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Mistletoebirds and Xylose: Australian Frugivores Differ in Their Handling of Dietary Sugars

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ABSTRACT

Carbohydrate-rich mistletoe fruits are consumed by a wide range of avian species. Small birds absorb a large portion of water-soluble nutrients, such as glucose, via the paracellular pathway. D-xylose, a pentose monosaccharide, is abundant in some nectars and mistletoe fruits consumed by birds, and it has been suggested that it is most likely absorbed via the paracellular pathway in birds. We measured apparent assimilation efficiency (AE*) and bioavailability (*f*) for D-xylose and D- and L-glucose in three frugivorous Australian bird species. Mistletoebirds, silvereyes, and singing honeyeaters showed significantly lower AE* for D-xylose than for D-glucose. Across two diet sugar concentrations, silvereyes and singing honeyeaters significantly increased *f* of both L-glucose (a metabolically inert isomer of D-glucose commonly used to quantify paracellular uptake) and D-xylose on the more concentrated diet, probably because of increased gut processing time. By contrast, mistletoebirds (mistletoe fruit specialists) did not vary *f* of either sugar with diet concentration. Mistletoebirds also showed higher *f* for D-xylose than L-glucose and eliminated D-xylose more slowly than silvereyes and singing honeyeaters, demonstrating differences in the handling of dietary xylose between these species. Our results suggest that D-xylose may be absorbed by both mediated and nonmediated mechanisms in mistletoebirds.

Introduction

Mistletoe fruit is used by a wide range of bird species, with at least 30 bird species recorded feeding on Australian mistletoe fruits (Reid 1986, 1989; Barker and Vestjens 1990; Higgins et al. 2001). The composition of the fleshy fruit pulp varies among species, but most mistletoe fruits are high in carbohydrates, lipids, and protein (Walsberg 1975; Herrera 1987; McPherson 1987; Witmer 1996; López de Buen and Ornelas 2001; Barea 2008), with 24%–74% of the dry mass comprised of soluble carbohydrates (Godschalk 1983; Lamont 1983; Restrepo 1987; Snow and Snow 1988; Gedalovich-Shedletzky et al. 1989). The sugar composition varies between species, with fructose, rhamnose, mannose, glucose, arabinose, galactose, and xylose present in varying amounts in four Santalaceae and Loranthaceae species studied to date (table 1 and references therein).

Xylose is a pentose monosaccharide that has been reported as one of the most abundant sugars in the fruit of several species of the North American mistletoe families Santalaceae and Loranthaceae and is present in one species of Australian Loranthaceae mistletoe, *Amyema preissii* (table 1). Xylose is also abundant in nectar sugar of two genera of Proteaceae, comprising up to 39% of the total nectar sugar by weight in *Protea* and *Faurea* nectars examined (Nicolson and Van Wyk 1998). The high concentration of xylose in these mistletoe fruits and floral nectars suggests that this sugar could be an important dietary component for nectarivores and frugivores. However, few vertebrates are able to tolerate high concentrations of xylose in food, and it is unclear whether many vertebrates are able to directly metabolize it (Lotz and Nicolson 1996; Franke et al. 1998; Jackson et al. 1998a, 1998b). Xylose appears to be absorbed to some degree in nectar-feeding birds, indicated by reasonably high apparent assimilation efficiencies (AE*) in two southern African species: the Cape sugarbird (*Promerops cafer*; AE* ~53%) and the Cape white-eye (*Zosterops pallidus*; AE* ~61%; Franke et al. 1998; Jackson et al. 1998a, 1998b), although assimilation of sucrose and hexoses including glucose and fructose are still much higher, with AE* close to 100% in most nectarivores and frugivores tested to date (Lotz and Schondube 2006; Fleming et al. 2008; Napier et al. 2008a). Some birds have been shown to exhibit osmotic diarrhea (resulting from unabsorbed xylose) after ingesting pure xylose solutions (Lotz and Nicolson 1996; Jackson et al. 1998b; Jackson and Nicolson 2002). While nectar-feeding bird species tested to date have shown avoidance of pure xylose solutions (Lotz and Nicolson 1996), a southern African mammal pollinator, the Namaqua rock mouse (*Aethomys namaquensis*), freely consumed xylose solutions with an AE* of 97% (Johnson et al. 1999). However, xylose has nutritional value only if it is able to be absorbed

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Table 1: Relative sugar composition (% dry mass \pm SEM) of mistletoe fruit viscin

Family and species	Harvest region	Total sugar (% dry mass)	Sucrose	Glucose	Fructose	Rhamnose	Raffinose	Xylose	Galactose	Arabinose	Mannose	Source
Santalaceae:												
<i>Viscum album</i>	France			61.6	1.1	.9		4.0	12.5	10.9	10.4	1
<i>Phoradendron californicum</i>	USA	51 \pm 2		14.4	Trace	2.3		36.1	15.6	19.4	11.1	2
<i>Arceuthobium americanum</i>	Canada	55 \pm 4		6.8	Trace	5.7		39.5	20.3	24.4	3.4	2
Loranthaceae:												
<i>Phthirusa pyrifolia</i>	Canada*	24 \pm 1		43.5		4.4		32.7	5.4	11.8	2.2	2
<i>Amyema preissii</i>	WA		+7.4	+34.4	+58.2		+	+	+			3
<i>Amyema miquelii</i>	WA		5.0	38.3	56.7							3

Source. 1, Azuma et al. 2000; 2, Gedalovich-Shedletzky et al. 1989; 3, K. R. Napier, D. J. Perry, P. S. Solomon, P. A. Fleming, and T. J. McWhorter, unpublished data.

Note. Viscin is the mucilaginous tissue that surrounds the endosperm and embryo of mistletoe fruit. Sugar compositions for *Amyema* species (K. R. Napier, D. J. Perry, P. S. Solomon, P. A. Fleming, and T. J. McWhorter, unpublished data) were obtained from high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), with a plus sign indicating presence, as determined by GC-MS. Sucrose, glucose, and fructose were the only three sugars detected with confidence by HPLC. WA, Western Australia.

*Fruits obtained from plants grown in a greenhouse.

and metabolized. The mechanisms of absorption of this sugar are therefore of direct interest in interpreting how animals deal with the presence of xylose in nectar and fruit.

There are two routes for sugar absorption at the intestine: via carrier-mediated transport through the intestinal epithelial cells themselves (the transcellular route) or diffusion between these cells (the paracellular route). Small birds and bats with reduced intestinal absorptive surface area as an adaptation to flight (Caviedes-Vidal et al. 2007) absorb a large portion of their water-soluble nutrients (e.g., glucose) via the paracellular pathway (reviewed by McWhorter 2005). Paracellular absorption provides a nonsaturable absorptive mechanism that matches absorption capacity to acute changes in dietary nutrient concentration (Afik et al. 1997; Ferraris 2001). The relative contribution of paracellular to total glucose absorption in nectarivorous birds increases with an increase in diet sugar concentration, an effect that may be largely due to increased digesta retention time of more energy-dense nectars in the intestine (McWhorter et al. 2006; Napier et al. 2008b). Frugivorous birds and bats also rely extensively on the paracellular pathway for the absorption of glucose (Tracy et al. 2007; McWhorter et al. 2010), but the effects of diet energy density and thus digesta transit or residence times have not been assessed in frugivorous species to date.

The mechanism of absorption of xylose appears to vary among species and probably includes both paracellular and carrier-mediated components. There is evidence that intestinal absorption of xylose in chickens, hamsters, rats, frogs, rabbits, and cows occurs at least in part by active carrier-mediated transport; it requires energy expenditure, and it is Na⁺ dependent and inhibited by phlorizin (Salem et al. 1965; Alvarado 1966; Lassen and Csaky 1966; Heyman et al. 1980; Scharrer and Grenacher 2000). However, in humans, xylose appears to be absorbed only via passive (i.e., paracellular) diffusion (Ohkohchi et al. 1986; Fine et al. 1993). It has been suggested that xylose is likely to be absorbed via the paracellular pathway in

nectarivorous birds (Jackson and Nicolson 2002), although no direct uptake measurements have been done.

We investigated the AE* of D-xylose (assumed to be absorbed by paracellular mechanisms) and D-glucose (absorbed by both paracellular and transcellular pathways) in three bird species: a mistletoe fruit specialist, a generalist frugivore, and a generalist nectarivore that also takes mistletoe fruit. We also explored the mechanism(s) of absorption by assessing the bioavailability of radiolabeled L-glucose (an isomer of D-glucose that is not metabolized and is absorbed only by the paracellular pathway) and D-xylose. We predicted, first, that all three study species would exhibit high AE* for D-glucose but lower AE* for D-xylose (as shown in previous studies; e.g., Lotz and Nicolson 1996; Franke et al. 1998; Jackson et al. 1998b). Because L-glucose is absorbed only via the paracellular pathway (Chang and Karasov 2004), a high bioavailability of L-glucose is indicative of significant paracellular uptake of D-glucose. Therefore, we further predicted that if D-xylose is also absorbed solely by the paracellular pathway, both L-glucose and D-xylose bioavailability would increase with diet sugar concentration.

Material and Methods

Study Species

Our study species were three Australian bird species that are known to ingest mistletoe fruit (Chaffer 1966; Paton and Ford 1977; Bernhardt 1984; Forde 1986; Reid 1986). The mistletoe-bird (*Dicaeum hirundinaceum*) is a specialized frugivore that feeds primarily on mistletoe fruit (Richardson and Wooller 1988). The short, specialized alimentary tract of the mistletoe-bird is typical of specialized frugivores; mistletoebirds possess smaller and less muscular gizzards than insectivorous birds of similar sizes, and the gizzard, proventriculus, and duodenum are all in the same plane, which allows the more direct and rapid processing of the large number of mistletoe fruit that are ingested (Richardson and Wooller 1988).

The silvereye (*Zosterops lateralis*) is a generalist frugivore (Wilkinson 1931; Thomas 1980; Richardson and Wooller 1986), and the singing honeyeater (*Lichenostomus virescens*) is considered a generalist nectarivore (Collins and Morellini 1979; Richardson and Wooller 1986). The digestive tract of honeyeaters is less specialized than that of the mistletoebird (Richardson and Wooller 1988). Silvereyes and singing honeyeaters show no distinct morphological adaptations for frugivory, with intestine lengths expected for nectarivorous birds of their size and more muscular gizzards that fall between those of specialist frugivores and those of specialist insectivores (Richardson and Wooller 1986; Stanley and Lill 2002). The junction of the gizzard and duodenum is also on a separate plane from that of the proventriculus and gizzard in these species (Richardson and Wooller 1988).

The transit time of mistletoe seeds is shorter in Australian specialist mistletoe feeders, such as the mistletoebird and painted honeyeater (*Grantiella picta*), than in more generalist frugivores and honeyeaters. For example, the mean (\pm SE) gut passage time of *Amyema quandong* mistletoe seeds by the spiny-cheeked honeyeater (*Acanthagenys rufogularis*), an opportunistic mistletoe fruit feeder in Australia, is 40.57 ± 0.60 min, nearly three times longer than for the mistletoebird at 13.67 ± 0.48 min (Murphy et al. 1993) and nearly twice as long as painted honeyeaters at 24.43 ± 1.27 min (Barea 2008). Keast (1958) also reported longer voiding times in silvereyes feeding on *Amyema miquelii* and *Amyema gaudichaudii* fruits (30–80 min) compared with mistletoebirds (25–60 min). Seed transit times for silvereyes feeding on *Coprosma quadrifida* and *Rhagodia parabolica* fruits (slightly smaller than *Amyema preissii* fruit) average 18 and 31.5 min, respectively (French 1996; Stanley and Lill 2002).

Silvereyes (body mass [M_b] = 9.0 ± 0.4 g) and singing honeyeaters (M_b = 28.9 ± 4.1 g) were captured on the grounds of Murdoch University, Perth, Western Australia (32°04'S, 115°50'E) by mist netting in May 2009 and January 2010, respectively. There is no measure of sexual dimorphism by plumage in either species, and we did not conduct genetic sexing, so sex is not known for these species. Mistletoebirds (M_b = 8.7 ± 0.6 g) were captured on a private property at York, Western Australia (31°50'S, 116°44'E) in December 2010 and January and March 2011. All birds were acclimatized to outdoor captive conditions for at least 2 wk before the commencement of experimental trials. All three species were fed a maintenance diet of Wombaroo nectarivore mix (Wombaroo Food Products, Glen Osmond), which contains sucrose as the main sugar type, supplemented with additional sucrose or equal parts of glucose and fructose for a total sugar content of ~25% w/w dry matter. Silvereyes and mistletoebirds were also fed a variety of fleshy fruits daily (e.g., mistletoe fruit, watermelon, grapes, apricots).

Birds were housed in individual outdoor aviaries (116 cm \times 160 cm \times 210 cm) and confined to individual cages (47 cm \times 54 cm \times 41 cm) within the aviary for apparent assimilation efficiency (AE*) and gut passage time experiments. During pharmacokinetic experiments, birds were housed individually in opaque plastic cages (42 cm \times 54 cm \times 50 cm) with an automatic lighting regime (12L : 12D) and a one-way

mirror to minimize disturbance during sample collection. Excreta were collected from waxed paper that was rolled through slots in the bottom of the cage, allowing samples to be collected immediately upon defecation.

All animal care and experimental procedures were approved by the Murdoch University Animal Ethics Committee (approval R2175/08).

Apparent Assimilation Efficiency

Seven silvereyes, eight singing honeyeaters, and five mistletoebirds (three male, two female) fed ad lib. from D-glucose : D-xylose (4 : 1) solutions at three concentrations (0.25, 0.5, 1 mol L⁻¹ total sugar) for 24 h through inverted, stoppered syringes. Each bird fed from each solution with sugar concentration randomized and trials commencing within 30 min after sunrise (0500–0716 hours Australian Western Standard Time). Maintenance diet was removed 1 h before sunrise to ensure that all ingested food was voided before trials commenced. Trays were placed under experimental cages to collect excreta, and small containers of liquid paraffin were placed directly below feeders to collect any diet spilled. Food intake was recorded over 24 h by weighing feeders (0.01 g). Dried excreta were reconstituted with a recorded amount of distilled rinse water. AE* was estimated separately for glucose and xylose as

$$AE^* = \frac{\text{sugar}_{\text{in}} - \text{sugar}_{\text{out}}}{\text{sugar}_{\text{in}}}, \quad (1)$$

where sugar_{in} (mg) is the concentration (mg mL⁻¹) of sugar in the ingested diet multiplied by the volume of solution ingested (mL), and $\text{sugar}_{\text{out}}$ (mg) is the sugar concentration (mg mL⁻¹) in the total volume of excreta plus rinse water (mL).

Glucose assays. Two replicates of each excreta sample (100 μ L) were incubated at room temperature (~21°C) for 15 min with 500 μ L of hexokinase-glucose-6-phosphate dehydrogenase enzymatic assay reagent (G3293, Sigma Aldrich, Castle Hill). Standard reagent blanks were included for each excreta sample. Absorbance was then measured at 340 nm relative to distilled water by spectrophotometry (ultraviolet mini 1240, Shimadzu Scientific Instruments, Balcatta).

Xylose assays. Two replicates of each excreta sample (50 μ L) and two standard reagent blank samples were incubated at room temperature (~21°C) for 10 min with 200 μ L of triethanolamine/MgCl₂ buffer solution, 200 μ L of nicotinamide adenine dinucleotide/adenosine triphosphate solution, and 10 μ L of hexokinase suspension (D-xylose assay kit, Megazyme, Wicklow) in order to remove any D-glucose present in the sample. Absorbance (A1) was then measured at 340 nm relative to distilled water by spectrophotometry. The xylose detection reaction was then initiated by the addition of 10 μ L of β -xylose dehydrogenase/xylose mutarotase solution and incubated for 10 min. Absorbance (A2) was then measured at 340 nm relative

to distilled water by spectrophotometry, with the absorbance difference calculated ($A_2 - A_1$).

Pharmacokinetic Experiments

Bioavailability of L-glucose and D-xylose was measured using [^{14}C] and [^3H] radiolabeled L-glucose ($180.16 \text{ g mol}^{-1}$) and D-xylose ($150.13 \text{ g mol}^{-1}$), administered orally and by intramuscular injection to eight silvereyes, eight singing honeyeaters, and five mistletoebirds (three male, two female), as described below. Oral and intramuscular trials were performed in separate experiments. To vary food intake rate, birds received two diet sugar concentrations (0.25 and 1 mol L^{-1} hexose solutions) in separate feeding experiments. Both the order of trials and the treatments given were randomly assigned and followed published protocol (McWhorter et al. 2006; Napier et al. 2008b). Bioavailability (f) was calculated as

$$f = \frac{P \times S \times K_{el}}{I}, \quad (2)$$

where P is the steady-state feeding concentration of radiolabeled sugars in plasma (disintegrations per minute [dpm] mg^{-1} of plasma), S is the probe distribution space of radiolabeled sugars in plasma (mg of plasma), K_{el} is the elimination rate constant for the removal of radiolabeled sugars from plasma and its excretion in urine (min^{-1}), and I is the ingestion rate of radiolabeled sugars (dpm min^{-1} ; Karasov and Cork 1994; McWhorter et al. 2006; Napier et al. 2008b).

For intramuscular administration, each bird was injected into the pectoralis muscle with $\sim 40 \mu\text{L}$ of a solution of 175 mmol L^{-1} NaCl containing $500 \text{ KBq } [^{14}\text{C}]\text{-L-glucose}$ per mg M_b , or $2,220 \text{ KBq } [^3\text{H}]\text{-D-xylose}$ per mg M_b . The osmolality of the intramuscular injection solution was controlled at approximately 350 mmol kg^{-1} , so that the solution was isosmotic with avian blood (Goldstein and Skadhauge 2000). After intramuscular administration, excreta were collected continuously for $\sim 2 \text{ h}$, followed by collection of a small blood sample from the brachial vein. The parameters for the mono- and biexponential models were derived for each individual by nonlinear curve fitting of the concentration of radiolabeled sugars in excreta after intramuscular administration versus time, by use of the Marquardt-Levenberg algorithm (Marquardt 1963). For oral administration, birds fed in separate trials from a hexose solution containing radiolabeled sugars ad lib. for $\sim 3 \text{ h}$ ($^3\text{H-D-xylose}$: 120 and 200 KBq mL^{-1} ; $^{14}\text{C-L-glucose}$: 60 and 90 KBq mL^{-1} for 0.25 and 1 mol L^{-1} hexose diets, respectively). One small blood sample was collected 3 h after introduction of the radiolabeled diet; four silvereyes, eight singing honeyeaters, and four mistletoebirds (three male, one female) reached steady state with regard to radiolabel ingestion and excretion by 90 min , after returning to steady-state feeding within 30 min after introduction of the radiolabeled diet (measured through the collection of excreta approximately every 15 min ; data not shown). The final analyses were conducted on these individuals only, since four silvereyes and one mistletoebird did not return

to steady-state feeding despite repeated attempts at oral trials, violating this assumption (Napier et al. 2012).

Gut Passage Times

Eight silvereyes, eight singing honeyeaters, and five mistletoebirds (three male, two female) were offered fresh, ripe mistletoe fruits on branches, either *A. miquelii* (singing honeyeaters) or *A. preissii* (mistletoebirds and silvereyes), depending on time of year and seasonal availability (the bird species were not held simultaneously because of space and logistic constraints). Birds were then observed via video camera, and the gut passage time of mistletoe fruits was determined by recording the time of ingestion and defecation of mistletoe fruits and calculating the difference; it was assumed that fruits were defecated in the order they were ingested (Karasov and Levey 1990; Murphy et al. 1993; Witmer 1994). Trials commenced within 1 h after sunrise ($0500\text{--}0716$ hours Australian Western Standard Time) and lasted for up to 3 h , until all ingested seeds had been defecated. Maintenance diet was removed 1 h before sunrise to increase appetite for the mistletoe fruit (Murphy et al. 1993). Gut passage time was calculated for each fruit defecated during the feeding trial by viewing of the video footage, with up to two trials per bird conducted on separate days.

Statistical Analysis

Proportional AE^* data were arcsine square root transformed (Zar 1999) before analysis. Differences in AE^* , total sugar intake, and pharmacokinetic parameters between sugar type, sugar concentration, and species were assessed by three-way repeated-measures ANOVA and two-way repeated-measures ANOVA with sugar concentration and sugar type as repeated measures, with Tukey-Kramer post hoc tests for unequal sample sizes as required. Data are reported as means $\pm 1 \text{ SD}$ throughout. Statistical analyses were performed with Statistica (StatSoft 2007) and SPSS (SPSS, Chicago). Statistical significance was accepted for $\alpha < 0.05$.

Results

Apparent Assimilation Efficiency

All three species modulated their sugar intake with changes in diet concentration (i.e., exhibited compensatory feeding), ingesting the same mass of sugar irrespective of diet concentration (three-way repeated-measures ANOVA; sugar concentration: $F_{4,68} = 0.605$, $P = 0.660$). A separate analysis examining AE^* revealed a significant three-way interaction term (sugar concentration \times sugar type \times species; $F_{4,34} = 3.1$, $P = 0.029$), which indicated that the three bird species were handling the sugars differently. Glucose AE^* was higher than xylose AE^* (sugar type: $F_{1,17} = 733.8$, $P < 0.001$). Glucose AE^* was extremely high (averaged over all concentrations; mistletoebirds: $99.77\% \pm 0.39\%$; silvereye: $99.85\% \pm 0.14\%$; singing honeyeater: $99.85\% \pm 0.14\%$) and did not differ with species or diet concentration (fig. 1; post hoc analyses shown). Xylose

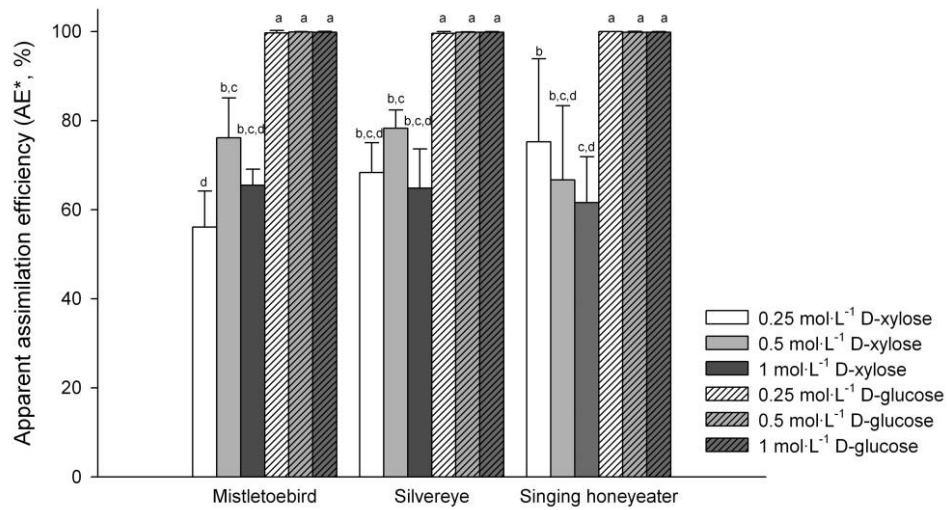


Figure 1. Apparent assimilation efficiency (AE*) of D-xylose (solid bars) and D-glucose (hatched bars) when fed solutions containing 80% D-glucose and 20% D-xylose at three total sugar concentrations (0.25 [white], 0.5 [light gray], and 1 [gray] mol L⁻¹) for mistletoebirds ($n = 5$), silvereyes ($n = 7$), and singing honeyeaters ($n = 8$). Values are presented as means \pm 1 SD. Letters refer to results of Tukey-Kramer post hoc tests for unequal sample sizes (three-way repeated-measures ANOVA).

AE* ranged from $56.08\% \pm 8.10\%$ to $78.3\% \pm 4.07\%$ (fig. 1). Diet concentration did not have a significant effect on xylose AE* for silvereyes, but singing honeyeaters assimilated significantly less xylose on the 1 mol L⁻¹ diet compared with the 0.25 mol L⁻¹ diet, and mistletoebirds assimilated significantly less xylose on the 0.25 mol L⁻¹ diet compared with the 0.5 mol L⁻¹ diet (fig. 1).

Pharmacokinetic Experiments

Pharmacokinetic data were available for all singing honeyeaters ($n = 8$) tested but only four out of the original eight silvereyes and four of the original five mistletoebirds, as a result of silvereyes and one mistletoebird failing to recommence steady-state feeding within a sufficient time frame. These individuals were therefore excluded from pharmacokinetic analyses because of the violation of steady-state feeding conditions (Napier et al. 2012). Over the 3-h trial period, birds drank approximately three times the volume of the dilute diet (0.25 mol L⁻¹) compared with the more concentrated diet (table 2). The mean steady-state concentration (P) of L-glucose and D-xylose in plasma was relatively high in all species, indicating significant absorption of both compounds (table 2). The elimination of both L-glucose and D-xylose after intramuscular injection did not fit a biexponential model significantly better than a monoexponential model for all individual birds of all species (glucose: $F < 3.29$, $P > 0.1$; xylose: $F < 4.49$, $P > 0.06$), indicating single-compartment kinetics. Xylose was eliminated faster than glucose in singing honeyeaters, but the opposite was the case for mistletoebird (two-way repeated-measures ANOVA; K_d ; sugar type \times species: $F_{2,13} = 240$, $P < 0.001$; table 2). Probe distribution space (S) did not differ between glucose and xylose

for mistletoebirds and silvereyes but did for singing honeyeaters (table 2).

In terms of bioavailability (f) of the two sugars, there was a significant species \times sugar type interaction (three-way repeated-measures ANOVA; sugar type \times species: $F_{2,13} = 7.65$, $P = 0.006$), indicating that the three species handled glucose and xylose differently; we therefore analyzed the data for each species separately by two-way repeated-measures ANOVA (sugar and diet concentration as the repeated-measures factors). Mistletoebirds did not vary either glucose or xylose f with diet concentration (concentration: $F_{1,3} = 0.006$, $P = 0.941$), while the other two species did (silvereyes concentration: $F_{1,3} = 97.69$, $P = 0.002$; singing honeyeaters concentration: $F_{1,7} = 33.38$, $P = 0.001$; fig. 2). Furthermore, mistletoebirds showed higher f for xylose compared with glucose, while the opposite pattern was observed for singing honeyeaters (no significant effect for silvereyes; fig. 2).

Gut Passage Times

Mistletoebirds were the only species to ingest mistletoe fruits whole after removing the exocarp. None of the eight singing honeyeaters ingested mistletoe fruit over their two 3-h trials (i.e., 6-h observations for each individual bird). One of eight silvereyes ingested small amounts of *Amyema preissii* fruit pulp (two out of 14 available fruits; in one trial) by prizing open the exocarp of the fruit using its beak in a pliers-like motion to open a small hole into a wider split that it could feed from, but it did not consume any seeds. When the seed was removed from the exocarp, the bird stuck it to the mistletoe branch and continued to eat the fruit pulp.

The average gut passage time of *A. preissii* ingested by mis-

Table 2: Parameters used to determine bioavailability (f) of radiolabeled L-glucose and D-xylose in mistletoebirds, silvereyes, and singing honeyeaters at two diet concentrations (0.25 and 1 mol L⁻¹ hexose solutions)

	Mistletoebirds ($n = 4$)		Silvereyes ($n = 4$)		Singing honeyeaters ($n = 8$)	
	L-glucose	D-xylose	L-glucose	D-xylose	L-glucose	D-xylose
Feeding rate (mg min ⁻¹):						
.25	48.44 ± 8.14		26.99 ± 4.95	26.42 ± 9.55	65.16 ± 32.97	
1	16.08 ± 6.93		5.72 ± 2.66	9.611 ± 3.61	22.28 ± 5.78	
Intake rate (I ; dpm min ⁻¹):						
.25	76,215 ± 12,811	86,764 ± 14,583	67,002 ± 16,493	99,876 ± 52,152	597,098 ± 303,068	61,976 ± 30,509
1	43,132 ± 40,343	63,568 ± 27,376	16,289 ± 6,503	25,860 ± 8,544	218,258 ± 59,577	31,294 ± 9,709
Steady-state plasma (P ; dpm mg ⁻¹ plasma):						
.25	282 ± 156	832 ± 156	707 ± 160	1,310 ± 465	2,335 ± 667	157 ± 56
1	101.89 ± 56	658 ± 256	500 ± 193	653 ± 291	1,668 ± 456	142 ± 63
Elimination constant (K_{el} ; min ⁻¹) ^a	.044 ± .005 ^A	.014 ± .003 ^{BC}	.021 ± .009 ^{BC}	.025 ± .009 ^{BC}	.014 ± .009 ^C	.022 ± .005 ^B
Probe distribution space (S ; mg plasma) ^a	2,100 ± 1,062 ^B	4,015 ± 1,772 ^{AB}	1,456 ± 590 ^B	1,631 ± 1,117 ^B	6,268 ± 2,243 ^A	3,631 ± 2,033 ^B
Bioavailability (f %):						
.25	32.88 ± 19.72 ^B	51.98 ± 23.46 ^A	29.70 ± 12.69 ^D	53.31 ± 16.24 ^{BD}	36.53 ± 13.35 ^B	25.06 ± 17.13 ^D
1	32.92 ± 27.84 ^B	54.15 ± 14.75 ^A	81.81 ± 17.23 ^{AB}	89.44 ± 12.07 ^A	65.22 ± 21.93 ^A	36.86 ± 14.89 ^B

Note. Values are presented as means ± 1 SD. Feeding rate: mistletoebirds and singing honeyeaters were presented both L-glucose and D-xylose in a single solution with double-labeled isotopes, while silvereyes were fed L-glucose and D-xylose in separate feeding trials. Letters refer to significant differences obtained from two-way repeated-measures ANOVA (between species: K_{el} and S ; within species: f) and Tukey-Kramer post hoc tests for unequal sample sizes. dpm, disintegrations per minute.

^aCalculated only once for each bird while feeding on 0.25 mol L⁻¹ hexose solution.

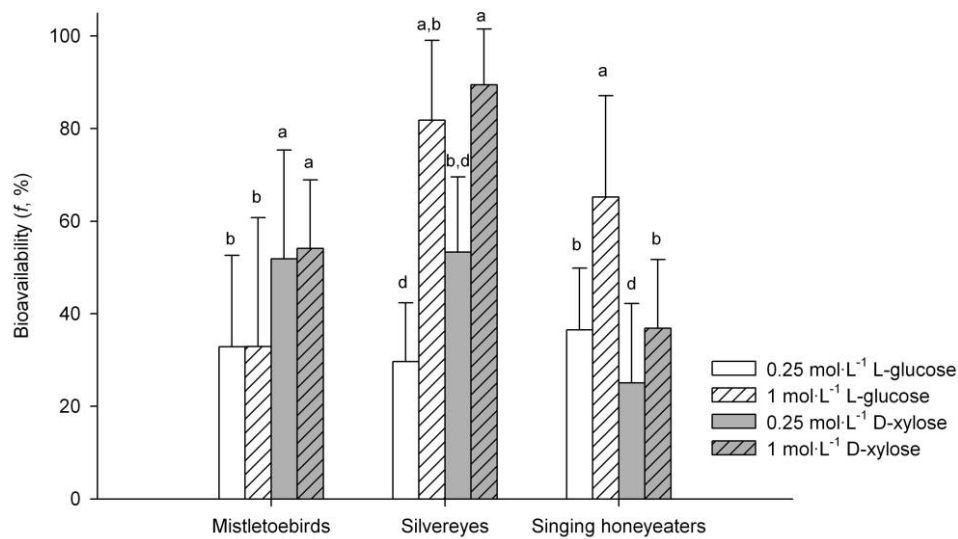


Figure 2. Bioavailability (f , %) of radiolabeled L-glucose (white) and D-xylose (gray) at two diet concentrations (0.25 [solid bars] and 1 [hatched bars] mol L⁻¹) for mistletoebirds ($n = 4$), silvereyes ($n = 4$), and singing honeyeaters ($n = 8$). Values are presented as means \pm 1 SD. Superscripts refer to significant differences between diets within species (two-way repeated-measures ANOVA with Tukey-Kramer post hoc tests for unequal sample sizes).

tletobirds was 16.45 ± 7.21 min and ranged from 2.0 to 40.0 min ($n = 177$; total number of trials $n = 8$). The number of fruits presented to individual birds ranged from 22 to 32, with 84%–100% ingested by birds and 0%–16% of fruits manipulated but dropped before consumption. The average feeding period (71.99 ± 16.73 min) ranged from 49.0 to 98.0 min, while the total trial period (first feeding to last defecation) averaged 87.95 ± 18.89 min (range, 54.0–109.0 min). The average number of fruits in a bird's intestine over the total trial period was 5.49 ± 2.49 (range, 1–12). The average number of seeds defecated over the total trial period was 3.08 ± 1.03 (range, 1–5).

Discussion

Xylose was only first reported as a nectar sugar in southern African Proteaceae by Van Wyk and Nicolson (1995), but we know very little about its prevalence and presence in other plant nectars across the globe. Xylose is also evident in the fruits of some mistletoe species (table 1 and references therein). Xylose may therefore be more prevalent in nectar and fruit diets for birds than we currently are aware of, and birds that have evolved with a high-xylose diet are likely to have developed mechanisms of handling this sugar. Mistletoe birds clearly process xylose differently from the other two frugivores tested in this study. While there was no clear pattern between the species in terms of the AE* of xylose, the three species handled the absorption of glucose and xylose differently: silvereyes and singing honeyeaters showed significantly greater bioavailability for both sugars on the more concentrated diet, but mistletoebirds did not vary f with diet sugar concentration. Furthermore, mistletoebirds showed higher f for D-xylose compared with L-glucose,

while the opposite pattern was observed for the singing honeyeaters (no significant effect for silvereyes). We discuss these findings first in terms of the assimilation of these sugars, followed by their mechanisms of absorption.

Apparent Assimilation Efficiency

All three species assimilated significantly less D-xylose than D-glucose. Because mistletoebirds consume mistletoe fruit (previous studies reveal several species of mistletoe contain large quantities of xylose sugar; table 1 and references therein), these birds should have been better able to deal with xylose than the other two bird species. However, we did not find this, since mistletoebirds did not demonstrate higher D-xylose AE* values than the other two species.

All three species exhibited compensatory feeding; that is, they were able to adjust their intake rate so they ingested a similar total mass of sugar over the range of sugar concentrations. Sugar concentration did not affect D-xylose AE* in silvereyes, but varying effects of concentration were demonstrated in mistletoebirds and singing honeyeaters, with no clear discernible pattern. The AE* experiments integrate digestive function over a longer time period in comparison to the pharmacokinetic measures of bioavailability and so may be more sensitive to variation in feeding rate and frequency than the latter. It is also possible that varying amounts of microbial degradation of xylose in the excreta collection pans may contribute to this variation, but the absolute causes of the varying effects of diet concentration between bird species on the D-xylose AE* values are unclear.

We also found slightly higher AE* of D-xylose in our study

species (56%–78%) compared with other bird species studied to date (53%–61%; Franke et al. 1998; Jackson et al. 1998b) but much lower than the Namaqua rock mouse (~97%; Johnson et al. 1999), suggesting that, like other bird species studied, xylose is not absorbed efficiently by these Australian birds.

Mechanism of Sugar Absorption

Silvereyes and singing honeyeaters showed greater bioavailability of both L-glucose and D-xylose on the more concentrated diet, where slower gut passage time would promote increased absorption via the paracellular route (likely because of increased contact time of digesta with absorptive surfaces), as has been previously described in three specialized nectarivores to date (McWhorter et al. 2006; Napier et al. 2008b). On the other hand, mistletoebirds did not vary *f* with diet concentration for either sugar. This could suggest that absorption of these compounds known to be absorbed by nonmediated (i.e., paracellular) mechanisms is somehow decoupled from digesta retention time in this species. Although we were unable to directly compare the transit rates of mistletoe fruit between our study species, mistletoebirds have previously demonstrated much faster transit rates of mistletoe fruit through their highly specialized intestinal tracts compared with silvereyes and honeyeaters (Keast 1958; Murphy et al. 1993; French 1996; Stanley and Lill 2002; Barea 2008). We are not aware of any measurements of digesta retention time in mistletoebirds feeding on liquid diets, but it is highly likely that mistletoebirds, like many other species (Lopez-Calleja et al. 1997; Levey and Martinez del Rio 1999; McWhorter and Lopez-Calleja 2000; McWhorter et al. 2006; Wilson and Downs 2011), do vary retention time with energy density. A high capacity for mediated glucose uptake (not quantified in this study) might mean that mistletoebirds can meet their energy needs without an increased reliance on paracellular uptake. Indeed, the significantly lower *f* of L-glucose for mistletoebirds in comparison to silvereyes and singing honeyeaters suggests a decreased reliance on the paracellular pathway in mistletoebirds. One other specialized frugivore, the cedar waxwing, has three times the active transport of glucose compared with the American robin (Karasov and Levey 1990). This allows cedar waxwings to maintain a high and efficient absorption of sugars, even at relatively high intake and processing rates of fruit (Witmer and van Soest 1998). If mistletoebirds have relatively high capacity for mediated carbohydrate uptake, digesta retention time may vary less with energy density than in other species studied, providing a potential mechanism to explain the apparent decoupling of *f* and diet sugar concentration in this species.

The paracellular space discriminates according to molecular size, similar to a sieve (Friedman 1987; Chediack et al. 2003). Therefore, if D-xylose and L-glucose were both absorbed via the paracellular pathway, we would expect higher *f* values for D-xylose on the basis of its lower molecular mass (Chediack et al. 2003). Because of these differences in molecular mass between D-xylose and L-glucose and the fact that diffusion in water declines with molecular weight ($MW^{0.5}$), the bioavail-

ability values of D-xylose can be corrected by a decrease of 8.7% ($[(180.16^{0.5} - 150.13^{0.5})/180.16^{0.5}] \times 100$) to assess the effects of molecule size on absorption. Mistletoebirds were the only species to demonstrate a significantly higher *f* for D-xylose than L-glucose, and this difference is not solely accounted for by differences in molecular mass (i.e., average D-xylose *f* values are >8.7% higher than L-glucose; table 2). This suggests that xylose could possibly be absorbed by both mediated and paracellular routes in these birds, possibly in the same manner as D-glucose (i.e., both paracellular and carrier-mediated active transport). Further work is required to determine whether xylose is actively transported by membrane proteins in these birds.

Evidence also suggests that in some other animals, D-xylose is actively transported along with D-glucose, although the affinity of the transporter for D-xylose is much lower than that of D-glucose (Salomon et al. 1961; Bihler et al. 1962; Csaky and Lassen 1964; Alvarado 1966; Ohkohchi and Himukai 1984). In chickens, Savory (1992) reported that D-xylose was absorbed slower than D-glucose and D-galactose but faster than D-arabinose and D-mannose, which concurs with results previously reported in chicks (Wagh and Waibel 1967), rats (Kohn et al. 1965), and humans (Wood and Cahill 1963), suggesting that the absorption rates of these sugars may depend on their relative contributions of active and passive (transcellular and paracellular) transfer mechanisms (Savory 1992).

Metabolism of Xylose

Xylose is beneficial as a source of energy to these birds only if it is able to be metabolized either by gastrointestinal microbes or directly by bird tissues (discussed in depth in Jackson and Nicolson 2002). Animals such as the Namaqua rock mouse, through the cecal fermentation chamber in its gut, are able to ferment xylose to convert it into a source of energy (Johnson et al. 2006). The cecal enlargement demonstrated in chicks fed xylose suggests a similar process of bacterial fermentation (Schutte 1990; Schutte et al. 1992). While frugivorous and nectarivorous birds such as mistletoebirds and honeyeaters tend to have rudimentary or small caeca (Richardson and Wooller 1986, 1988), the presence of intestinal microbes (that may be able to metabolize xylose) has not been studied in these birds. Xylose is also able to be catabolized by certain mammalian tissues, with mammalian cells demonstrated to be able to survive with xylose or xylitol as their sole energy source (Deme-trakopoulos and Amos 1978), but to date, this has not been investigated in birds.

The presence of xylose in nectar and fruit remains puzzling because of the aversion shown by birds and rodent pollinators (Jackson and Nicolson 2002). Although the Namaqua rock mouse is able to efficiently absorb and metabolize xylose, it is the least preferred sugar in preference tests (Johnson et al. 1999). A coevolutionary explanation for xylose as an attractant for pollinators and dispersers therefore remains contentious (Jackson and Nicolson 2002), and the study of the potential catabolism of xylose by intestinal microbes or systemic catabolism in birds certainly warrants further investigation.

Conclusion

Mistletoebirds showed higher bioavailability for xylose compared with glucose, exactly the opposite pattern that was observed for singing honeyeaters (data for silvereyes were not statistically significant). This implies that mistletoebirds may be absorbing D-xylose by both mediated and nonmediated mechanisms. Mistletoebirds also eliminated xylose more slowly than silvereyes and singing honeyeaters, suggesting that xylose may have been incorporated into cells or used in biochemical pathways in mistletoebirds; it might also reveal a delay due to xylose passing through the gut enterocytes on its way to the circulation.

The observation that mistletoebirds did not vary f with diet sugar concentration, unlike silvereyes and singing honeyeaters, is also very intriguing. While possible explanations may include the decoupling of retention time and absorption of compounds absorbed by nonmediated mechanisms, a decreased reliance on the paracellular pathway, relatively less modulation of digesta retention time with diet energy density, or the presence of intestinal microbes in this species, we did not measure digesta mean retention time, mediate carbohydrate uptake, or quantify the intestinal microbiome of birds in this study.

These data build on our understanding of the handling of sugars in frugivorous Australian birds, with new insight in particular to a specialized frugivore. While mistletoebirds do not assimilate more xylose than the more generalist frugivores assessed in this study, they may absorb xylose differently. These three species therefore reveal differences in how they handle dietary sugars.

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