

Energetic costs and implications of the intake of plant secondary metabolites on digestive and renal morphology in two austral passerines

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Abstract Seed-eating birds have a diet of high nutritional value; however, they must cope with plant secondary metabolites (PSM). We postulated that the detoxification capacity of birds is associated with a metabolic cost, given that the organs responsible for detoxification significantly contribute to energetic metabolism. We used an experimental approach to assess the effects of phenol-enriched diets on two passerines with different feeding habits: the omnivorous rufous-collared sparrow (*Zonotrichia capensis*) and the granivorous common diuca-finch (*Diuca diuca*). The birds were fed with one of three diets: control diet, supplemented with tannic acid, or supplemented with *Opuntia ficus-indica* phenolic extract (a common food of the sparrow but not the finch). After 5 weeks of exposure to the diets, we measured basal metabolic rates (BMR), energy intake, glucuronic acid output and digestive and kidney structure. In both species, detoxification capacity expressed as glucuronic acid output was higher in individuals consuming phenol-enriched diets compared to the control diet. However, whereas sparrows increase energy intake and intestinal mass when feeding on phenol-enriched diets,

finches had lower intestinal mass and energy intake remains stable. Furthermore, sparrows had higher BMR on phenol-enriched diets compared to the control group, whereas in the finches BMR remains unchanged. Interspecific differences in response to phenols intake may be determined by the dietary habits of these species. While both species can feed on moderate phenolic diets for 5 weeks, energy costs may differ due to different responses in food intake and organ structure to counteract the effects of PSM intake.

Keywords Birds · BMR · Gut size · Kidney · *Opuntia ficus-indica* · Plant secondary compounds

Introduction

Birds that eat seeds with a high nutrient density (Klasing 1998; Sabat et al. 2013; Ríos et al. 2014) often consume high amounts of ‘Plant Secondary Metabolites’ (PSM), such as cyanogenic glycosides, saponins, alkaloids, tannins and other phenolic compounds (Díaz 1996; Karasov and Martínez del Río 2007; Ríos et al. 2012a). These compounds enable plants to protect themselves from consumers by acting as allelochemicals, causing deterrence (Matson et al. 2004; Ríos et al. 2012a, b) or physiological consequences to consumers (Foley et al. 1995; Dearing et al. 2005; Kohl and Dearing 2011; Au et al. 2013; Kohl et al. 2015). For example, tannins have been described that form complexes with dietary proteins in the intestine, thus reducing digestibility, and some also can be degraded in compounds that act as toxins upon being absorbed (Hagerman et al. 1992, Foley and McArthur 1994, Lowry et al. 1996; Niho et al. 2001).

Once inside the body, PSM detoxification and elimination cause a high energy cost for birds (Guglielmo et al.

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1996), which, in turn, could be reflected in higher basal metabolic rates as shown by several studies conducted with mammals (Thomas et al. 1988; Silva et al. 2004). This increase in metabolic rate could be because the organs involved in detoxification (the kidney and liver) contribute significantly to energy metabolism (Maldonado et al. 2009). Most PSM, particularly phenols and terpenes, are absorbed in the small intestine (Green et al. 2005; McLean and Duncan 2006; Skopec et al. 2010) and the detoxification pathway can be divided into two stages: the biotransformation process, mainly regulated by enzymatic action to increase PSM water solubility (Jakubas et al. 1993; Karasov et al. 2012), such as the glucuronide transformation that occurs in intestinal cells, the liver and the kidney (Foley et al. 1995; Shipkova et al. 2001); and secondly the subsequent excretion of these metabolites, where the renal function plays a central role (Foley and Moore 2005; Karasov and Martínez del Rio 2007).

Consumers require the concentrations of PSM in the blood to be relatively low to feed on an ongoing basis (Torregrossa and Dearing 2009). The detoxification process requires the elimination of the conjugated PSM via urine (Foley et al. 1995), and also entails an increase in the amount of ammonia in body fluids (Jakubas et al. 1993; Foley et al. 1995), a compound that requires a large amount of water to be eliminated (Roxburgh and Pinshow 2002). Both processes challenge the kidney to adequately filter the byproducts of biotransformation from the blood and maintain the water balance, respectively. Therefore, given that the processes of detoxification and elimination require significant amounts of water (Dearing et al. 2001), water loss related to detoxification is more challenging for birds inhabiting arid environments. In fact, scarcity of water in the surrounding environment as well as preformed water in desert seeds (Díaz 1996; Ríos et al. 2012a) would create a trade-off between the need to eliminate metabolic waste and maintain adequate hydration. This is evidenced by the degree of specialization of several granivorous bird species to arid environments (Bartholomew and Cade 1963; MacMillen 1990), which may eventually lead to further development of the renal system, such as highly developed medullary tissue. Indeed, we have documented that granivorous passerines have a higher level of development of the renal medulla than insectivorous passerines inhabiting semi-arid environments (Barceló et al. 2012). We suggested that the renal morphology exhibited by the birds could either be due to less water volume in seeds than in insects (Díaz 1996), increased renal activity for the elimination of PSM in seeds, or for both these reasons. In general, adjustments to the kidney's capabilities have been documented for birds that inhabit arid environments or environments

with a high degree of water stress (Casotti and Richardson 1992; Sabat et al. 2006; Peña-Villalobos et al. 2013). Thus, we expected that consumption of high amounts of PSM would lead to larger kidneys with a large fraction of renal tissue allocated to the medulla.

Birds have developed behavioral and physiological strategies that allow them to cope with PSM (Schaefer et al. 2003; Matson et al. 2004; Karasov et al. 2012; Zungu and Downs 2015) such as limiting the amount of food consumed (Gilardi et al. 1999; Ríos et al. 2012a), developing metabolic pathways for PSM detoxification (Jakubas et al. 1993, Guglielmo et al. 1996; Green et al. 2005), increasing the saturation level of one of the PSM metabolic pathways (Ríos et al. 2012b) and avoiding absorption through gut microbiota activity (Kohl 2012). However, to our knowledge, no study has been conducted from a systemic perspective to evaluate the effects of subchronic PSM intake in an integrative manner incorporating the renal and digestive physiology of passerines. Accordingly, in this study we have explored how the interaction between (a) the intake of PSM, (b) the osmoregulation capabilities, and (c) the detoxification capacity and the energy expenditure that this mechanism implies, differ between passerines with different diets.

The rufous-collared sparrow (*Zonotrichia capensis*) and the common diuca-finch (*Diuca diuca*) are two of the most conspicuous passerines in the Mediterranean environments of central Chile (Sabat et al. 1998). The first is an omnivorous species, which has a greater diversity of seeds, insects, and fruits in its diet, including *Opuntia ficus-indica*; while the second is a strictly granivorous bird (López-Calleja 1995; Sabat et al. 1998; Ramírez-Otárola et al. 2011). As such, these two bird species offer a suitable model for studying the role of PSM in shaping the food habits of South American wild passerines and the relationship with the bird's morphology, physiology and energetics. We hypothesized that (a) metabolic expenditure (cost) linked with dietary PSM detoxification will differ between both species, and therefore, (b) the ability to cope with PSM will be closely related to the feeding ecology of the birds. As the natural diet of the sparrows is comprised of items with a higher amount of PSM, we expected this species could modify its energy budget, renal structure and consequently its food intake to deal with the energetic cost and water requirement associated with PSM waste excretion. In turn, because finches' natural diet is composed of seeds low in PSM content, we predicted this species would not exhibit such physiological and morphological changes. As a result, we expected this species would be more affected by the toxic effect of PSM, manifested as deterrence and diuresis, producing a decrease of body mass during acclimation.

Materials and methods

Bird capture and maintenance in the laboratory

Rufous-collared sparrow and common diuca-finch (henceforth referred to simply as the sparrow and the finch, respectively, except in instances when they could be confused with other species of sparrow or finch) used in this study were captured during the fall and winter of 2012 at Quebrada de la Plata (33°30'S, 70°54'W), central Chile. This study site has a Mediterranean climate characterized by hot dry summers (mean precipitation = 5.5 mm) and cold rainy winters (mean precipitation = 214 mm) (mean annual precipitation = 356 mm, di Castri and Hajek 1976). A total of 21 adults per species were collected using mist nets (Ecotone). The birds were transported to the laboratory, housed in individual cages (30 × 30 × 40 cm), kept under a constant temperature (25 °C) and light regime [12 h:12 h (light:dark)] and provided with milled wheat seeds and water ad libitum, and once a week the diet of sparrows was supplemented with *Tenebrio molitor* larvae. After 2 days of habituation to laboratory conditions, the 21 individuals of each species were randomly assigned to one of the three dietary treatments (including a control) for 5 weeks; this is the time that has been shown to produce morphological changes in the kidney (Sabat et al. 2004; Aldea and Sabat 2007). Individuals were weighed weekly to assess their body condition during feeding trials.

Experimental diets

Two diets with added PSM and a control diet were prepared based on milled (particle size ≤ 0.5 mm, Moulinex) commercial wheat seeds. The first experimental diet was supplemented with tannic acid (TA) (Sigma-Aldrich) as a standard phenol, or model substrate, at a concentration of 2 % w/w. This concentration was chosen because it represents a level that maintains a high survival probability in individuals of both species during a subchronic exposure (Ríos 2011). The second diet (OE) was performed by adding known concentrations of an extract of *Opuntia ficus-indica* fruit (hereinafter *Opuntia* fruit), where the final concentration was 0.5 %w/w of total phenols in the diet. The total concentration of phenols in *Opuntia* fruit are of around 2 % and the proportion of this fruit consumed in summer by the common diuca-finch is approximately 30 % (López-Calleja 1995). Therefore, 0.5 % concentration of phenols approximated the phenolic load in free-living common diuca-finches. The alcoholic extract of *Opuntia* fruit was obtained according to Tequida-Meneses et al. (2002) with fruits from the same area, which were crushed and dried at 40 °C. Fruit dry matter (17 % of total) was grounded in

an electric grinder (particle size ≤ 0.5 mm, Moulinex), then dissolved to 6 % mass-volume with a methanol:ethanol (30:70) mixture that was left macerating for 48 h in darkness. The total mixture was then filtered and solvents were evaporated under reduced pressure with a rotary evaporator within a temperature range of 50–60 °C. A final extract of *Opuntia* fruit was obtained (7 % of total) and total content of phenols was measured with Folin-Ciocalteu reagent (Merck, Germany) following the colorimetric assay adapted by Ainsworth and Gillespie (2007). Briefly, the *Opuntia* extract concentrate was diluted with distilled water fifty times prior to measurement to fall into the range of the tannic acid's standard (0–200 mg/l). Then, 100 μ l of the diluted extract, standard or blank sample was mixed with 200 μ l of 10 % (vol/vol) Folin–Ciocalteu reagent and vortexed in a microtube. Immediately afterwards, 800 μ l of 700 mM Na₂CO₃ was added to each tube and incubated at room temperature for 2 h. 200 μ l of each sample was transferred to a microplate and its absorbance measured at 765 nm in a Multiskan GO microplate spectrophotometer (Thermo Scientific, USA). In the case of the blank sample, the extract was replaced with distilled water. Assays were done in triplicate.

To prepare both the TA and the OE diets, known amounts of tannic acid or *Opuntia* fruit final extract were weighed and diluted in absolute ethanol to facilitate the proper mixture with the dry milled wheat used as base food of both the control and PSM diets. The ethanol was allowed to evaporate under a bell jar for 36 h at room temperature with no exposure to light. The control diet in this experiment was likewise treated with ethanol, but without the addition of PSM. The control diet and each treatment diet with PSM were stored in hermetic bags and kept at –25 °C until used.

Feeding trial: food intake and digestibility measurements

After 5 weeks of acclimation to the TA, OE or control diet, cages were covered in the lower half to avoid birds spilling food out, and daily food intake was estimated as the difference in mass between the dry food offered and that which remained the day after, daily excreta was collected from the tray under the cage. Excreta and remaining food were dried at 50 °C in an oven for 48 h and weighed. A sample of excreta of each bird and of each diet type was ground and gross energy was determined using a Parr adiabatic bomb calorimeter (Parr Instrument Co., IL, USA). Apparent metabolizable energy coefficient (AMEc, Guglielmo and Karasov 1993; the term apparent is used because in birds energy from undigested matter cannot be separated from endogenous energy from urine) was calculated

as the difference between daily energy intake (daily food intake times the average energy density of a diet) and daily excreta energy output (dry mass of excreta times the average energy density of excreta of each bird), divided by daily energy intake. Daily water consumption (DWC, in milliliters) was measured, offsetting the loss by evaporation using non-experimental drinkers out of the cage. All measurements were taken for 4 days and averaged to a daily value.

Glucuronic acid in excreta

The glucuronidation pathway is thought to be a major route of phenolic detoxification in birds (Jakubas et al. 1993; Guglielmo et al. 1996; Ríos et al. 2012b). Therefore, on the final day, glucuronic acid was analyzed in the excreta of each bird, for all three diets. Glucuronic acid was measured following the colorimetric assay described by Blumenkantz and Asboe-Hansen (1973) and adapted by Ríos et al. (2012b). Briefly, 100 mg of ground, lyophilized, excreta was vigorously mixed with 10 ml of 0.01 M borate buffer (pH ca. 9.5) by vortex followed by 30 min of centrifugation at 1000 rpm. The supernatant was filtered (#1 Whatman filter paper) and 20 μ l was added into a culture tube and diluted to 200 μ l with distilled water. The culture tube was placed in an iced water bath and 3 ml of 0.0125 M sodium tetraborate-sulfuric acid solution was added and mixed using a vortex and returned to the iced water bath. Tubes were then heated in a water bath at 100 °C for 10 min. After cooling, 20 ml of the 0.5 % aqueous NaOH reagent 3-phenylphenol (Sigma Chemical Co., St. Louis, USA) was added to one set of samples. For the blank sample, the reagent was replaced by 20 ml of 0.5 % NaOH. Assays were made in triplicate. A standard curve was made with known concentrations of glucuronic acid (Sigma Chemical Co., St. Louis, USA). Absorbance was measured at 520 nm in a Shimadzu Mini-UV spectrophotometer.

Basal metabolic rate

BMR was determined using standard flow-through respirometry methods after 5 weeks of acclimation in post-absorptive (6 h after feeding) resting birds during the inactive phase (night) and within the thermoneutral zone of the species. Birds were weighed, put in a dark metabolic chamber (1 l) and then placed in a controlled temperature cabinet (Sable Systems, Henderson, NV, USA) at a constant temperature (30 ± 0.5 °C). We were confident that the ambient temperature of 30.0 °C is within the thermoneutral zone for both species, as we had previously measured oxygen consumption at temperatures ranging from 15 to 35 °C (Sabat et al. 2006; Sabat et al. 2010). The metabolic chamber received dried air at 500 ml/min from a mass

flow controller. The excurrent air passed through columns of Driedrite, CO₂ absorbent granules of Baralyme, and Driedrite before passing through an O₂-analyzer, model Turbo Foxbox (Sable Systems, Nevada, USA) calibrated with a known mix of oxygen (20 %) and nitrogen (80 %) that was certified by chromatography (INDURA, Chile). The mass flowmeter of the Turbo Foxbox was calibrated monthly with a volumetric (bubble) flow meter. The measurement and calibration followed protocols established by Tieleman and Williams (2000). Due to the fact that water vapor and CO₂ were scrubbed before entering the O₂ analyzer, oxygen consumption was calculated as per Withers (1977): $VO_2 = [FR \times 60 \times (FiO_2 - FeO_2)] / (1 - FiO_2)$, where FR is the flow rate in ml/min, and Fi and Fe are the fractional concentrations of O₂ entering and leaving the metabolic chamber, respectively. Body mass (Mb) was measured before the metabolic measurements using an electronic balance (± 0.1 g). Output from the oxygen analyzer (%) and the flow meter were digitalized using a Universal Interface II (Sable Systems) and recorded using EXPEDATA data acquisition software (Sable Systems). Our sampling interval was 5 s. Birds remained in the chamber for at least 6 h until a visual inspection of the recorded data allowed us to determine that steady-state conditions had been reached. We averaged O₂ concentration of the excurrent air stream over a 20 min period after steady-state was reached (following Tieleman et al. 2002).

Renal and digestive tract morphometric

At the end of this experiment, all the birds were killed by CO₂ asphyxiation. Individuals were dissected and had their digestive tract and kidney excised, measured (± 0.05 cm) and weighed (± 0.1 mg). Kidneys were preserved in paraformaldehyde-glutaraldehyde (4 %) and processed by light microscopy. The kidneys were then fixed and dissected to assess the proportion of renal cortex and medulla and the amount, mass and length of medullary cones following Barceló et al. (2012). Mass measurements were also taken for the gizzard, heart, and liver.

Data analyses

To test the effect of species, dietary treatment, and the interaction between these factors on the response variables, we performed a factorial ANCOVA with body mass (Mb) as covariate. However, given that ANCOVA reduces degrees of freedom in one (reducing power), when our analyses exhibited a non-significant effect of Mb, this term was dropped from the model. Therefore, we used a factorial ANOVA to test for differences in body mass, renal medulla, daily water intake, energy intake, AMEc and glucuronic acid output, and an factorial ANCOVA for test for differences in

organ masses and lengths and BMR. Because, physiological and morphological variables represent a power function of body mass (Kleiber 1961; Lasiewski and Dawson 1967), BMR and organ masses were log-transformed before statistical analysis. After ANOVA or ANCOVA, we performed a post hoc (LSD) Fisher test, to check for specific differences among treatments. To test for significant relationships between variables we performed linear regression analysis (LRA). Besides, we performed a test for homogeneity of slopes to evaluate whether the slope for glucuronic output as a function of phenols intake differed significantly between species. We used Statistica version 7.0 (StatSoft 2004) for all analyses, and a value of $P < 0.05$ was considered significant. All values were reported as mean \pm standard deviation (SD).

Results

Body mass and organ masses

After 5 weeks of acclimation to experimental diets, a significant effect for both species and the interaction between species and diets on body mass were found (Table 1). The a posteriori Fisher test revealed that only finches reduced body mass in response to OE diet in comparison with control and TA treatments (Table 1).

The LRA revealed that the heart ($r^2 = 0.73$, $P < 0.0001$), gizzard ($r^2 = 0.27$, $P = 0.001$), liver ($r^2 = 0.43$, $P < 0.001$), cloaca mass ($r^2 = 0.19$, $P = 0.007$), cloaca length ($r^2 = 0.23$, $P = 0.003$), small intestine (SI) mass ($r^2 = 0.63$, $P < 0.0001$) and SI length ($r^2 = 0.26$, $P < 0.001$) were significantly and positively correlated with Mb. ANCOVA revealed that SI mass and length was affected by the interaction between species and diet, but no effect for diet was found. The a posteriori test, showed that finch individuals acclimated to TA had significantly lower SI mass and length than those from the control group ($P = 0.01$, $P = 0.16$; Table 1). On the contrary, sparrows feeding on the TA diet had a significantly higher SI mass than those fed with the control diet ($P = 0.04$, Table 1). Furthermore, ANCOVA revealed that finches had a higher heart mass and SI length than sparrows (Table 1). The remaining organ masses were not statistically affected by species, diets or the interaction between them ($P > 0.1$ in all cases; Table 1).

Kidney morphology and water consumption

Kidney mass was positively correlated with body mass (LRA; $r^2 = 0.5$, $P < 0.0001$). The factorial ANCOVA revealed that kidney mass did not differ among diets, species or in the interaction between diet and species. (Table 1). Nevertheless, the percentage of renal medulla

was significantly affected by species and diet, but not by the interaction between factors (Table 1). Daily water intake was significantly affected by species, but not by diet (Table 1). However, we found a significant effect of the interaction between species and diet. The a posteriori test, revealed that finches fed on the OE consumed 123 % more water than those fed on control diets (Fisher test, $P = 0.01$). In turn, no effect of the dietary treatment on daily water intake was found for sparrows (Table 1).

Basal metabolic rate and energy intake

Basal metabolic rate was positively correlated with body mass (LRA; $r^2 = 0.34$, $P < 0.002$). The results of the factorial ANCOVA revealed there was a significant effect of species ($F_{1,32} = 5.02$, $P = 0.03$), diet ($F_{2,32} = 4.07$, $P = 0.03$) and the interaction between them ($F_{2,32} = 4.22$, $P = 0.02$) on bird's BMR. The a posteriori Fisher test showed that sparrows fed TA and OE diets had a higher BMR than those fed the control diet (TA $P = 0.01$; OE: $P = 0.004$), whereas finches did not exhibit significant differences in BMR among dietary treatments ($P > 0.05$, Fig. 1).

Daily energy intake was not affected by body mass (LRA $r^2 = 0.02$, $P = 0.45$). A factorial ANOVA revealed that in both species, daily energy intake was significantly higher in individuals feeding on PSM diets compared to the control diet (a posteriori Fisher test, <0.05 , Table 1). In the case of sparrows, daily energy intake was 76 and 43 % higher than the control diet for the TA and OE diets respectively, whereas finches energy intake was 9 and 23 % higher for TA and OE diets respectively (Table 1). Moreover, daily energy intake was positively correlated with BMR in sparrows ($r^2 = 0.36$; $P = 0.015$; Fig. 2), but not in finches ($r^2 = 0.15$; $P = 0.16$; Fig. 2).

AMEc was not significantly affected by species, diet nor by the interaction between factors (Table 1). In finches, we found a tendency towards increased AMEc with small intestine length (LRA $r^2 = 0.25$, $P = 0.051$) and small intestine mass ($r^2 = 0.21$, $P = 0.09$; Fig. 3). This tendency was not however evident in sparrows (SI length $r^2 = 0.01$, $P = 0.7$; SI mass: $r^2 = 0.16$, $P = 0.15$; Fig. 3).

Glucuronic acid output

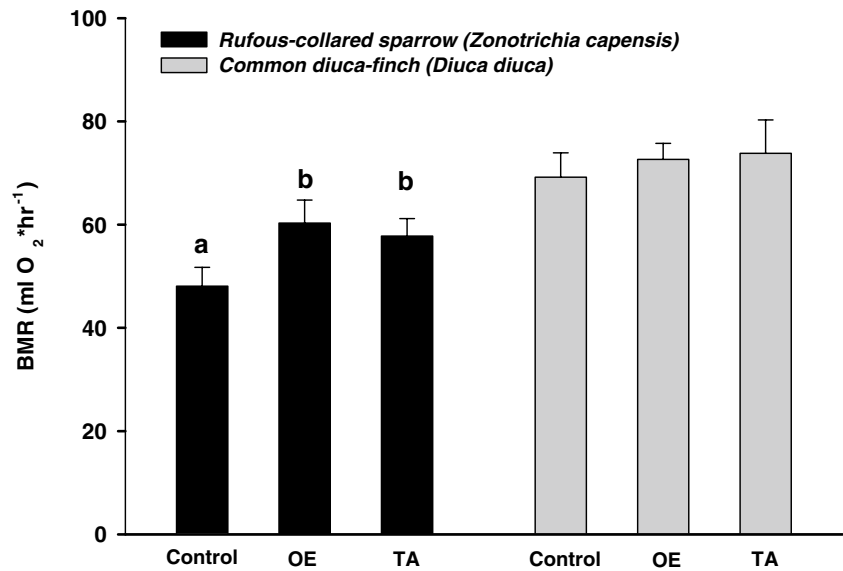
Glucuronic acid output was not significantly correlated with body mass ($r^2 = 0.06$, $P = 0.2$). A factorial ANOVA revealed that, in both species, glucuronic acid output was higher in individuals fed on PSC-enriched diets than in those fed the control diet (Table 1). Moreover, in both species, glucuronic acid output was similarly affected by TA and OE diets ($P = 0.9$, Table 1). Accordingly, we found a significantly positive correlation between phenols intake and glucuronic acid for both species (LRA;

Table 1 Values of measured variables in the three dietary treatments for both species

	Rufous-collared sparrow (<i>Z. capensis</i>)			Common diuca-finch (<i>D. diuca</i>)			Species (df), F, P	Diet (df), F, P	Species x diet (df), F, P
	Control	TA	OE	Control	TA	OE			
	Control	TA	OE	Control	TA	OE			
Body mass (g)	17.93 ± 0.72 ^a	18.53 ± 1.5 ^{a,b}	19.89 ± 1.57 ^b	32.52 ± 2.04 ^A	31.38 ± 0.89 ^A	29.41 ± 3.3 ^B	(1,34), 432, <0.001	(2,34), 0.3, 0.74	(2,34), 6.26, 0.005
Daily energy intake (Kcal)	13.95 ± 1.71 ^a	23.91 ± 4.8 ^b	19.59 ± 1.95 ^b	16.78 ± 5.61	18.3 ± 3	20.88 ± 4.6	(1,24), 0.12, 0.72	(2,24), 6.35, 0.006	(2,24), 3.29, 0.05
AMEc (%)	68.09 ± 8.69	64.43 ± 7.95	61.80 ± 5.95	70.46 ± 9.83	62.24 ± 3.62	68.89 ± 5.16	(1,24), 0.81, 0.37	(2,24), 1.67, 0.21	(2,24), 0.98, 0.39
Glucuronic acid (mg/day g)	0.64 ± 0.15 ^a	2.18 ± 0.24 ^b	1.73 ± 0.45 ^b	0.66 ± 0.04 ^A	1.17 ± 0.34 ^B	1.57 ± 0.15 ^B	(1,26), 2.5, 0.13	(2,26), 9.65, <0.001	(2,26), 1.88, 0.17
Daily water intake (ml)	30.02 ± 5.85	31.77 ± 6.56	23.09 ± 3.26	13.10 ± 4.28 ^A	17.88 ± 2.37 ^A	29.33 ± 1.98 ^B	(1,25), 5.06, 0.03	(2,25), 0.6, 0.56	(2,25), 4.31, 0.02
Small intestine mass (g)	0.66 ± 0.03 ^a	0.79 ± 0.04 ^b	0.74 ± 0.06 ^{a,b}	1.22 ± 0.13 ^A	1.01 ± 0.1 ^B	1.10 ± 0.08 ^{A,B}	(1,30), 0.007, 0.93	(2,30), 0.21, 0.81	(2,30), 5.03, 0.01
Small intestine length (cm)	15.39 ± 0.45	16.05 ± 0.28	15.13 ± 0.17	21.15 ± 2.42 ^A	17.48 ± 0.5 ^B	18.13 ± 0.41 ^B	(1,30), 7.88, 0.009	(2,30), 1.54, 0.23	(2,30), 2.89, 0.07
Cloaca mass (g)	0.05 ± 0	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	(1,30), 0.17, 0.68	(2,30), 0.24, 0.79	(2,30), 1.7, 0.19
Cloaca length (cm)	0.66 ± 0.06 ^a	0.69 ± 0.05 ^{a,b}	0.79 ± 0.03 ^b	0.66 ± 0.05 ^A	0.57 ± 0.02 ^{A,B}	0.50 ± 0.03 ^B	(1,30), 12.77, 0.001	(2,30), 0.15, 0.86	(2,30), 5.08, 0.01
Heart (g)	0.23 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.41 ± 0.02	0.43 ± 0.01	0.35 ± 0.03	(1,30), 17.09, <0.01	(2,30), 1.64, 0.21	(2,30), 2.77, 0.07
Liver (g)	0.76 ± 0.04	0.64 ± 0.03	0.81 ± 0.06	0.93 ± 0.07	1.03 ± 0.08	0.89 ± 0.09	(1,30), 0.003, 0.95	(2,30), 0.21, 0.81	(2,30), 2.57, 0.09
Gizzard (g)	0.89 ± 0.12	0.95 ± 0.09	0.96 ± 0.12	1.03 ± 0.17	1.10 ± 0.09	0.96 ± 0.08	(1,30), 0.26, 0.61	(2,30), 1.65, 0.21	(2,30), 0.89, 0.42
Kidney (g)	0.23 ± 0.01 ^{a,b}	0.21 ± 0.01 ^a	0.25 ± 0.01 ^b	0.31 ± 0.02	0.32 ± 0.02	0.30 ± 0.02	(1,26), 0.85, 0.37	(2,26), 0.23, 0.79	(2,25), 0.81, 0.46
Renal medulla (%)	3.78 ± 0.36 ^{a,b}	2.97 ± 0.48 ^a	4.97 ± 0.45 ^b	5.14 ± 0.48	4.37 ± 0.52	5.36 ± 0.38	(1,25), 8.4, 0.008	(2,25), 5.5, 0.01	(2,25), 0.81, 0.47

Mean ± standard deviation. Significant differences are denoted with different symbols within each species. In bold the significant values of factorial ANOVA/ANCOVA

Fig. 1 Basal Metabolic Rate of *Z. capensis* (black) and *D. diuca* (gray) acclimated to contrasting PSM diets. Letters denote significant differences within species according to ANCOVA. Results expressed as mean \pm standard error. TA tannic acid 2 % diet; OE *Opuntia ficus-indica* phenolic extract diet



sparrow $r^2 = 0.29$, $P = 0.027$; finch $r^2 = 0.31$, $P = 0.04$). Moreover, the slopes for glucuronic output versus phenols intake between species were statistically indistinguishable (ANCOVA; species \times phenols intake: $F_{1, 27} = 0.31$, $P = 0.57$, Fig. 4), whereas the intercept differed significantly between species (ANCOVA; $F_{1, 27} = 36.73$, $P < 0.0001$, Fig. 4). The same pattern was found when we analyzed the glucuronic acid per gram of animal mass as a function of phenols intake; we observed a positive and significant relationship between these variables (LRA; sparrow $r^2 = 0.25$, $P = 0.04$, finch $r^2 = 0.56$, $P = 0.03$), with similar slope and intercept between species (homogeneity of slopes: $F_{1, 27} = 0.02$, $P = 0.88$; intercept: $F_{1, 27} = 25.5$, $P < 0.0001$). The difference in the excreted glucuronic acid per gram of animal reached 300 % in sparrows when consuming PSM, whereas in finches it was only 200 % (Table 1).

Discussion

Digestion, energy costs and detoxification

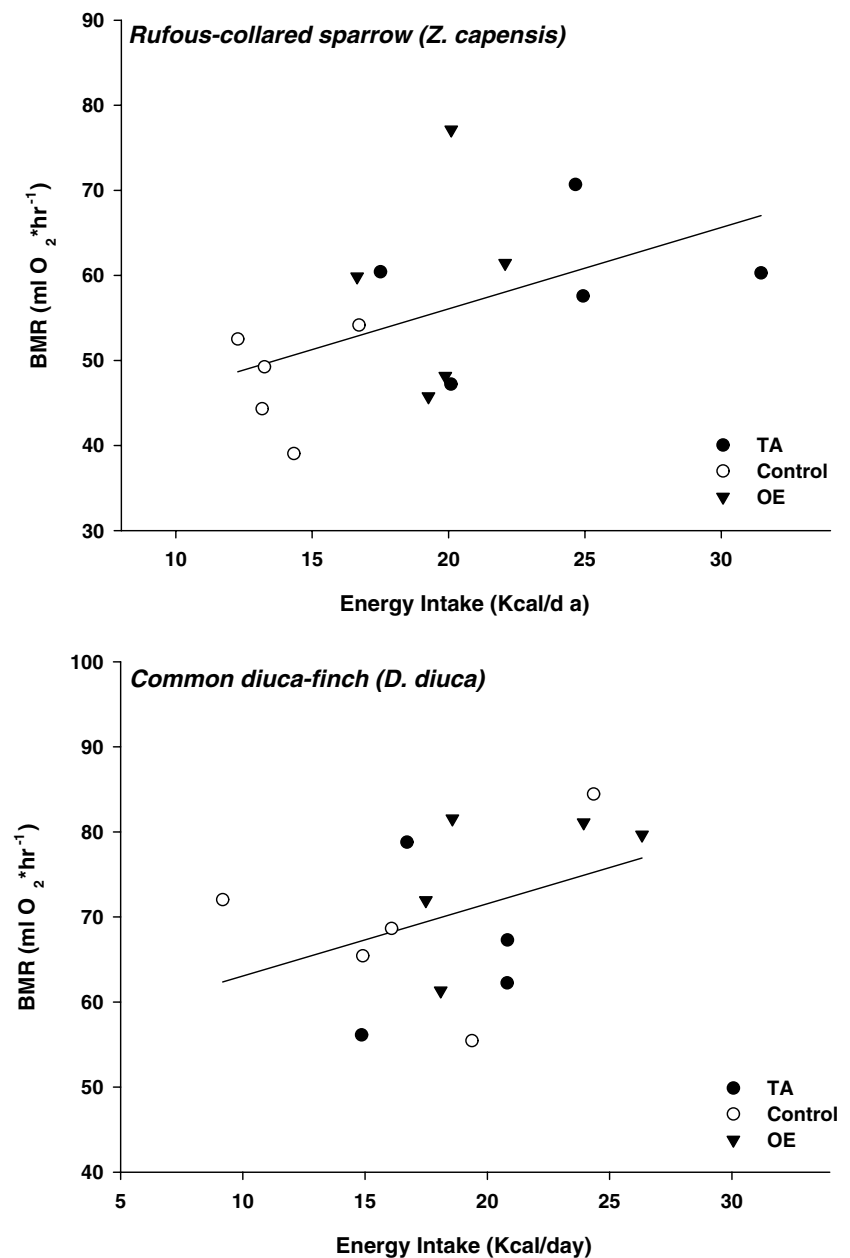
In spite of the fact that glucuronic acid excretion responds in the same way against the phenols intake for both birds (Fig. 4), sparrows at the maximum level of exposure ingested 25 % more phenols than finches, this was caused mainly by higher rates of food intake, which in sparrows exposed to PSM-enriched diets had in average 61 % higher energy intake over the control, while in finches it was just 16 % (Table 1). This gives the first difference in how the two species differently confront the presence of PSM in the diet. According to Foley and McArthur (1994), animals can use two types of strategies to react to consumption of PSM,

depending on if the costs that the consumer pays are pre or post absorptive. Pre-absorptive costs are defined as those in which the PSM prevents a proper ingestion or dilutes the quality of the diet. Post-absorptive costs are those related to costs in detoxification and the excretion of conjugated PSM.

In the present study, post-absorptive effects are more evident in sparrows. Thus, by comparing the different experimental diets, it is seen that this species increases daily energy intake and BMR when fed on both PSM diets, thus keeping its digestibility and AMEc seemingly constant. Hagerman et al. (1992) note that tannic acid may be absorbed and degraded by the body to avoid forming complexes with proteins in mammal's intestines in order to maintain digestibility. This would permit the consumption of food containing PSM, at the expense of an associated energy cost to process toxins. This behavior would fit with the model proposed by Illius and Jessop (1995) in which an animal facing tannic acid consumption should increase its food intake, as a larger amount of energy available for detoxification mechanisms could then be obtained. The increase in food intake has also been observed in other challenged birds that were forced to increase their daily energy expenditure (Van Gils et al. 2008; McWilliams and Karasov 2014; Barceló et al. 2016). This increase in energy intake could be linked to the detoxification process; which involves oxidation, hydroxylation, sulfation or conjugation with glucuronic acid, ornithine, or amino acids, and implies an expenditure of energy for the action of enzymes and synthesis of metabolites, while the kidney actively eliminates the detoxification byproducts (Karasov and Martínez del Río 2007).

An important determinant for avian BMR is the mass of metabolically active organs, such as the intestine and

Fig. 2 Correlation between BMR and daily energy intake for the three contrasting PSM diets. *Z. capensis* (above) and *D. diuca* (below). Abbreviation as in Fig. 1

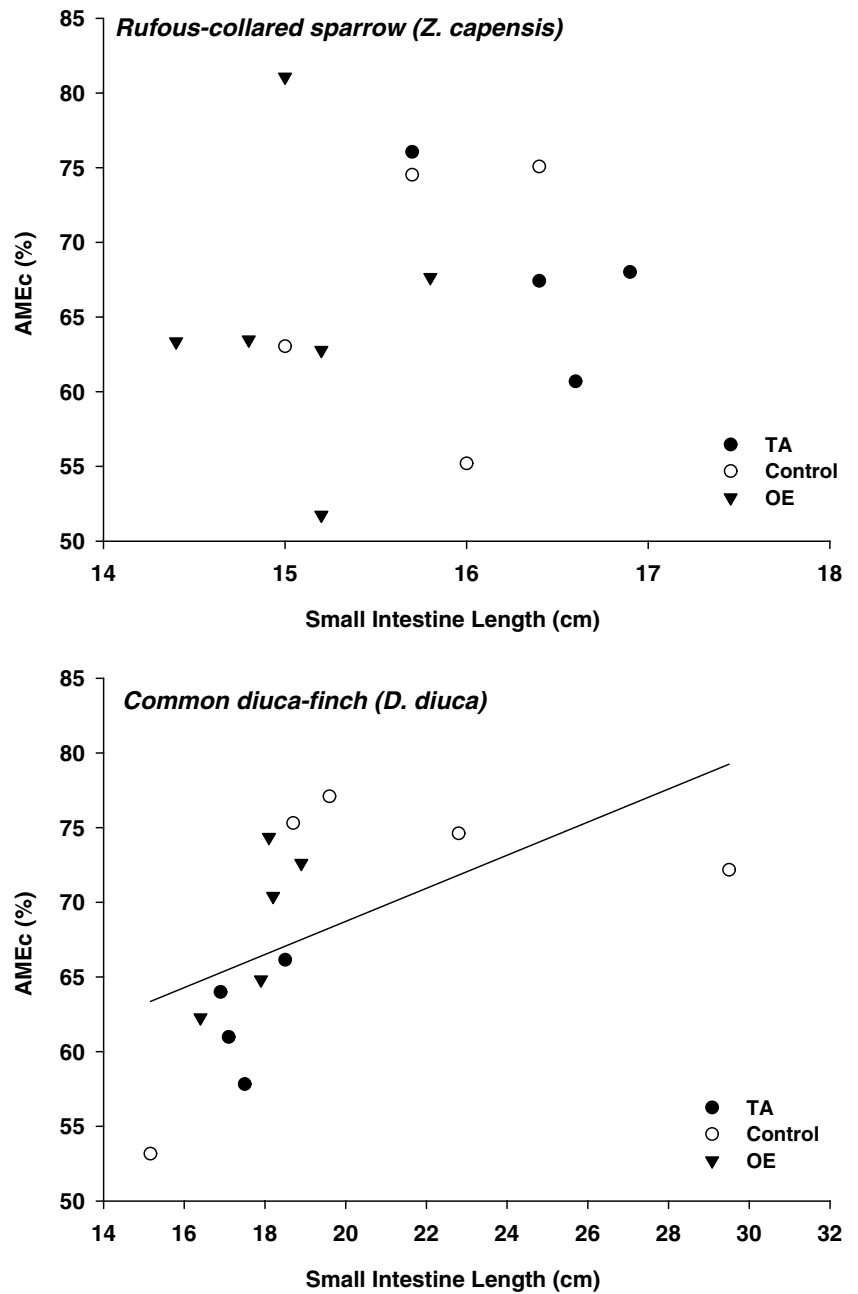


kidney and the specific metabolic activity of these organs (Chappell et al. 1999; Vézina and Williams 2005; Swanson 2010; Zheng et al. 2013). Accordingly, PSM intake in sparrows may be associated with an increase in BMR coupled with an increase in the energy intake and the mass of metabolically active tissue (i.e., kidney and small intestine). These results suggest that the significant higher rates of energy expenditure in PSM acclimated sparrows (Fig. 2) is associated with the increased detoxification demand, which in turn comprises both the energy cost per se, but also the long-term effect of increasing the mass of the kidney and small intestine. It is also possible that the rate of metabolism in sparrows would increase as the

kidney, liver and intestine tissues increment enzymatic activities and thus higher tissue-specific rates of energy expenditure. Further studies are needed to evaluate to what extent the differences in BMR imposed by detoxification of PSM affect the mass-specific metabolic capabilities of the internal organs.

On the other hand, there are several ways to avoid the absorption of ingested PSM that have been documented that could be part of a pre-absorptive strategy, such as the formation of a complex with digestive enzymes (Hagerman et al. 1992; Illius and Jessop 1995), the action of active glyco-protein transporters in the gut epithelial cells (Green et al. 2005; Sorensen and Dearing

Fig. 3 Correlation between apparent metabolizable energy coefficient (AMEc) and length of small intestine in *Z. capensis* (above) and *Diuca diuca* (below) reacting to variable PSM in three different diets. Abbreviation as in Fig. 1



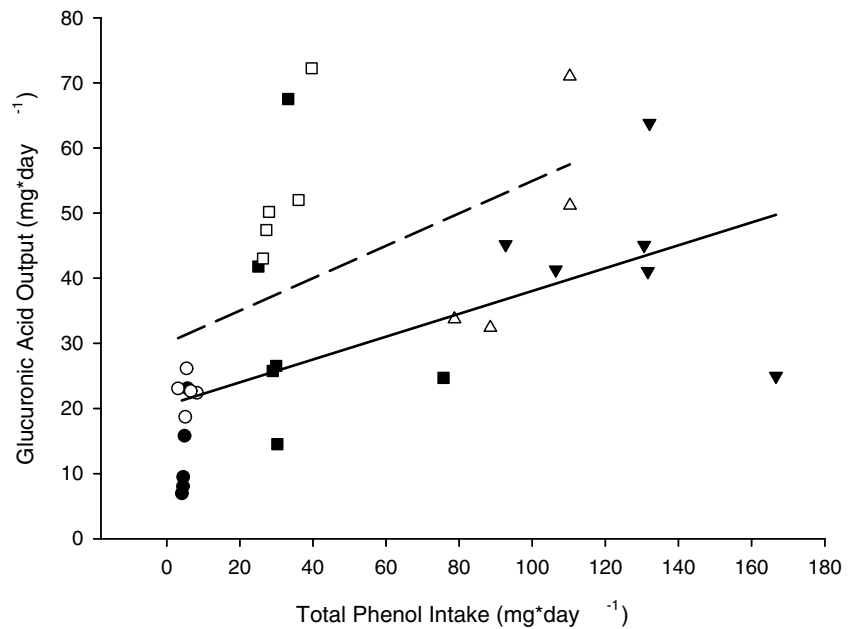
2006), and intestinal microbial metabolism (Kohl 2012). In this study, finch individuals exposed to PSM-enriched diets have lower total mass and length of their gut, which could result in less surface area exposed, lowering PSM absorption. This phenomenon can be explained by the increased paracellular permeability that exists in birds (Caviedes-Vidal et al. 2007), which allows a facilitated absorption of water soluble compounds, nutrients and PSM (Karasov et al. 2012). Our results would support the existence of a trade-off between the energy present in passively absorbed nutrients and the metabolic costs

to remove PSM absorbed collaterally (Karasov et al. 2012).

Kidney morphology and water balance

According to several authors (Bartholomew and Cade 1963; MacMillen 1990; Barceló et al. 2012) strict granivores, as the common diuca-finch, should have a higher tolerance to dehydration and more advanced development of the renal structure and its functions. This capability would allow this species to not rely on foods high in preformed

Fig. 4 Glucuronic acid output regression against total phenol intake by rufous-collared sparrow (*Z. capensis*; black) and common diuca-finch (*D. diuca*; open) feeding on diets with different phenolic composition: tannic acid (triangles), *Opuntia* extract (squares) and control diet (circles)



water, such as the *Opuntia* fruits, which is what occurs for sparrows (López-Calleja 1995). Thus, the need to include dietary items with a higher water content is what would lead the sparrows to include *Opuntia* fruits in its diet, with consequent costs generated by the PSM intake. In this vein, Ríos et al. (2012a, b) found that the average rufous-collared sparrow was more tolerant to PSM than the common diuca-finch and the strict graminivorous (i.e., gramineae seed-eating specialists) many-colored chaco-finch (*Salta-tricula multicolor*).

One important item in the detoxification capacity of PSM, at least for mammals, is water intake (Dearing et al. 2001). The reasoning is that the osmotic load caused by detoxification by-products or the diuretic effects of these compounds should increase water intake (Dearing et al. 2001; Mangione et al. 2000). In our study, effects on different attributes related to water homeostasis, such as water consumption and size of the cloaca, were observed in finch individuals, and the smaller size of the whole intestine to prevent further PSM absorption in finches (Table 1) could also have an impact on water balance. The cloaca mass and length decreases along with lower intestinal sizes which also may affect the water absorption capacity (Schmidt-Nielsen 1997; Braun 2003). The higher rates of water intake in finches feeding on the OE diet, not observed in sparrows, would be a way to compensate for the decrease in water reabsorption capacity of the cloaca, as an indirect response to the presence of PSM in their diets. These results agree with those reported by Dearing et al. (2001) in which the specialist rodent *Neotoma stephensi*, accustomed to eating food with high levels of PSM, did not alter its drinking behavior when given a diet containing PSM; by contrast

Neotoma albigula, a species that usually eats low amounts of PSM in their natural diet, showed PSM intake regulation by substantially increasing water intake.

Additionally, the effects of PSM-enriched diets intake on kidney morphology are more pronounced in sparrows than in finches (Table 1). The apparent use of a post-absorptive strategy by the sparrows could explain, in part, such a pattern. The increased PSM intake by sparrows must be filtered and excreted by the kidney (Foley et al. 1995), which would produce a greater impact on the organ. In this sense, this species has considerable variations in all of the morphological variables related to renal function (Table 1). Nevertheless, the effect on the kidney morphological traits seems to differ between the two PSM-enriched diets. This could be explained by the different nature of the PSM given in each of the diets. In this sense, the *Opuntia* fruit final extract used to form the OE diet includes a greater variety of phenolic compounds as well as alkaloids (Lee et al. 2003; Saleem et al. 2006). Although total concentration of phenols was lower in the OE than in the TA diet, a potential synergistic activity of total PSM mixture content in the OE diet could produce a greater effect on renal morphology.

In this line, the higher water intake in finches, not observed in sparrows, could explain the difference in renal trait response to *Opuntia* extract between both species. Whereas the excretion of by-products of PSM metabolism seems to be coupled to increasing total water intake in finches; in sparrows, it appears to be achieved through modifications in kidney structure, and where this morphological plasticity has been observed it contributes to urine concentration in this species (Sabat et al. 2009).

Conclusions

This study confirms that different mechanisms may be triggered in response to PSM intake in birds. We suggested that these different strategies would be related to the evolutionary ecology of natural populations. Ríos et al. (2012b) state that the common diuca-finch is a less tolerant species to PSM intake. However, our study revealed that this may vary according to the evolutionary history that is related to the natural diet, as shown by previous studies in mammals (Mangione et al. 2000; Samuni-Blank et al. 2014). The observed differing strategies used by the sparrows and the finches to cope with PSM consumption allow them to consume these foods without an apparent decline in their health condition; we only observed a slight reduction in body mass for finches fed with the OE diet. The impact of PSM intake is multi-systemic and various parameters not included should be taken into account in future research. A deeper analysis of energy costs through measurements of daily energy expenditure, surplus energy and maximum metabolic rate, as well as a study of the water balance (e.g., concentration and composition of the urine), could be useful for better understanding the physiological effects of and their limitations to PSM intake in birds. Finally, our results revealed that the organismal response can be differentiated both in direction and magnitude depending on the kind of PSM consumed and also on the physiology and ecology of the consumer. Thus, it is of major importance to perform studies with other ecologically relevant PSM. These may contribute to the specific knowledge of the effects of consuming diets with PSM and go some way towards explaining food preferences among birds.

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