digestive modulation of intestine mass appears to be variable among avian taxa, for example, in yellow-rumped warblers (*Dendroica coronata*) and pine warbler pine warblers (*Dendroica pinus*), intestine mass was significantly higher in birds maintained on an insect based diet relative to birds maintained on a seed based diet (Afik *et al.,* 1995 Levey *et al.,* 1999). However, in house sparrows (*Passer domesticus*), and Feral pigeons (*Columba livia*) intestine mass was not significantly variable with diet (Caviedes-vidal *et al.,* 2000, Caviedes-vidal *et al.,* 2005).

In accordance with the adaptive modulation hypothesis which generally suggests that, the digestive enzyme activity of an animal is proportional to the relative substrate level in its diet (Diamond and Hammond, 1992), multiple studies to date have demonstrated a positive relationship between digestive enzyme activity modulation and relative dietary substrate quantity in a number of species. The typical digestive enzymes investigated include the pancreatic enzymes; proteases, amylase and lipase, as well as the intestinal brush border membrane enzymes; aminopeptidase-N (APN) , maltase and sucrase (Hulan and Bird, 1972, Karasov, 1996, Levey *et al*., 1999, Lee *et al.,* 2002, Kohl *et al.,* 2016).

The capacity to modulate digestive enzymes appears to vary among avian taxa. For example, while species such as mallards (*Anas platyrhynchos*) and pine warblers (*Dendroica*

pinus) biochemically modulate both intestinal peptidase (APN) and disaccharidases (maltase and sucrase) activity, relative to the respective substrate quantity (Levey *et al.,* 1999, Kohl *et al.*, 2017), a number of passerine species, such as the European starling (*Sturnus vulgaris*), yellow-rumped warbler (*Dendroica coronata*) and house sparrow (*Passer domesticus*), appear unable to modulate disaccharidases (maltase and sucrase) when maintained on a high carbohydrate diet (Martinez del Rio, 1990, Afik *et al.*, 1995 , Sabat *et al.*,1998,Caviedes-vidal *et al*., 2000). Additionally, chickens (*Gallus gallus*) and quails (*Coturnix coturnix*) appear unable to modulate aminopeptidase-N when maintained on a high protein diet (Kohl *et al.*, 2017). Notwithstanding excellent examples of interspecific and phylogenetic variation in enzyme activity in birds (Martinez del Rio *et al.*, 1990, Kohl *et al.*, 2011), it is not yet clear whether modulation of dietary enzymes follows a phylogenetic pattern, a dietary category pattern or neither. However it is worth highlighting that, carbohydrase activity modulation relative to substrate quantity has been negative in all studied passerines to date, except in one species, the pine warbler (*Dendroica pinus*) (Levey *et al.,* 1999, Ciminari *et al.*, 2005).

Dietary flexibility allows birds to often adjust their diets in response to food resources readily available to them (McWilliams and Karasov, 2001). Thus, dietary flexibility is likely to confer selective advantages in unpredictable habitats (Karasov, 1990, McWilliams and Karasov, 2001, McWilliams and Karasov, 2014, Lee *et al.*, 2002). For this reason, the capacity to modulate digestive physiology in response to changes in food availability is likely to be particularly important for birds inhabiting deserts (Maclean, 1984), which usually present extreme climate conditions characterised by very hot summers, mild winters and very low precipitation (Maclean, 1984, Goudie, 2002). Birds residing in such areas are

often faced with consequential physiological challenges, including unpredictable spatial and temporal variation in food abundance (Serventy, 1971, Maclean, 1984).

To the best of my knowledge, studies investigating dietary flexibility, specifically, in passerine species has been conducted in northern hemisphere species such as the yellow-rumped warblers (*Setophaga coronata*), pine warbler (*Dendroica pinus*), white-throated sparrow (*Zonotrichia albicollis*) and house sparrow (*Passer domesticus*) (Afik *et al*., 1995, Levey *et al.*, 1999, Caviedes-Vidal *et al.*, 2000, McWilliams and Karasov, 2014), no study has been conducted in African passerine bird species. To begin addressing the lack of data from Afrotropical taxa, I investigated dietary flexibility in white-browed sparrow-weavers (*Plocepasser mahali*), a ~40-g ploceid passerine wide-spread in the arid zones of southern and east Africa (Fry and Keith, 2004). The diet of this species consists of a combination of protein-rich insects and carbohydrate-rich seeds (Ferguson, 1988), but the dietary composition (proportion of seeds to insects) varies between seasons (Maclean, 1973, Ferguson, 1988).

A study conducted by Ferguson (1988) in the Free State province of South Africa showed that there is a seasonal variation in the proportion of insects to seeds consumed by *P. mahali*, with arthropods contributing up to 36% in summer and 26% in winter. Despite the seasonal variation in terms of the ratio of insect and seeds consumed (Ferguson, 1988, Maclean, 1973), the extent of dietary flexibility in this species remains unknown. In this study I examined phenotypic flexibility of digestive traits in *P. mahali* from the Kalahari Desert in response to diets of varying nutritional content. My study addressed two questions: First, what are the limits to dietary flexibility in response to dietary variation? Second, to what extent does dietary modulation of digestive organ sizes and digestive enzyme activities occur in response to changes in dietary level of insects and seeds?

I addressed these questions based on, whole animal, organ and cellular physiological levels. The first experiment investigated the maintenance of body mass in birds gradually acclimated to diets of different composition [100% seed, 100% insect and the control diet (70% seeds: 30% insects)]. The limit to dietary flexibility here was determined as a point during the acclimation process where individuals lost a maximum of 15% of body mass or presented any signs of poor health (Levey *et al.*, 1999).The second experiment investigated the modulation of digestive organs, as well as the modulation of dietary enzyme activity relative to a diet that consisted of mealworms or commercial wild bird seed as a main constituent (100% seed diet and 100% insect diet).

For experiment one, considering that *P. mahali* typically eats a mixed diet with 26-36% insects (Ferguson 1988). I predicted a limited ability to maintain M_b during the gradual acclimation to the 100% seed and 100% insect diets. However, birds acclimated to the control diet (70% seeds: 30% insects) were predicted to either maintain or gain M_b, because the composition of the control diet is similar to the natural diet of *P. mahali*, which ranged from 26% insects in winter to 36% insects in summer (Ferguson, 1988).

For experiment two, based on data observed from previous studies, I expected the gizzard mass of *p. mahali* to be higher in the seed diet group in order to compensate for the higher hardness/coarseness of the seed diet (Starck, 1999, Dekinga *et al.,* 2001, Starck and Rahman, 2003). However, assuming large differences in food intake rate among the diet groups, I predicted that small intestine mass, liver mass and pancreas mass would remain unmodulated for both diet groups. As regards intestinal enzymes, in accordance with the adaptive modulation theory, I predicted that APN activity would be higher in the higher protein insect diet group. Furthermore, I predicted that maltase and sucrase activity would remain unmodulated in all diet groups as observed in most previously studied passerine

species (Afik *et al*., 1995; Levey *et al.*, 1999; Caviedes-Vidal *et al.*, 2000; McWilliams and Karasov, 2014, Caviedes-Vidal *et al.*, 2000).

CHAPTER 2 MATERIALS AND METHODS

2.1 STUDY SPECIES AND STUDY SITE

I caught white-browed sparrow-weavers (*Plocepasser mahali*) in the Kalahari Desert at Murray Game Ranch, Askham, Northern Cape, South Africa (26° 58' 5" S, 20° 46' 51" E) during autumn (April) 2017 (n=45, M_b at capture = 40.34 ± 3.04 g). The capture site is an arid area with daily maximum air temperatures of up to 43°C, and average annual rainfall of up to 50mm in summer (October to March) (Meteoblue, 2019). To capture the birds, handheld nets were placed over the entrances of roost nests after sunset. The birds were immediately placed in cloth bags following capture, and kept within the field station facilities until sunrise. They were subsequently housed in outdoor aviaries (2.5 m × 2.5 m × 4 m) at the field station for a week, where food (wild bird seed; Marltons Wild Mixed Bird Seed) and live mealworms (*Zophobas morio*) and water were provided *ad libitum*. Thereafter, the birds were transported by road in modified pet crates (0.67 m × 0.51 m × 0.47 m) to the University of Pretoria's Small Animal Physiology Research Facility (S25° 45' 10" E28° 14' 46") where they were housed for the duration of the experiment from May to October 2017.

2.2 BIRD HOUSING AND CARE

Birds were housed in two separate climate-controlled rooms in individual cages (0.6 m × 0.4 m × 0.4 m) at a constant air temperature of 27 °C and a photoperiod of 12 L:12 D. Feeding bowls were placed at the centre of each cage. Water dispensers were placed on the side of each cage at a similar height. The cages were modified to prevent mealworms escaping or seeds being scattered. The birds were allowed an acclimation period of two

weeks while being maintained on an *ad libitum* diet consisting of both commercial wild bird seed (Marltons Wild Mixed Bird Seed) and live mealworms (*Zophobas morio*). I determined the wet and dry mass of the mealworms (1 g wet mass = 0.414 g dry mass) and commercial wild bird seed (1g wet mass = 0.921g dry mass) by drying to constant mass at 55°C (Ecotherm economy, Labotec, Midrand, Gauteng, South Africa).

Following the initial 2-week acclimation period to captivity, I quantified daily food requirement and seed: insect ratio preference. I continued to provide an *ad libitum* diet to all birds, the food was weighed before feeding and orts (left over food particles) were collected every 24 hours after feeding, and subsequently oven-dried and weighed. Daily intake for each bird was the difference between food provided and the orts collected, in dry mass equivalent values. The final average daily food consumed during *ad libitum* feeding was 5 g dry mass consisting of 70% seeds and 30% mealworms. The birds were maintained on this diet for an additional acclimation period of two weeks prior to the onset of experiment one, while monitoring body mass and health status of the birds

2.3 PROXIMATE ANALYSIS OF DIET COMPONENTS

The macronutrient composition of the seeds and mealworms was determined at the University of Pretoria Nutrilab (nutrilab@up.ac.za) (table 1). Crude fat was measured by ether extraction, and crude protein was measured as 6.25 X N content, measured by Kjeldahl method. Starch was measured by Amyloglucosidase/ α-Amylase method, and Crude carbohydrate was calculated by difference from measures of total dry mass, ash, crude fat, and crude protein.

Table 1: Proximate analysis of diet components fed to white-browed sparrow-weavers

Component, units	**Seeds**	**Mealworms**
Ash content, %	3.7	5.2
Crude protein, %	10.2	46.9
Crude fat, %	4.8	35.7
Crude carbohydrate[1] %	81.4	12.2
Starch, %	59.2	n.d.[2]
Gross energy, kJ/g	**18.7**	**29.5**

Notes:

[1]Calculated crude carbohydrate = 100% - ash – fat – protein

[2] Not detected

2.4 EXPERIMENT ONE: LIMITS TO DIETARY FLEXIBILITY

To determine the limits to dietary flexibility, I randomly assigned each sparrow-weaver to one of three experimental diet groups: seed, insect or control (n=15 per group). A 5g dry mass equivalent meal consisting of 70% seeds and 30% mealworms was initially provided to all birds daily. I then progressively increased the ratio of either seed or insect for the seed and insect treatments, respectively, following a series of diet trials (trials A,B,C,D; table 2), each lasting 5-7 days, whereas, the control group continued to receive a diet with the same composition as the initial diet. Orts were collected from a sheet of paper that lined the floor of each cage, on the fourth day of diet trials. The orts were subsequently oven dried at 55 C° (Ecotherm economy, Labotec, Midrand, Gauteng, South Africa). Thereafter, the mealworm and seed components of the orts, were separated using forceps and weighed (model SP602US, Scout Pro, Ohaus, Pine Brook NJ, USA). The mass of the dried seeds and mealworms, was subsequently subtracted from the corresponding 5g dry mass equivalent meal, in order to estimate the exact amount and ratio of the diet components consumed per individual bird. Each bird was weighed (± 0.01 g) prior to feeding, on the fifth day of each diet trial (model SP602US, Scout Pro, Ohaus, Pine Brook NJ, USA), and body mass difference (body mass lost or gained) for each diet group (n=15) was calculated as the difference between the final average group mass at the end of a diet trial, and the initial average group mass preceding each diet trial. An individual's limit to dietary flexibility was determined as a point during experiment one diet trials where the individual lost a maximum of 15% of its body mass relative to its initial body mass. Following experiment one, the birds were reassigned to the control diet (5 grams dry mass consisting of 30% % mealworms and 70% seed) for 10 days.

Table 2: Ratios of seed: insect administered to *P. mahali* during experiment one diet trials. Values are based on dry mass provided.

Diet Group	Trial A (day 0-7)	Trial B (day 7-14)	Trial C (day 14-19)	Trial D (day 19-24)
Seed	70:30	80:20	100:0	100:0
Control	70:30	70:30	70:30	70:30
Insect	70:30	50:50	20:80	0:100

2.5 EXPERIMENT TWO: MORPHOLOGICAL AND PHYSIOLOGICAL BASIS FOR DIETARY FLEXIBILITY

Digestive organ modulation

Once the birds were re-acclimated to the control diet following experiment one, in order to minimize the amount of birds euthanized as per ethical clearance agreement, only 24 birds were randomly selected from the group (n=45) and allocated to one of two experimental diets in experiment two, (n=12), which consisted of either a 100% seed diet for the seed diet group or a 100% insect diet for the insect diet group, and the rest of the birds (n=21) were released back to their natural habitat. These two groups were maintained on their respective diets for 8 days, and subsequently euthanized by cervical dislocation. Digestive organs; intestine, liver, gizzard, and pancreas, were dissected out and immediately kept in ice cold

ringer solution (NaCl 0.17 M). The organs were dry blotted using absorbent paper and weighed on a 4-decimal place balance (model AS 220/C/2 SADWAG, Poland). A metal ruler was used to measure the length of the small intestine, and the small intestine was subsequently partitioned into the distal, medial and proximal sections, the intestine sections were cut open longitudinally and the lumen contents were removed and stored. Vernier callipers were used to measure the width of each section. The various small intestine sections and organs were stored in separate cryovials, snap-frozen in liquid N_2, and subsequently stored in a -80°C freezer (Glacier, -86 degree low temperature freezer, Lasec, South Africa) for 9 months prior to the digestive enzyme assays.

Enzyme assays

Small intestine samples were thawed at room temperature (25°C) and preserved on ice as a precaution against protein denaturation. The intestine sections were subsequently weighed on a 4-decimal place balance (model AS 220/C/2 SADWAG, Poland). The mass of each intestine section was used to estimate the appropriate volume of homogenizing buffer required (8 ml. g^{-1} tissue) (buffer: Mannitol 350 mM, in 1mM Hepes: KOH, pH 7). The intestine sections were homogenised with the homogenizing buffer, for 30 seconds (Omni 5100 homogenizer setting 6, Kennesaw, GA, USA). Assays were separately conducted for the proximal, medial and distal segments of the small intestine, in triplicates along with a blank for each intestine section, using the colorimetric method developed by Dahlqvist (1984) and modified by Martinez del Rio (1990).

Aminopeptidase-N assay

Aliquots of 250 µL of aminopeptidase-N assay mix (L-alanine-P-nitroaniline 2 mM) were pipetted into 1.5 ml snap-cap eppendorf tubes. Thereafter, 2.5 µL of the homogenate was pipetted into the aminopeptidase-N assay mix (L-alanine-P-nitroaniline 2 mM), 2.5 µL was confirmed by preliminary measurements as the preferred homogenate volume to produce linear results. The tubes were subsequently incubated in a 40°C water bath (Sigma Techware, USA) for 20minutes. The tubes were placed at room temperature (25°C) following incubation, and 750 µL acetic acid glacial 2N, was immediately pipetted into each tube to stop the reaction. The tubes were subsequently centrifuged at 10000 rpm for 3 minutes in order to spin down the remaining tissue (C2500-230V, Labnet prism microcentrifuge, USA). Thereafter, 200 µL of the assay contents were pipetted from each tube into a 96-well flat bottom plate, and the absorbance of each assay was read at 380nm using a micro-plate reader (Biotek, A.D.P South Africa, Gauteng, South Africa), which was considered the optimal wavelength for p-nitroalanine in earlier studies (Afik *et al.,* 1995, Levey *et al.,* 1999, Caviedes-Vidal *et al.,* 2000 and Kohl *et al.,* 2016). Aminopeptidase- N activity was calculated based on the absorbance above background using a p-nitroaniline standard curve. A calibration standard curve for aminopeptidase- N was established using a series of p-nitroaniline (0.0625 g/L) standard solution dilutions; 250 µL of each dilution was pipetted into empty 1.5-mL eppendorf tubes along with 750 µL of acetic acid glacial 2N and 2.5 µL of thawed homogenate. Thereafter, 200 µL of the mixture contents were pipetted from each tube into a 96-well flat bottom plate, and the absorbance of each mixture was read at 380nm using a microplate reader (Biotek, A.D.P South Africa, Gauteng, South Africa).

Preliminary kinetic measurements in the middle intestine indicated that the chosen substrate concentrations (2mM) were saturating (V_{max}).

Dissacharidase assay (maltase and sucrase activities)

As a measure of carbohydrate digesting ability we measured activity against the substrates maltose and sucrose. We chose these because of the vast amount of comparative data available for maltase and sucrase activity. We considered isomaltase activity, however the substrate is quite expensive and variation in isomaltase activity would be strongly correlated with variation in sucrase activity because both activities come from the same enzyme, sucrase-isomaltase, another potential carbohydrase is trehalase, however, trehalase activity is apparently very low or nil in most birds (Martinez del Rio, 1990). Thawed intestinal tissue homogenates were diluted (498 µL of homogenising buffer + 2 µL of homogenate). Thereafter, 30 µL of maltose or sucrose, along with 30 µL of the diluted homogenate was pipetted into the 1.5 ml snap-cap tubes and incubated in a 40°C water bath for 20 minutes (Sigma Techware, USA). I subsequently pipetted 400 µL of stop/develop mix (Sigma GAGO20, Sigma-Aldrich chemistry) to each tube, and returned the tubes into incubation for an additional 30 minutes. The tubes were thereafter removed from the water bath and kept at room temperature (25°C), then 400 µL of H_2SO_4 was pipetted into each tube in order to stop the assay reaction. All samples were subsequently centrifuged (C2500-230V, Labnet prism microcentrifuge, USA) at 10000 rpm for 3 minutes in order to spin down the remaining tissue, and 200 µL was pipetted from the assays, into a 96-well flat bottom plate. Absorbance was read at 540nm (Biotek, A.D.P South Africa, Gauteng, South Africa). Maltase and sucrase activity was calculated based on the absorbance values above background as well as a Glucose Standard solution 5.5 Mm calibration curve. For calibration curve, 30 µL of each

dilution combination along with 400 µL of stop/develop mix (Sigma GAGO20,Sigma-Aldrich chemistry) was pipetted into empty eppendorf tubes, and immediately incubated at 40 C° (Sigma Techware, USA) for 30 minutes. After incubation, 400 µL H_2SO and 30 µL of homogenizing buffer was added to each tube. Thereafter, 200 µL of the reaction was pipetted from each tube into a 96-well flat bottom plate, absorbance was read at 540 nm (Biotek, A.D.P South Africa, Gauteng, South Africa).

2.6 DATA ANALYSIS

In experiment one, ANOVA was used to assess the effect of diet [insect, seed and control (n=15)] on quantity of food consumed in diet trials A, B, and C (table 2), Food intake analysis was not conducted for diet trial D because orts were not collected following diet trial D . Paired t-test, where each pair was the average initial M_b and final M_b of a group [insect, seed and control (n=15)], was used to analyse average M_b change for each diet group between the initial (trial A, table 2) and final stages (trial D, table 2) of experiment one. A post-hoc test was used to compare final average M_b (trial D) between the diet groups (n=15). In experiment two, ANCOVA was used to assess the effect of diet group on digestive organ size (gizzard, liver, and pancreas, intestine mass and intestine length) with body mass as a covariate. ANOVA was used to assess the effect of diet group on digestive enzyme activity (APN, Maltase and Sucrase), for each of the intestine sections separately (proximal, medial and distal). A t-test was used to compare summed enzyme activity between the insect and seed diet group (n=15), for APN, maltase and sucrase separately. We also analysed the data for maltase and sucrase activity using non-parameteric Wilcoxon test. Model assumptions including independence, homogeneity of variance, normality of error and linearity were

tested using appropriate tests in R studio. Values are presented as means ± S. D. (*n* = sample size).

CHAPTER 3 RESULTS

3.1 EXPERIMENT ONE: LIMITS TO DIETARY FLEXIBILITY

The quantity of food consumed was similar among all diet groups in diet trials A ($F_{2, 42}$=1.40, P= 0.260) and B ($F_{2, 42}$=0.14, P= 0.870). Daily food consumption varied by diet in trial C ($F_{1, 42}$=5.78, P= 0.006), and post-hoc tests indicated differences between control and seed diet groups (t=-3.15, P= 0.008), and between seed and insect diet groups (t= -2.68, P=0.027), with no difference between the control diet group and insect diet group (t= 0.47, P=0.886).

Diet group emerged as a significant predictor of average M_b change (ΔM_b; lost or gained) between the beginning of trial A and end of trial D, corresponding to the progressive diet shifts to a seed-only or insect-only diet, ($F_{1, 2}$=10.56, P= 0.001), seed diet group (ΔM_b = -1.69, S.D = 2.51, t-value = -2.52), insect diet group (ΔM_b = 3.63, S.D = 4.34, t-value = 3.13) and control diet group (ΔM_b = 1.95, S.D = 2.53, t-value = 2.88). Daily average M_b loss for birds in the seed diet group was equivalent to (~0.07 % of M_b per day), the control diet group gained (~0.08% of M_b per day) and the insect diet group gained (~0.15 % of M_b per day), (table 3), furthermore, a post hoc test indicated that, in the final stage of experiment one (trial D), there was a significant difference in M_b amongst individuals in the seed diet group and insect diet group (t= -4.50, P =0.001) as well as seed diet group and control diet group (t=-4.08, P= 0.010). However, there was no variation in M_b between insect diet group and control diet group (t=1.41, P=0.341) (table 3). Body mass loss for all birds in experiment one was below the experimental limit of <15%.

3.2 EXPERIMENT TWO: MORPHOLOGICAL AND PHYSIOLOGICAL BASIS FOR DIETARY FLEXIBILITY

Digestive organ modulation

Diet group was not a significant predictor of body mass ($F_{1, 22}$ =0.019, P=0.88), but did significantly affect gizzard and liver mass. Individuals in the seed diet group had both larger gizzards ($F_{1, 22}$ =6.188, P= 0.008) and livers ($F_{1, 22}$ = 3.487, P=0.050). However, there were no differences between diet groups in pancreas mass ($F_{1, 22}$ =2.018, P= 0.159), intestine mass ($F_{1, 22}$ = 0.814, P=0.457), and intestine length ($F_{1, 22}$ = 0.448, P= 0.645) (Figure 2).

Enzyme assay

Diet affected APN activity, with the insect diet group having significantly higher APN activities overall along the intestine ($F_{1, 22}$=16.2, P=0.001). The effect of diet varied along the length of the intestine (diet x position interaction: $F_{2, 044}$=3.8, P=0.03). All combinations of paired comparison by diet and position are apparent in the post-hoc comparisons in (Figure 3). APN activity summed over the entire intestinal length was 2.6-fold higher in insect eaters (10.2 ± 1.6µmol/min) than seed eaters (3.8 ± 0.7 µmol/min) (P=0.001, *t*-test) (Figure 6).

Maltase activities were not normally distributed, even after transformations. Therefore, we analysed the data using the non-parameteric Wilcoxon test. Diet had an effect in only one region, the distal section where birds eating seeds had lower maltase activity (*Z*= -3.67, P=0.0002) (Figure 4). In other regions there was no significant dietary effect (all P >

0.05). Maltase summed activity over the entire intestine was somewhat lower in seed eaters than insect eaters (P = 0.02, *t*-test; Figure 6).

Sucrase activities per gram tissue were generally very low compared with other avian species, and were not normally distributed, even after transformations. Therefore, we analysed the data using non-parametric Wilcoxon test. Diet had an effect in only one region, the distal section where birds eating seeds had lower maltase activity (*Z*= -3.8, P=0.0001). In other regions there was no significant dietary effect (all P's > 0.05) (figure 5). Generally, sucrase activity also did not vary with intestinal position, except for a single low value in the distal gut of seed-eaters (χ^2 = 18.1, df= 2, P=0.0001). Sucrase activity summed over the entire intestinal length was not different from insect eaters (P=0.5, *t*-test) (Figure 6).

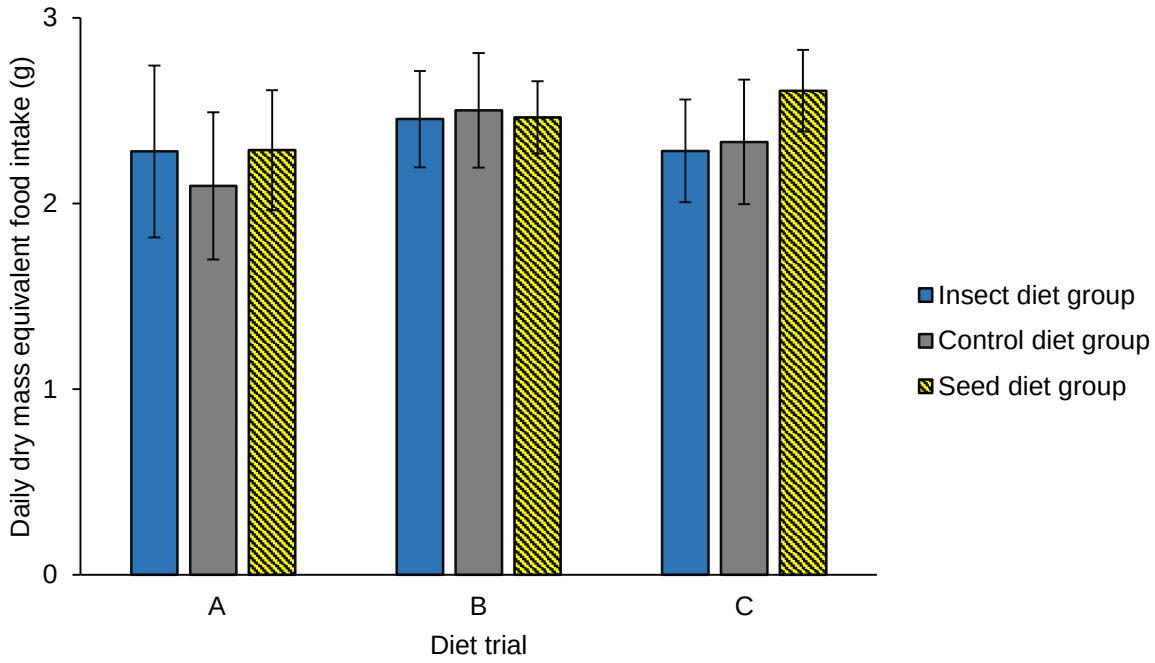

Figure 1: Daily amount of food consumed by *P. mahali* in dry mass equivalent values, for birds maintained on one of three experimental diet groups [seed (yellow, filled and barred) , control (grey, filled) or insects (blue, filled)], n=15, during feeding trials A, B and C of experiment one, error bars represent S.D. The quantity of food consumed was similar among all diet groups in diet trials A and B, and varied by diet in diet trial C.

Table 3: Average M_b of *P. mahali* for the respective diet groups during the initial and final stages of experiment one, and average group mass loss or gained, following progressive diet shifts to a seed-only or insect-only diet, values are represented as S.D.

Diet group	Initial group mass(g)	Final group mass(g)	Average group body mass difference(g) (final mass- initial mass)
Seed	**42.18** ± 3.67	**40.49** ± 2.22	**-1.69** ± 2.51
Control	**41.56**± 3.03	**43.52** ± 2.14	**+1.95** ± 2.53
Insect	**40.64** ± 3.97	**44.28** ± 5.17	**+3.63** ± 4.34

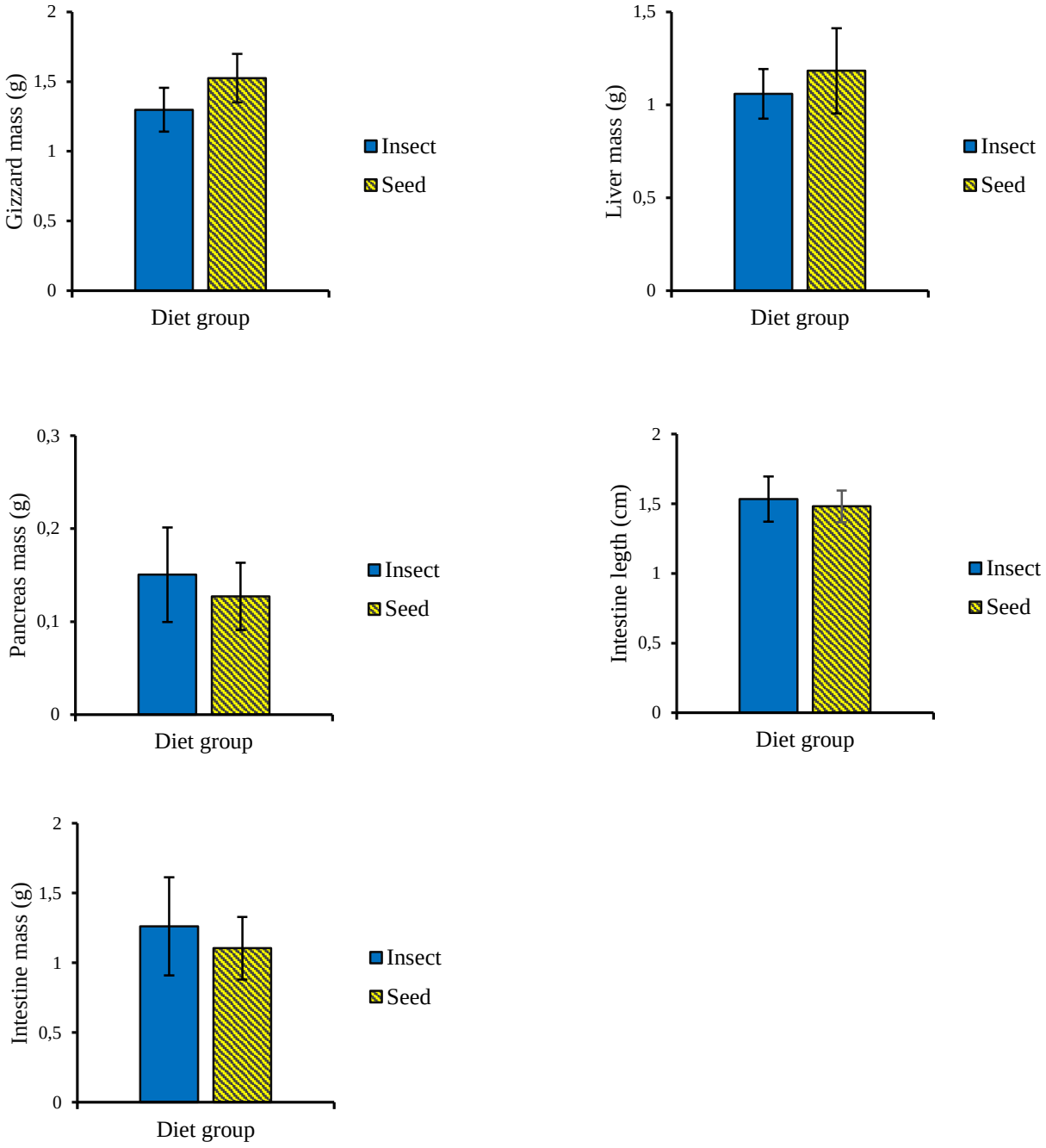

Figure 2. Digestive organ sizes (y-axis) of *P. mahali* after eight days maintenance on a seed-only diet (yellow, filled and barred) or insects-only diet (blue, filled) (x- axis) Error bars represent S.D. Gizzard and liver mass was significantly higher for birds in the seed diet group, whereas, pancreas mass and intestine length was statistically similar among the two diet groups.

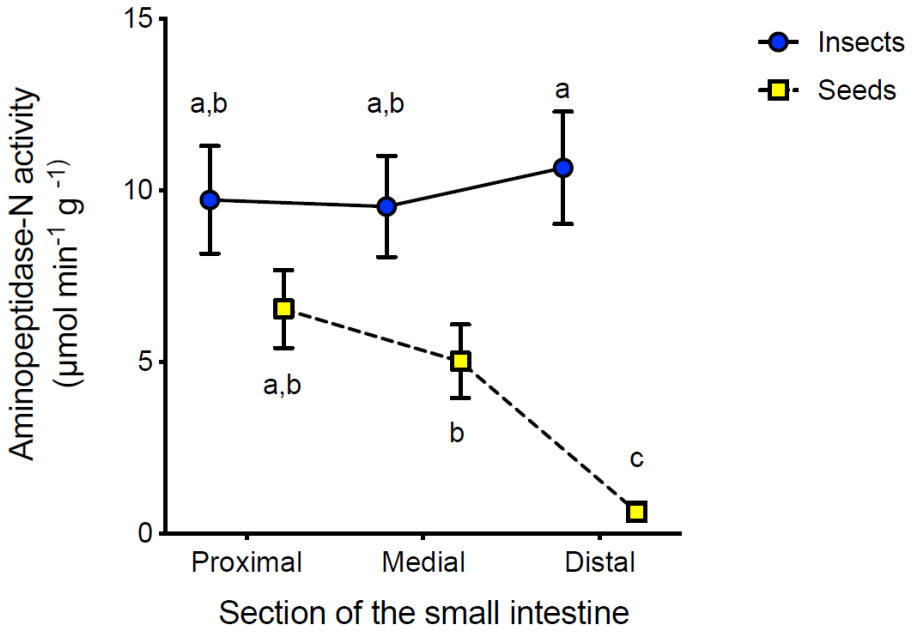

Figure 3. Aminopeptidase-N activity (y-axis, measured @ 2mM substrate concentration) along the small intestine of *P. mahali* (x axis) following 8 days on a seed- only diet (dashed line, yellow, filled, squares) or insects-only diet (thick line, blue, filled, circles). Aminopeptidase-N activity was significantly higher for birds in the insect diet group overall, especially in the distal intestine section. Aminopeptidase-N activity varied with intestinal position in seed-eaters but not insect-eaters. Values that share the same letters are not different by the multiple comparisons test.

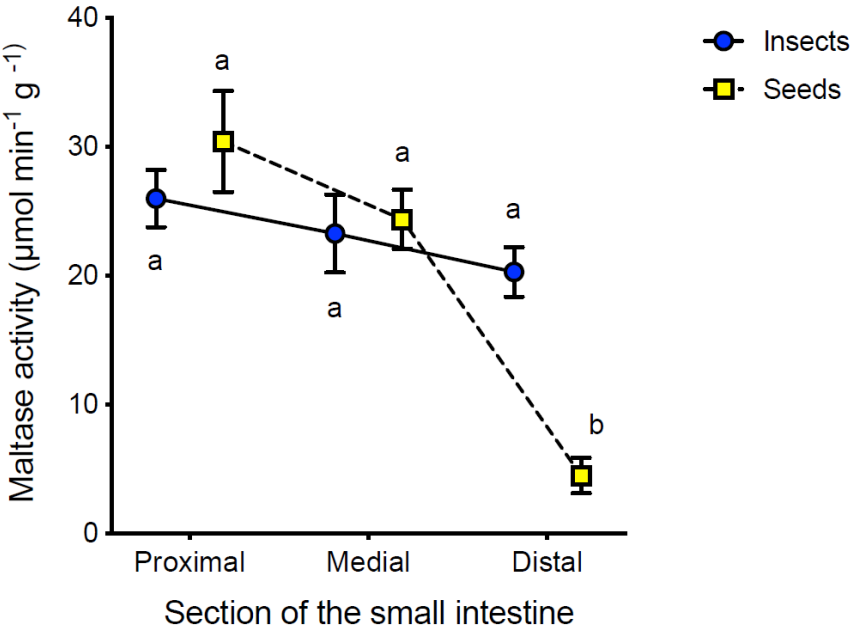

Figure 4. Maltase activity along the small intestine sections (x axis) after 8 days on a seed-only diet or insects-only diet, insect diet group and seed diet group symbols and colours as in Fig. 3). There was no significant variation in maltase activity between the diet groups in the proximal and medial sections, but activity the distal section was significantly higher in the insect diet group. In terms of variation, within each diet group, maltase activity variation between the intestine sections, was statistically similar in the insect diet, and significantly variable in the seed diet group.

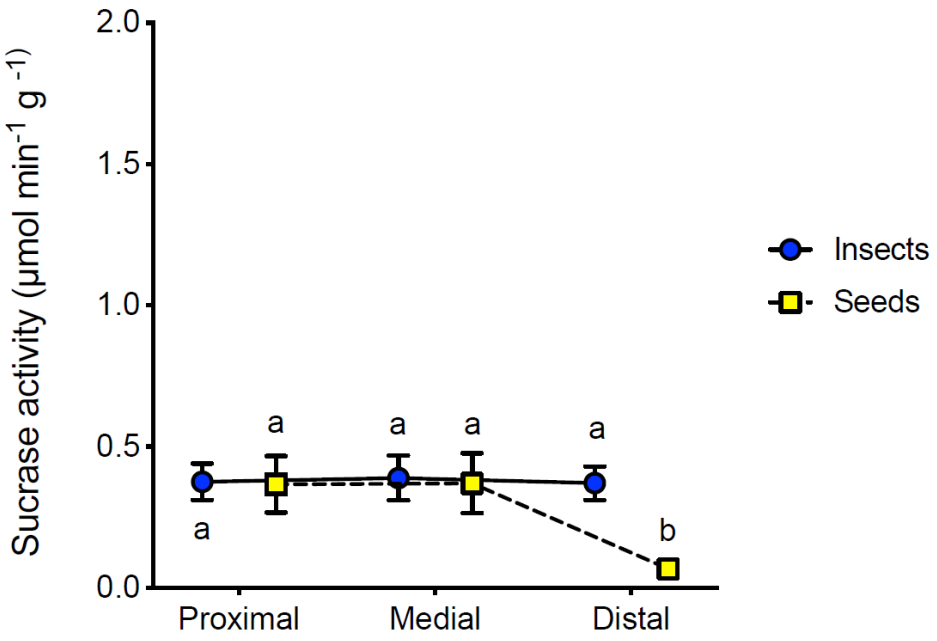

Figure 5. Sucrase activity along the small intestine sections (x axis) after 8 days on a seed-only diet or insects-only diet, insect diet group and seed diet group (symbols and colours as in Fig. 3). There was no significant variation in sucrase activity among the diet groups in the proximal and medial sections, but activity the distal section was significantly higher in the insect diet group. In terms of variation, within each diet group, for both diet groups, sucrase activity was statistically similar, between the intestine sections.

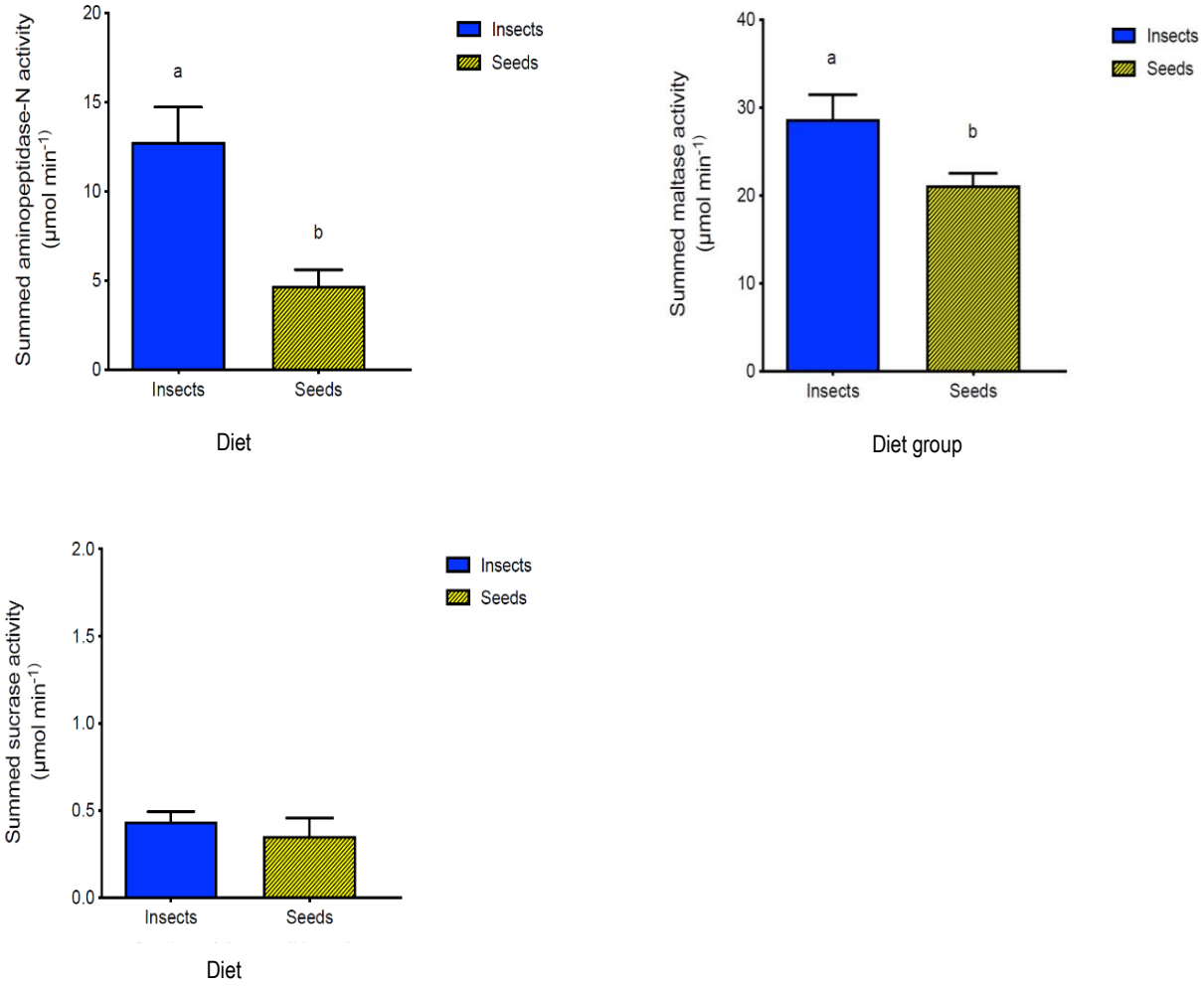

Figure 6. Summed enzyme activity of Aminopeptidase-N activity (y-axis), measured at 2mM substrate concentration, maltase activity and sucrase activity (x-axis), along the entire small intestine length of *P. mahali* following 8 days maintenance on a seed- only diet (yellow, filled and barred) or insects-only diet (blue, filled). Birds in the insect diet group had significantly higher summed Aminopeptidase-N activity and Maltase activity.

CHAPTER 4 DISCUSSION AND CONCLUSIONS

My results reveal that an Afrotropical passerine bird exhibits dietary flexibility that, in broad terms, is qualitatively and quantitatively consistent with that shown by north-temperate species. Sparrow-weavers acclimated to an insect-only diet gained mass, as did individuals maintained on the 70:30 seed: insect control diet. Individuals acclimated to a seed-only diet, however, showed a slight mass loss equivalent to ~0.07 % of M_b per day. The dietary flexibility in the sparrow-weavers involved modulation of both gut morphology and digestive enzyme activity. Flexibility in digestive organ morphology did not involve changes in intestine length, intestine mass, or pancreas mass, but instead comprised significantly higher gizzard and liver mass in birds fed a seed-only diet. Modulation of digestive enzymes involved the predicted significant up-regulation of APN activity among birds maintained on the insect diet, both on a per-gram intestine basis (Figure 3) and summed over the entire intestinal length (Figure 6). The activities of disaccharidases, maltase and sucrase, remained unmodulated in the proximal and medial sections of the small intestine, but unexpectedly, were significantly lower in the distal section of the small intestine among birds in the seed diet group (Figures 4 and 5). We doubt that this relates to gut atrophy associated with lower plane of nutrition in seed eaters, because there were no significant diet-dependent declines in either body mass or entire intestine mass in the distal gut. However, because over longer time periods we do suspect that the 100% seed diet may not be nutritionally adequate, it is plausible that some gut atrophy in poorly nourished birds may have occurred, as has been found in other animals (German *et al.*, 2010).

4.1 EXPERIMENT ONE: LIMITS TO DIETARY FLEXIBILITY

Birds fed the insect and control diets increased M_b by ~0.15 % and ~ 0.08% of M_b per day, respectively, whereas birds in the seed diet group lost ~0.07% M_b per day. The slight mass loss by birds fed a seed-only diet suggests seed alone does not completely meet the nutritional requirements of this species (table 2). Diet composition in wild *P. mahali* is consistent with this notion: the composition of the control diet in the present study approximated that of conspecifics at Bloemhof Dam (27°40' S 25°39' E), whose diets ranged from 26% insects in winter to 36% insects in summer (Ferguson, 1988). However, the ~ 0.08% increase in M_b per day by in the control group may also reflect in part the greater quantity of food consumed during diet trial C (Figure 1) relative to the seed and insect diet groups. Similar results have been reported for yellow-rumped warblers (*Dendroica coronata*) and pine warblers (*D. pinus*) (Afik *et al.*, 1996, Levey *et al.*, 1999), whereby birds maintained on an insect diet maintained higher M_b relative to birds maintained on a seed-diet. However, unlike *P. mahali* in the present study, *D. pinus* exhibited signs of poor health during acclimation to a seed diet, including erected feathers and M_b losses equivalent to ~15% of capture mass (Levey *et al.*, 1999). Although sparrow-weavers acclimated to the seed diet lost ~0.07% M_b per day, which is equivalent to 4 % of their original M_b, the birds appeared to be in similarly good health as individuals in the insect and control groups. It is worth adding that not all passerine species regulate M_b in a similar way to that observed in *P. mahali*, *D. coronata* and *D. pinus*. House sparrows (*Passer domesticus*) and European starlings (*Sturnus vulgaris*), for example, were able to either maintain or gain M_b following

acclimation to insect- and seed-only diets (Martinez del Rio *et al.*, 1995, Caviedes- Vidal *et al.*, 2000).

4.2 EXPERIMENT TWO: MORPHOLOGICAL AND PHYSIOLOGICAL BASIS FOR DIETARY FLEXIBILITY

Modulation of digestive organ morphology

The modulation of gizzard size in *P. mahali*, with birds feeding on a seed-only diet significantly increasing gizzard mass, is consistent with the findings of previous studies, which reported increases in gizzard size among birds maintained on diets with a high fibre content (Savory and Gentle, 1976, Kehoe *et al.*, 1987, Starck, 1999, Starck and Rahman, 2003, Piersma and Drent, 2003). Up-regulation of gizzard size occurs in order to increase its grinding capacity for mechanical digestion of large and/or hard food items (Starck, 1999, Dekinga *et al.*, 2001, McWilliams and Karasov, 2001, Starck and Rahman, 2003). In Japanese quails (*Coturnix japonica*), gizzard size can be up regulated by ~ 230% after approximately four weeks of acclimation to experimental diets (Starck, 1999, Starck and Rahman, 2003). The acclimation period in the present study was eight days, and I thus do not have the data to evaluate whether gizzard size in *P. mahali* was modulated to the maximum extent possible.

The livers of sparrow-weavers maintained on a seed diet were significantly larger than those of individuals maintained on the insect diet. The direction of liver size modulation in *P. mahali* contrasts with that observed in nestling house sparrows (*Passer domesticus*) and Japanese quails (*Coturnix japonica*) (Starck, 1999, Rott *et al.*, 2017). Nestling house sparrows maintained on a high protein diet had larger liver and pancreas sizes than individuals maintained on a high carbohydrate diet. It was suggested by these authors that

this observation is associated with, increasing the digestive capacity of nestling house sparrows maintained on a high protein diet, to process excess amino-acids post-absorption (Starck, 1999, Rott *et al.,* 2017). Japanese quails (*Coturnix japonica*) maintained on diets with varying dietary fibre content showed down-regulation of liver mass with increasing dietary fibre content (Starck, 1999), A subsequent study revealed that liver atrophy in Japanese quails occurred as a result of mobilization of lipids, possibly to fuel metabolic costs associated with modulation of gut size, increased gizzard mass and intestine length. (Starck and Rahman, 2003). However, in another study, the direction of liver size modulation in captive mallards (*Anas platyrhynchos)* (Kehoe *et al.,* 1987) was similar to that I observed in *P. mahali*. The livers of mallards were significantly larger when they were consuming diets consisting of relatively high fibre content (Kehoe *et al.,* 1987). Potential underlying mechanisms included increased glycogen storage, lipid content and bile production (Kehoe *et al.,* 1987). The most obvious explanation for the changes in liver size in *P. mahali* is that, as an accessory digestive organ that stores carbohydrates in the form of glycogen (Donlik and Blyumental, 1967), liver size would be expected to be significantly greater among birds in the seed diet group on account of the high carbohydrate content (table 1). My data thus support the argument by Kehoe *et al.* (1987) that glycogen storage was a source of liver size increases in mallards.

Most of the previously mentioned studies investigating dietary flexibility did not report modulation of pancreas size, with the exception of the pine warbler study and the nestling house sparrows (*Passer domesticus*) study (Levey *et al.,* 1999, Brzek *et al,* 2013, Rott *et al.,* 2017). When nestling house sparrows (*Passer domesticus*) were maintained on a higher protein diet, they showed significant up-regulation of the pancreas size (Rott *et al.,* 2017). However, for pine warblers maintained on diets with varying levels of carbohydrate,

protein and lipid, there was no modulation of pancreas size, this observation was repeated in sparrow weavers as well. Further studies are required in order to draw conclusions on pancreas modulation in digestive physiology of birds.

Contrary to my predictions, intestine length remained similar for both diet groups. Up-regulation of intestine length improves nutrient extraction efficiency by increasing the retention time (Karasov, 1996) and this phenomenon has been reported in a number of avian taxa (Kehoe *et al.*, 1987, Starck, 1993, Starck, 1999, Starck and Rahman, 2003). However, a number of previous studies also reported an absence of modulation of intestine length in avian taxa in response to high dietary fibre (Afik *et al.*, 1995, Levey *et al.*, 1999, Kohl *et al.*, 2017). There even appears to be variation within genera: whereas the intestine length of pine warblers (*Dendroica pinus*) was significantly higher in a seed diet group relative to an insect diet group (Levey *et al.*, 1999). In yellow-rumped warblers (*Dendroica coronata*) intestine length did not vary between diet groups (Afik *et al.*, 1995), contrastingly, the wet intestine mass of yellow-rumped warblers varied significantly between the two diets. The authors suspected that contrasting results occurred due to imprecision in their measurement (Afik *et al.*, 1995). However, further investigation revealed that the intestine mass (wet mass per cm of the intestine) was indeed similar among the two diets (Afik *et al.*, 1995). Additionally, pine warblers were maintained on the experimental diets for an 8-fold longer period than yellow-rumped warblers, 54 days *versus* seven days respectively, which could have contributed to the discrepancy in the results observed among the two species (Afik *et al.*, 1995 Levey *et al.*, 1999). In the present study, I maintained birds on the experimental diet for eight days, a period similar to that in the yellow-rumped warbler study (Afik et al. 1995). These observation suggest that, in addition to diet quality, acclimation period may be an important determinant of the magnitude of digestive organ modulation.

Modulation of digestive enzyme activity

As predicted, APN enzyme activity was significantly higher for birds on the insect diet (47 % protein) compared to birds on the seed diet (10 % protein), a finding supporting my predictions and consistent with those of previous studies that reported significant up-regulation of APN activity in passerine species (Afik *et al.,* 1995, Levey *et al.,* 1999; Martinez del Rio *et al.,* 1995; Sabat *et al.,* 1998, Caviedes-Vidal *et al.,* 2000, McWhorter *et al.,* 2009). In sparrow-weavers, modulation of APN activity along the length of the small intestine differed between the two diet groups: APN activity was approximately constant along the small intestine in birds on the insect diet, but was 7-fold higher in the proximal section relative to the distal section in birds fed a seed diet. Passerines show considerable interspecific variation in patterns of APN modulation along the small intestine (Afik *et al.,* 1995, Levey *et al.,* 1999; Martínez del Rio *et al.,* 1995; Sabat *et al.,* 1998, Caviedes-Vidal *et al.,* 2000, McWhorter *et al.,* 2009). For example, APN activity in house sparrows was highest in the distal section and lowest in the proximal section for both insect and seed diet groups, whereas pine warbler APN activity was highest in the medial section. Furthermore, some studies reported a lack of modulation of APN enzyme activity in avian species with digestive tracts that include ceca (Biviano *et al.,* 1993, Ciminari *et al.,* 1998, Ciminari *et al.,* 2003, Kohl *et al.,* 2017). It has been suggested that the secretion of protein into the cecum promotes microbial growth (Ciminari *et al.,* 2001). Since passerines lack ceca, their guts are instead adapted to efficiently digest protein by up-regulating APN activity when necessary, a trait that appears to be phylogenetically conserved (Ciminari *et al.,* 2001, McWhorter *et al.,* 2009). My data for *P. mahali* are broadly consistent with this notion.

No adult passerine species studied to date increased disaccharidase activity when maintained on relatively high carbohydrate content diet, with the exception of pine warblers (Afik *et al.,* 1995, Martínez del Rio *et al.,* 1995; Sabat *et al.,* 1998, Levey *et al.,* 1999, Caviedes-Vidal *et al.,* 2000, McWhorter *et al.,* 2009). Similar to most adult passerines, *P. mahali* did not up regulate carbohydrase activity when fed high carbohydrate seeds. Also, the sucrase activities were quite low compared to most studied avian species. More studies are required to assess whether *P. mahili* is similar to other avian species that lack sucrase activity (e.g., the starling *Sturnus vulgaris*; Martínez del Rio *et al.,* 1995). Furthermore, for *P. mahali,* it remains surprising that dissacharidase activity in the distal segment was significantly higher in the insect diet group (figure 4, 5). To the best of my knowledge, this pattern has not been reported in any previously studied avian species, however, because it occurred in only a single intestinal region its reliability remains to be confirmed.

Additionally, it has been suggested by Levey *et al.*, (1999) that the modulation of disaccharidases in passerine species requires a relatively longer turnover period than suggested in earlier studies (Afik *et al.,* 1995, Martínez del Rio *et al.,* 1995), which might explain the ability of pine warblers to modulate disaccharidases enzyme activity when maintained on a fruit-based diet (Levey *et al.,* 1999). Sparrow-weavers, however, were maintained on the experimental diets for just eight days compared to 54 days in the pine warbler study. Moreover, for *P. mahali* the carbohydrate content of the seed diet was 7 X that of the insect diet, whereas in the pine warbler study, dietary carbohydrate in the fruit diet was 4- fold higher relative to the insect diet (Levey *et al.,* 1999).

The ability of passerines to modulate dissacharidase activity might be species-specific, or perhaps related to the carbohydrate content of a particular diet. Since my study and similar previous studies followed a similar enzyme assay protocol developed by

Dahlqvist (1984) (Afik *et al.*, 1995, Levey *et al.*, 1999; Martínez del Rio *et al.*, 1995; Sabat *et al.*, 1998, Caviedes-Vidal *et al.*, 2000, McWhorter *et al.*, 2009). I suspect that, as observed with APN modulation, for passerine species, there is interspecific variation in patterns of maltase and sucrase modulation along the small intestine, which explains the ability of *P. mahali* to modulate maltase and sucrase activity in the distal segment.

4.3 CONCLUSIONS

The ability of *P. mahali* to maintain body mass despite variability in diet composition and the modulation of digestive morphology and physiology relative to diet composition, suggests that phenotypic flexibility in digestive function is functionally linked to the seasonal variation in diets consumed by *P. mahali* (Ferguson, 1988). This may also be true for other arid-zone species such as a close relative to *P. mahali*, the sociable weaver (*Philetairus. socius*), in which the ratio of insects to seeds consumed varies seasonally within populations, and spatially between populations (Ferguson, 1988, Ferguson, 1989). The proportion of insects consumed by sociable weavers in South Africa's Free State province, for instance, is higher in dry periods relative to wet periods; 49% in summer and 16% in winter (Ferguson, 1988, Ferguson, 1989), In the Kalahari Desert population, however, insects contributed up to 80% of the diet, while plant matter seldom contributed more than 50% of the overall diet (Maclean, 1973, Ferguson, 1988, Ferguson, 1989). It seems likely that, like *P. mahali*, species such as sociable weavers are phenotypically flexible and able to adjust digestive physiology in response to fluctuating diets.

In arid-zone birds, dietary flexibility may also be indirectly related to water balance in addition to changes in food availability. Arid-zone larks such as the Dune Lark

(*Calendulauda erythrochlamys*) often increase the insect content of their diets during hotter periods of the year, presumably on account of the higher water content (Dean and Ryan 2005). In environments characterised by scarce and unpredictable food resources, the modulation of gut function to maximise nutrient extraction from food is likely to be under strong selection, and desert larks may thus be another good model taxon for exploring dietary flexibility.

To the best of my knowledge, this is the first study investigating phenotypic flexibility of digestive morphology and physiology in an African passerine. My data support my main prediction that *P. mahali* will exhibit limited positive phenotypic flexibility in their digestive physiology. I suspect that the ability of *P. mahali* to modulate some of digestive physiology characteristics explains their ability to maintain energy and nutrient balance despite variable diet compositions observed in different populations or over different seasons. Generally, results from this study are consistent with previous work on dietary flexibility in that dietary flexibility in gut function is likely to be wide-spread if not ubiquitous in birds whose diets vary among populations or among seasons (Afik *et al.,* 1995, Martínez del Rio *et al.,* 1995, Levey *et al.,* 1999, Caviedes-Vidal *et al.,* 2000, Lee *et al.,* 2002, Kohl *et al, 2017*). Also, flexibility may be expected to be strongly selected for in desert species, where food availability and type may vary more unpredictably and rapidly than in environments where food availability is more stable and predictable.

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