The feeding response of white-bellied sunbirds (*Cinnyris (Nectarinia) talatala*) to sugar concentration and viscosity of artificial nectar


Eckart Stolle

By

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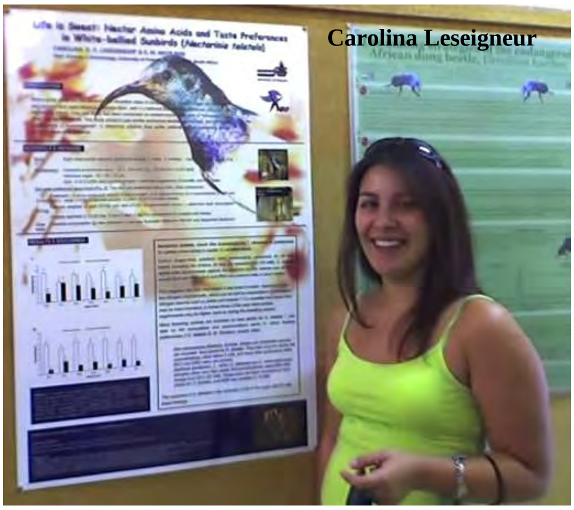

One must ask children and birds how cherries and strawberries taste.
- J. W. Von Goethe

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No bird soars too high if he soars with his own wings.

- William Blake

General Abstract

Plant nectar is a simple food and is easily digested by many different species of pollinators. Many compounds make up the composition of floral nectars, but the most abundant are sugars, generally dominated by sucrose and the hexoses, glucose and fructose. Nectar sugars have been measured for many plant species visited by hummingbirds, sunbirds and other passerines, revealing a range of concentrations. The nectars of passerine-pollinated flowers are generally dilute compared to those of bee-pollinated flowers. The question why bird nectars are so dilute has been addressed in many studies. Many hypotheses have been proposed, among them the relationship between viscosity and drinking by birds. The viscosity of sugar solutions increases exponentially with increasing concentration, and capillarity is inversely proportional to viscosity. Nectarivorous birds imbibe nectar by capillarity, and high sugar concentrations could impose constraints on their feeding efficiency. Feeding in nectarivorous birds, especially hummingbirds, has been mostly devoted to assessing sugar type preferences. However, concentration preferences have received less attention, and the effect of viscosity on feeding has not been examined separately from sugar concentration for any bird species. Do nectarivorous birds show a preference for specific concentrations at a broad and a fine scale of difference, given a specific sugar type? Does viscosity impose a feeding limitation on nectarivorous birds? Does it affect their feeding behaviour? Sunbirds and other nectar-feeding birds can choose amongst various flowering plant species at any one time. Their feeding responses may have important consequences on pollination ecology.

In this study, concentration preferences of white-bellied sunbirds were examined using paired solutions of either sucrose or equicaloric 1:1 mixtures of glucose and fructose, at a

both a broad and a fine scale of difference between pairs over the concentration range of 0.25 to 2.5 M. I hypothesized that sunbirds would prefer concentrations of 1 M and higher on sucrose solutions, while preferring concentrations less than 1 M on hexose solutions. On both sugar types at the broad scale, the higher concentration was significantly preferred up to 1 M, suggesting a preference for 1 M sugar solutions. At a finer scale, white-bellied sunbirds were able to discriminate 0.03 and 0.05 M (1 and 2% w/w) concentration differences between sucrose and hexose solutions respectively. This discrimination is similar to that reported at low concentrations for other passerine nectar-feeders, and at higher concentrations for hummingbirds.

To determine if high viscosity nectars limit the sugar intake of avian nectar consumers, white-bellied sunbirds were exposed to three different test series of sucrose solutions: control series (CS, pure sucrose 0.25 – 2.5 M), constant viscosity series (CVS, 0.25 – 0.7 M with increased viscosity equivalent to that of 1 M sucrose) and constant concentration series (CCS, 1 M with increased viscosities equivalent to that of 1.5, 2 and 2.5 M sucrose). Viscosities were artificially altered with Tylose ®. The sunbirds had reduced intake rates and gained less energy on more viscous sucrose solutions. Also, sunbirds did not alter their feeding behaviour (feeding frequency, feeding duration, total feeding duration and feeding interval) in any significant way when feeding on more viscous sucrose solutions. This lack of change in feeding behaviour led to lower sugar intake rates and sugar consumption. These results suggest that sunbirds suffer a preingestional limitation when consuming nectars with viscosities higher than those due to sugar concentration alone.

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Disclaimer

This thesis consists of a series of chapters that have been prepared as stand-alone manuscripts, for subsequent submission for publication purposes. Consequently, repetitions may occur between chapters.

Chapter 1

General Introduction

Carolina Leseigneur

The white-bellied sunbird

Sunbirds are specialized nectarivorous passerines and the Old World counterparts of hummingbirds (family Trochilidae). Sunbirds are important pollinators as they feed primarily on floral nectar, occurring throughout Africa and Asia (Skead 1967; Cheke et al. 2001). They are all typified by their small size (ranging between 6 and 22 g in body mass), long, thin curved bills, and highly energetic lifestyles. The sunbirds' primary diet is floral nectar from a variety of plant species, and many sunbirds species often form groups with other sunbirds and passerines at rich nectar sources (Skead 1967). They also consume pollen and glean foliage for small arthropods, especially during the breeding season (van Tets & Nicolson 2000; Hockey et al. 2005).

The white-bellied sunbird *Cinnyris (Nectarinia) talatala*, (family Nectariniidae) is one of the most common sunbirds in the greater Johannesburg and Pretoria area of South Africa (Skead 1967). It occurs over a wide range in north-eastern sub-Saharan Africa, and also to the west in northern Botswana and Namibia (Hockey et al. 2005). Throughout its range it has a preference for semi-arid savannas and woodlands (Hockey et al. 2005), especially dry *Acacia* thickets (Skead 1967).

Mature males of the species have a characteristic white breast and contrasting iridescent green and blue on the head, neck, mantle, chin, throat, upper breast and lesser and median wing coverts (Hockey et al. 2005). The flight feathers and tail tip are a dull black. Females are duller with the upper parts a grey-olive brown, and the tail a darker brown. The young resemble the female. The white-bellied sunbird occurs solitarily at times, but mostly in pairs and occasionally in groups (Cheke et al. 2001; Hockey et al. 2005).

White-bellied sunbirds habituate well to captivity, and learn to feed from an artificial feeder within three hours of capture (personal observation). This nectarivorous passerine has proven to be a highly suitable model animal to examine relationships between floral nectars and energy management, and the physiological adaptations of the birds in response to a simple, watery diet (for example, Fleming et al. 2004a; Köhler et al. 2006, Napier et al. 2008).

The nectars of bird-pollinated flowers

Plant nectar is a simple food consumed by a great variety of floral visitors and pollinators. Floral nectars contain many solutes, but the most abundant are sugars, generally dominated by sucrose and the hexoses glucose and fructose (Baker & Baker 1982; Baker &

Baker 1983; Nicolson & Thornburg 2007). Nectar sugars have been measured for many plant species visited by hummingbirds, sunbirds and other passerines, revealing a range of sucrose, hexose and even xylose concentrations (Pyke & Waser 1981; van Wyk & Nicolson 1995; Nicolson 2002, Nicolson & Fleming 2003a). There are however marked differences in the properties of nectar from specialized (specialized nectarivores) and generalized (occasional nectarivores) bird pollination systems (Johnson & Nicolson 2008). Flowers pollinated by specialized nectarivores, like hummingbirds and sunbirds, tend to be characterized by small volumes, high sugar concentrations and high sucrose content. In contrast, nectar from plants pollinated by generalist bird pollinators has large volumes, dilute sugar concentrations and low sucrose content (Johnson & Nicolson 2008). In general however, bird-pollinated floral nectars are more dilute than the nectars of bee-pollinated flowers (Pyke & Waser 1981; Nicolson & Fleming 2003a).

Sugar type preferences of bird pollinators

Sugar type preferences in birds have been studied for some species of Japanese quail (Harriman & Milner 1969), the Red Lory (Downs 1997) and several hummingbird and sunbird species (see below, reviewed by Lotz & Schondube 2006). Stiles (1976) conducted several experiments using Anna's hummingbird (*Calypte anna*) and three other species of hummingbirds (*Selasphorus rufus, Archilochus alexandri, Thalurania furcata*). Using pair-wise tests, he found that sucrose (S) at 30% w/v was preferred over an equicaloric mixture of the hexoses glucose (G) and fructose (F), which was preferred over glucose, while fructose was rejected by all birds (preference order from most to least preferred: S > G:F > G > F). However, given no other choice, hummingbirds will consume fructose over the course of a day in equal amounts (ml h^{-1}) to sucrose (Stiles 1976). The hierarchy of sugar preference was the same for all hummingbird species, and fructose was rejected by nearly all birds. Martínez del Rio (1990) offered 17.1% w/v solutions of sucrose, a 1:1 glucose and fructose mixture, and glucose and fructose alone, in pairs to three species of Mexican hummingbirds (*Amazilia rutila*, *Cynanthus latirostris*, *Chlorostilbon canivetii*). The birds showed the same hierarchy of preference as those studied by Stiles (1976), even though all birds digested all the sugars with very high and similar efficiencies. Further echoing these results, Hainsworth and Wolf (1976) found for five species of hummingbirds that sucrose alone was preferred when paired with glucose, and fructose alone was never preferred. However, none of these preferences were strong, possibly due to the fact that sugars were made up on a % w/w basis. Most articles and experiments use % w/w (g solute per 100 g solution) concentrations due to the wide use of refractometers. Refractometers are hand held devices which measure the refractive index of a solution placed onto the prism, which varies according to the nature of the solute, concentration and temperature (Corbet 2003). This can easily be converted to % w/v (g solute per 100 ml solution), by multiplying by the density of the sugar solution at the set concentration (Corbet 2003). This is based on the assumption that the solutions are equicaloric when measured as % w/w. However, because the molecular mass of sucrose (342.3 g mol^{-1}) is less than that of glucose and fructose combined (180.2 g mol^{-1} each), hexose solutions will have only 95% of the energy of a sucrose solution if mixed on a % w/w basis (Fleming et al. 2004b). Additionally, if one is examining whether nectarivorous birds select one sugar type over another depending on factors other than the energy value of solutions, then solutions should be presented on an equicaloric basis (Brown et al. 2008) Another cause of weak preferences could be short-term exposure to solutions, as feeders in Hainsworth and Wolf’s (1976) experiments were alternated in position every half hour. Similarly, frugivorous and nectarivorous bats consistently prefer sucrose over equicaloric solutions of glucose and fructose, and solutions of the single hexoses in pairwise tests, when concentrations are maintained at values equivalent to the nectar of bat-pollinated flowers (Herrera 1999). However, some bat species have no sugar type preferences when concentrations of paired test solutions have the same concentrations (Rodríguez-Peña et al. 2007). Interestingly, and in contrast to other studies on hummingbirds, broadtailed hummingbirds (*Selasphorus platycercus*) do not show any preference for sucrose or hexoses at 21ºC T_a over the range of 0.25 to 1 mol l^{-1} (molar, hereafter M; Fleming et al. 2004b).

However, for other avian nectarivores, preferences have been found to be different. For three species of nectarivorous tanagers (*Tachyphonus cristatus*, *Dacnis cayana* and *Chlorophanes spiza*), pairwise tests offering sucrose and glucose at 20% concentration yielded no preferences (Schaefer et al. 2003). Cape white-eyes (*Zosterops pallidus*), which are generalist passerine nectarivores/frugivores, prefer sucrose to both fructose and glucose though they do not discriminate significantly between fructose, glucose and equicaloric hexose mixture (Franke et al. 1998). Since xylose (X) has been found in some floral nectars of *Proteaceae* (van Wyk & Nicolson 1995), it has also been tested for some southern African bird species. Cape white-eyes avoid xylose, yielding a preference ranking of S > F = G = G:F > X (Franke et al. 1998). In the southern double-collared sunbird (*Cinnyris chalybeus*) the order of preference for 20% sugars presented in pairwise tests was S = G:F = F > G > X (Lotz & Nicolson 1996). The fact that fructose was preferred over glucose is surprising in view of hummingbird preferences. Cape sugarbirds (*Promerops cafer*) were also found to significantly prefer fructose over glucose at 20% in pairwise tests (Jackson et al. 1998).

Concentration preferences of bird pollinators

The limited research on nectar-sugar concentration preferences in birds shows that preferences tend to be for concentrations higher than those of natural nectars. Stiles (1976) found that when Anna’s hummingbirds were presented with choices between 15, 30 and 45% w/v or between 30, 45 and 60% concentrations in three-way preference tests, repeated for all three sugars (sucrose, glucose and fructose), the birds preferred the highest concentrations available for both sucrose and glucose, but favoured the lower concentrations for fructose. Tamm and Gass (1986) found similar results for rufous hummingbirds (*Selasphorus rufus*) using four-way feeder tests with sucrose solutions. The birds preferred more concentrated sucrose solutions when offered concentrations less than 45%, but preferred more dilute solutions when offered concentrations higher than 55%. Hummingbirds were found to maximize their gain (amount of energy per unit time) of nectar by selecting the more concentrated sucrose solutions between concentration pairs (Hainsworth & Wolf 1976). Interestingly, flower visiting bats (*Glossophaga soricina antillarum*) also prefer the more concentrated of two solutions when offered honey diluted with water to concentrations less than or equal to 50% w/w sucrose equivalents, SE (Roces et al. 1993). Concentration of sucrose differs from that of the hexoses glucose and fructose due to their different molecular masses: 30% w/w sucrose is 1 M, while 30% w/w of either glucose or fructose is 1.85 M (Weast 1980, see Appendix). Hence, concentrations are often made to be equivalent to that of sucrose. However, when concentrations are equal to or higher than 60% SE, bats prefer the more dilute solution, and also consume more free water which has a dilution effect. In another study, Saussure’s long-nosed bat (*Leptonycteris curasoae*) was found to prefer concentrated over dilute artificial nectar in paired tests (sucrose- or hexose-dominated) regardless of sugar type at various concentrations, whereas *Glossophaga soricina* showed no preference except for preferring the more concentrated

(27% w/v) hexose-dominated solution over a more dilute (18% w/v) sucrose-dominated solution (Rodríguez-Peña et al. 2007). In general, hummingbird and bat preferences seem to be for sugar concentrations much higher than those found in natural nectars. In the southern double-collared sunbird (*Cinnyris chalybeus*), preliminary results show that sucrose and fructose at 20 and 30% w/w were equally accepted and preferred over 10% solutions; but for glucose, 10 and 20% were equally accepted and preferred over 30% solutions (Lotz & Nicolson 1996).

In more recent studies, sugar type preferences have been found to be concentration-dependent. In white-bellied sunbirds a strong preference for hexose sugars at 0.1 M SE was found at 21ºC, while they were indifferent to hexose or sucrose solutions of 0.25 M or higher, although they significantly preferred sucrose solutions at 0.75 M (Fleming et al. 2004b). In the same study, broadtailed hummingbirds showed only a slight (non-significant) preference for hexoses on 0.25 M at 10ºC (as hummingbirds use torpor, two temperature extremes were used), but no preference for sucrose. Hummingbirds also showed no preferences for sucrose or hexoses at 21ºC (Fleming et al. 2004b). The findings on the white-bellied sunbird however are similar to data for the cinnamon-bellied flowerpiercer (*Diglossa baritula*) and the magnificent hummingbird (*Eugenes fulgens*) (Schondube & Martínez del Rio 2003). Sugar type preferences in the latter species were concentration-dependent, and preference for hexoses was found at lower concentrations (146 mM SE) in contrast to a preference for sucrose at higher concentrations (1168 mM SE). At intermediate concentration (584 mM SE) the birds were indifferent to hexose or sucrose solutions. The switch from preferring sucrose at high concentrations to hexose preference at low concentrations could be common among specialized nectarivorous birds (see Lotz & Schondube 2006). This interesting as hexose dominated nectars tend to be more dilute than sucrose dominated nectars (Nicolson 2002). But it is important to note that nectarivorous birds, particularly sunbirds, adjust the volume of nectar consumed according to sugar concentration (Downs 1997; Lotz & Nicolson 1999), and have been found to show perfect compensatory feeding on sucrose concentrations between 8.5 and 65% w/w (0.25 and 2.5 M) (Nicolson & Fleming 2003b).

Discrimination abilities of nectarivorous birds

All the studies mentioned above reported feeding preferences over broad scales of concentration. There is very limited data on preferences at a finer scale. Lloyd (1989) found that the greater double-collared sunbird (*Cinnyris afer*) and the malachite sunbird (*Nectarinia famosa*) selected the more concentrated of two sucrose solutions (0.25 to 1 M solutions, pairs differing in concentration by either 0.05, 0.1 and 0.2 M) at low concentrations, though at higher concentrations this discrimination decreased. For three species of tanagers (Family Dacnidinae), the difference between sugar solutions was reduced to 1% in pairwise tests (either glucose or sucrose), and the birds preferred the 6% over the 5% solution, though at 12 and 13% they did not show a preference (Schaefer et al. 2003). Thus discrimination varies with sugar concentration. Rufous hummingbirds (*Selasphorus rufus*) were tested for sucrose concentration preferences in four-way field trials (Blem et al. 2000). Concentrations ranged from 10 to 70% w/v, and for each test the four concentrations offered together differed by 10% increments. There were significant differences between all concentrations from 10 to 50%, but above 50% hummingbirds never showed a preference. In a separate experiment, concentration differences were reduced by 4 or 6%, and when a significant preference was obtained, the difference between concentrations was further reduced until a non-significant result was found. When concentrations approximated values typical of those found in hummingbird-pollinated flowers (approximately 20% on average), the birds could distinguish solutions differing by as little as 1% (Blem et al. 2000).

Viscosity of sugar solutions and effects on preingestional processing

The question why bird nectars, particularly those of passerine-pollinated flowers, are so dilute in sugar concentration has been addressed in several studies (for example, Nicolson 2002, Johnson & Nicolson 2008). Many hypotheses have been proposed (for a review see Nicolson 2002), among them the relationship between viscosity and drinking by birds which was first proposed by Baker (1975). Baker hypothesized that low concentration-low viscosity nectars were necessary for efficient extraction of nectar from flowers. Dynamic viscosity (measured in mPa.s) can be defined as the internal friction of a fluid which resists forces that cause change in its form (Weast 1980; Bourne 1982; Vogel 1994). The viscosity of sugar solutions increases exponentially with increasing concentration, and capillarity is inversely proportional to viscosity. Capillarity is defined as a physical phenomenon caused by surface tension that results in the surface of a liquid rising or falling in contact with a solid (Bourne 1982; Vogel 1994). Nectarivorous birds imbibe nectar by capillarity along their tongues, either down bifurcated “tubes” as in sunbirds and hummingbirds, or by collection on brush-tipped tongues as in honeyeaters (Schlamowitz et al. 1967; Skead 1976; Kingsolver & Daniel 1983; Paton & Collins 1989; Cheke et al. 2001; Downs 2004), and high sugar concentrations with high viscosities could impose constraints on the feeding efficiency of birds. The effect of viscosity on feeding has not been explicitly examined separately from sugar concentration for any bird species (but see Stromberg & Johnsen 1990).

The concentration of nectar sugars, as well as ambient temperature and other nectar solutes affect the viscosity of nectar (Heyneman 1983; Bourne 1982; Mathlouthi & Génotelle 1995). Models based on the feeding of hummingbirds propose that for large volumes that require several licks, both fluid loading and unloading of the tongue are required and higher concentrations maximize extraction efficiency in terms of energy content. Fluid loading occurs through capillarity up the grooves of the tongues of nectarivorous birds. The unloading phase requires muscular work of the tongue and related muscles (see Schlamowitz et al. 1976; Skead 1976; Downs 2004). Small volumes of nectar can be loaded on a single lick, and because of capillarity it requires no unloading phase (Heyneman 1983; Kingsolver & Daniel 1983; Gass & Roberts 1992). The viscosity of sucrose solutions increases exponentially as a function of concentration while the energy content increases linearly, and volumetric flow rate decreases with concentration (Weast 1980; Gass & Roberts 1992; Josens & Farina 2001). It is expected then that maximum energy intake rates are a compromise between energy density and viscosity. This implies that the most efficient energy intake by nectarivorous birds could fall at intermediate levels of sugar concentration (Baker 1975; Pyke & Waser 1981).

Studies on insects reveal that viscosity does impact feeding behaviour. In honeybees (*Apis mellifera*), trophallactic food transfer increases with sucrose concentration up to 30%, but is impeded at higher concentrations by the effect of viscosity, either because it is not energetically worthwhile for the donor or because of a direct physical limitation (Tezze & Farina 1999). In a nectarivorous ant (*Camponotus mus*), it has been found that foraging behaviour is affected by body size in conjunction with viscosity (large versus small workers varied in feeding time on different viscosity of solutions; Medan & Josens 2005). In the hovering hawk moth (*Macroglossum stellatarum*), intake rate was analyzed by separating the effects of concentration and viscosity (measured as kinematic viscosity – dynamic viscosity divided by the density of the solution) using Tylose ®, an inert polysaccharide (Josens & Farina 2001). Presented with different solutions at single feeders following a detailed experiment of varying concentrations and viscosities, it was found that both viscosity and concentration of sucrose in a solution influence the intake rate. For concentrations lower than 50% w/w with viscosities equivalent to 50%, hawk moths had higher ingestion rates. Solutions of equal viscosity should be ingested at the same rate if it was the only physical limitation, but this was not the case for hawk moths.

The effect of viscosity on the feeding variables of nectar-feeding birds has only been looked at to date by Stromberg & Johnsen (1990). They examined the independent effects of artificial sweeteners (including aspartame), sucrose solutions and viscosity (equal to that of sucrose solutions) in field experiments on the feeding preferences of black-chinned hummingbirds (*Archilochus alexanderi*). Artificial sweetener solutions were increased to sucrose equivalent viscosities with sodium carboxymethylcellulose (CMC), an anionic substance. Artificial sweeteners alone were first found to be ignored by the hummingbirds, and increasing their viscosity made no difference to their acceptance. A 20% sucrose solution was significantly preferred over a CMC solution of equal viscosity to 20% sucrose, and the authors concluded that sweet stimuli rather than the mechanical effect of high viscosity is required to induce feeding in black-chinned hummingbirds. However, these results are questionable for two important reasons. Firstly, CMC is anionic and ions can alter viscosity of fluids due to the charge they carry which may alter chemical bonds or form new bonds (Bourne 1982; Vogel 1994. Secondly, many artificial sweeteners, like aspartame, are comprised primarily of amino acids. Amino acids can also alter the viscosity of solutions, depending on concentration or molecular mass, or even the apparent specific volumes of the amino acids, which directly affect taste perception (Bourne 1982; Birch & Kemp 1989; Nicolson 2007). It has also been found that both hummingbirds and sunbirds avoid sugar solutions with high concentrations of amino acids, and are indifferent to them at lower concentrations (Hainsworth & Wolf 1976; Leseigneur et al. 2007).

Aim of study

In this study I will address the effects of concentration and viscosity of artificial nectars on the feeding response of white-bellied sunbirds (*Cinnyris talatala*).

Chapter 1 – Concentration preferences

Given a specific sugar type, will nectarivorous birds show a preference for specific concentrations, at both a broad and a fine scale of difference? White-bellied sunbirds, a specialized nectarivorous passerine, will be exposed to concentration pairs of sucrose and pairs of equicaloric glucose and fructose mixtures of different concentrations. Acuteness of discrimination at a fine scale of difference between concentrations will be assessed similarly. Since sugar type preferences have been shown to be concentration-dependent in some bird species (Schondube & Martínez del Rio 2003; Fleming et al. 2004b; Fleming et al. 2008), it is expected that there will be a difference in concentration preferences between sucrose and hexose sugars – sunbirds should prefer higher concentrations on sucrose solutions, but lower concentrations on hexose solutions. It is further expected that sugar (energy) intake will be constant over the concentration range as white-bellied sunbirds employ compensatory feeding (Nicolson & Fleming 2003b). At a finer scale of difference in concentration, I expect that sunbirds, like hummingbirds and some other passerines (Levey 1987; Lloyd 1989; Blem et al. 2000; Schaefer et al. 2003), will be able to discriminate between small differences in sugar concentration.

Chapter 2 – The effect of viscosity on feeding behaviour

Baker (1975) suggested that dilute (low sugar concentration) nectar of bird-pollinated flowers was necessary to increase the efficiency of nectar extraction from flowers by birds, as higher concentrations with higher viscosities may impede extraction efficiency. Does viscosity impose a feeding limitation on nectarivorous birds, and if so, how does it affect their feeding behaviour? I will determine dynamic viscosity values of artificial nectars containing pure sugars (sucrose and hexose mixture). The effect of viscosity will then be examined (independently of concentration) using a polysaccharide (Tylose ®), to determine differences in sugar (energy) consumption rates and feeding behaviour. The sunbirds will be exposed to three different treatment series as per Josens & Farina (2001), with varied concentrations and artificially increased viscosities. As white-bellied sunbirds use compensatory feeding (Nicolson & Fleming 2003b) and assuming that higher viscosity limits the amount of food ingested per unit volume, it is expected that sunbirds will consume solutions of low concentration with high viscosity at higher than normal rates. Concurrently, they should increase their feeding frequency, feeding duration per feeding event, total feeding duration and decrease the feeding interval between feeding events so as to compensate for increased viscosities behaviourally. However, if viscosity is the only physical limitation, solutions of equal viscosity should be ingested at the same rates.

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Chapter 2

Nectar concentration preferences of the white-bellied sunbird, *Cinnyris talatala* (Nectariniidae)

ABSTRACT

The responses of nectar-feeding birds to nectar sugar concentrations are much less studied than those to sugar types. Given a specific sugar type, do nectarivorous birds show a preference for specific concentrations at both a broad and a fine scale of difference? Concentration preferences of white-bellied sunbirds, *Cinnyris talatala*, were examined using paired solutions of either sucrose or equicaloric 1:1 mixtures of glucose and fructose. Preferences were first examined at a broad scale over the concentration range 0.25 to 2.5 M (0.25 or 0.5 M differences). On both sugar types, the higher concentration was significantly preferred up to 1 M, suggesting a preference for 1 M sugar solutions. Sunbirds ingested more sugar on the hexose mixtures than on sucrose on all concentrations. Discrimination between concentrations was then examined at a finer scale of 0.03 or 0.05 M differences (molar equivalents of 1 and 2% w/w). White-bellied sunbirds were able to discriminate 0.03 and 0.05 M concentration differences between sucrose and hexose solutions respectively, at both low and high concentrations. This discrimination is similar to that reported at low concentrations for other passerine nectar-feeders, and at higher concentrations for hummingbirds.

Introduction

Nectar-feeding birds have access to a wide range of nectar concentrations (Nicolson & Fleming 2003a; Nicolson & Thornburg 2007) and this has consequences for satisfying energy requirements and water balance. Nectar solutes are varied, but the most abundant are sucrose and the hexoses glucose and fructose (Baker & Baker 1982; Baker & Baker 1983; Nicolson & Thornburg 2007). There are marked differences in the properties of nectar from specialized and generalized bird pollination systems. Flowers pollinated by specialized nectarivores, like hummingbirds and sunbirds, tend to contain small volumes of nectar (10-30 µl), high sugar concentrations (15-25% w/w or 0.45-0.8 M sucrose equivalents, SE) and high sucrose content (40-60% total sugar). In contrast, nectar from plants pollinated by generalist bird pollinators has large volumes (40-100 µl), dilute sugar concentrations (8-12% or 0.23-0.37 M SE) and low sucrose content (0-5%) (Johnson & Nicolson 2008).

Because there are differences in the sucrose content of nectar, sugar type preferences have been intensively investigated in various nectar-feeding birds (for a review, see Lotz & Schondube 2006). In more recent studies sugar type preferences have been found to be concentration-dependent. In white-bellied sunbirds a strong preference for hexose sugars at 0.1 M (sucrose equivalent) was found, while the birds were indifferent to hexose or sucrose solutions of 0.25 M or higher, although they preferred sucrose solutions at 0.75 M (Fleming et al. 2004b). This finding is similar to studies on the cinnamon-bellied flowerpiercer (*Diglossa baritula*) and the magnificent hummingbird (*Eugenes fulgens*) (Schondube & Martínez del Rio 2003). A similar change of sugar preference with concentration occurs in two honeyeaters, the New Holland honeyeater (*Phylidonyris novaehollandiae*) and the red wattlebird (*Anthochaera carunculata*), and the rainbow lorikeet (*Trichoglossus* *haematonotus*) (Fleming et al. 2008). However, the preference for hexoses was only significant at very dilute concentrations (less than 0.25 M) for the two honeyeaters, whereas rainbow lorikeets demonstrated hexose preferences up to 0.75 M. Sugar type preferences in all these species are concentration-dependent, and the switch from preferring sucrose at high concentrations to hexose at low concentrations may be common among specialized nectarivorous birds (see Lotz & Schondube 2006).

The preferences of nectar-feeding birds for different food concentrations have received less attention. Most nectar of plants pollinated by hummingbirds, sunbirds and other passerines are considered dilute, averaging 8 to 25% w/w (0.23 – 0.8 M SE) in sugar concentration (Baker 1975; Pyke & Waser 1981; Nicolson 2002; Johnson & Nicolson 2008). However, in choice tests with sucrose solutions it has been consistently found that birds prefer the highest available concentrations. Presented with choices between 15, 30 and 45% or between 30, 45 and 60% w/v in three-way preference tests (30% w/v ≈ 0.88 M SE), Anna's hummingbirds (*Calypte anna*) showed a preference for the highest concentrations available (Stiles 1976). In four-way preference tests, Tamm & Gass (1986) found that rufous hummingbirds (*Selasphorus rufus*) preferred the highest solutions up to 45% w/w. Similarly, in four-way field preference tests, rufous hummingbirds consistently chose the highest concentrations available up to 50% w/v (Blem et al. 2000). In a passerine nectarivore, the bananaquit (*Coereba flaveola*), the results were similar: given a choice of paired feeders, they chose the highest sucrose concentrations up to 0.74 M; Mata & Bosque 2004).

Hexoses have been used less frequently in concentration preference tests, but hummingbirds also chose the highest concentrations of glucose, while the lowest concentrations of fructose solutions were favoured (Stiles 1976; Martínez del Rio 1990). Interestingly, similar results have been obtained for flower-visiting bats. Given paired feeders with diluted honey at various concentrations, *Glossophaga soricina antillarum* bats preferred the highest concentrations up to 50% w/w SE (approximately 1.8 M SE; Roces et al. 1993). Honey contains predominantly glucose and fructose, and most contain very little sucrose (Johannsmeier 2001). In another study, Saussure's long-nosed bat (*Leptonycteris curasoae*) was found to prefer concentrated over dilute artificial nectar in paired tests (sucrose- or hexose-dominated solutions) regardless of sugar type at various concentrations, whereas *Glossophaga soricina* showed no preference except for preferring the more concentrated (27% w/v) hexose-dominated solution over a more dilute (18% w/v) sucrose-dominated solution (Rodríguez-Peña et al. 2007). Preliminary results for the southern double-collared sunbird (*Cinnyris chalybeus*) showed that sucrose and fructose at 20 and 30% w/w (0.65 and 1 M SE) were equally accepted and preferred over 10% (0.33 M SE) solutions. But for glucose, 10 and 20% were equally accepted and preferred over 30% solutions (Lotz & Nicolson 1996).

Hummingbirds have been found to maximize their extraction efficiency of nectar (Hainsworth & Wolf 1976) as well as energy intake rates per foraging bout (leaving perch to visit a feeder and return to perch; Roberts 1996) by selecting the more concentrated sucrose solutions between pairs. In order to obtain the same amount of energy at various concentrations, choosing dilute nectars would involve more time spent foraging. Choosing more concentrated nectars would allow the birds more free time for other activities that may increase their fitness (Roberts 1996). This could help explain such choices in many nectar-feeding birds.

If concentration preferences can be established for a species, how fine are these preferences? It is essential to quantify discrimination abilities of species at a finer scale to further understand food choices and the mechanisms involved. For two African sunbird species in laboratory pairwise tests, Lloyd (1989) found that the birds selected the more concentrated of two sucrose solutions differing by as little as 0.05 M (2% w/w), up to 0.5 M. Levey (1987) found in pairwise laboratory trials that three tanager species (Family Dacnidinae) consistently chose the higher concentration of fruit pulps differing by only 2% in sucrose concentration (8, 10 and 12% or 0.23, 0.33 and 0.37 M SE). In a more recent study, three species of tanagers offered solid diet pairs (5 vs. 6%) of sucrose or glucose consistently chose the higher concentration (Schaefer et al. 2003). Blem et al. (2000) conducted four-way preference tests on rufous hummingbirds in the field. Concentration differences were reduced by 4 or 6% w/v, and when a significant preference was obtained, the difference between concentrations was further reduced until a non-significant result was found. When concentrations approximated values typical of those found in hummingbird-pollinated flowers (20% w/v) the birds could distinguish sucrose solutions differing by as little as 1%. These studies suggest that discrimination ability varies with concentration in nectarivorous birds. From the limited data available, passerines appear to have more acute discrimination abilities at low concentrations, whereas hummingbirds seem to discriminate best at higher concentrations.

Do nectarivorous birds have a preference for specific concentrations within a sugar type? If so, how acute is their discrimination ability between concentrations? In this study I assessed the concentration preferences of a specialized nectarivorous passerine, the white-bellied sunbird (*Cinnyris talatala*) using both sucrose and equicaloric hexose solutions. Sugar solutions were prepared using molar (mol l^{-1} or M) concentrations. Many sugar preference trials are based on sugars mixed on a % w/w (g of solute per 100 g of solution) basis, on the assumption that the solutions are equicaloric. However, because the molecular mass of sucrose (342.3 g mol^{-1}) is less than that of glucose and fructose combined (180.2 g mol^{-1} each), hexose solutions will have only 95% of the energy of a sucrose solution if mixed on a % w/w basis (Fleming et al. 2004b). The birds were exposed to concentration pairs of sucrose and equicaloric hexose (glucose and fructose) mixtures to determine their broad preferences. Fine scale concentration preferences were similarly assessed for both sugar types. Since sugar type preferences have been shown to be concentration dependent for some bird species (Schondube & Martínez del Rio 2003; Fleming et al. 2004b, Fleming et al. 2008), it is expected that there will be a difference in concentration preferences between sucrose and hexose solutions – sunbirds should prefer high concentrations for sucrose solutions while preferring low concentrations for hexose solutions. It is further expected that sugar (energy) intake will be constant over the concentration range as white-bellied sunbirds employ compensatory feeding (Nicolson & Fleming 2003b). At a finer scale of difference between concentrations, I expect that sunbirds, like hummingbirds and other passerines (Levey 1987; Lloyd 1989, Blem et al. 2000; Schaefer et al. 2003), will be able to discriminate between small differences in sugar concentration.

Materials & Methods

Birds and Maintenance

White-bellied sunbirds were captured by mist-netting at Jan Cilliers Park in Pretoria, South Africa. Seven males and three females (8.91 ± 0.25 g SE), all with full adult plumage, were captured during April 2006. Birds were maintained in an outdoor aviary (8 x 2 x 5 m), at the University of Pretoria’s Experimental Farm. They were fed a maintenance diet of 0.63

M sucrose mixed with a nutritional supplement for protein, vitamins and minerals (Ensure®, Abbott Laboratories, Johannesburg, South Africa). This diet and supplementary water were available to the birds *ad libitum* in inverted stoppered syringes (feeders), and water baths were also provided.

During the laboratory trials, the birds were maintained individually in cages of 40 x 42 x 28 cm in a climate-controlled (CC) room at 20 ± 2 ºC and 45% RH on a 12:12 h light: dark cycle (lights on at 07.00 h) as in previous studies on white-bellied sunbirds (for example, Nicolson & Fleming 2003b; Fleming et al. 2004a and b). Temperature and humidity were maintained by automated Siemens RLU 220 and 210 temperature and humidity regulators and an indoor air conditioner (Johnson Controls cr 722 CAREL). Dawn and dusk were simulated with 0.5 h of dimmed light at the beginning and the end of the photoperiod. The same maintenance diet was provided. Birds were allowed to acclimate to laboratory conditions for one week prior to the experiments. Birds were released at the end of the study.

Experiments

Solutions of sucrose and equicaloric 1:1 mixtures of glucose and fructose (hereafter referred to as hexose) ranging from 0.25 to 2.5 M were tested separately. The difference within each concentration pair was 0.5 M, except for the lowest concentration pair which differed by 0.25 M. Below 0.25 M the birds cannot maintain their energy balance at 20ºC (Nicolson & Fleming 2003b).

Sunbirds (*n* = 10) were simultaneously tested over two days per pairwise preference test. Feeders were weighed at the start and end of the 6 h experimental period (07.00 to 13.00 h) with a digital balance (± 0.01g, Mettler Toledo PB-602S, Microsep Ltd., Johannesburg) in order to determine the amount of food ingested. White-bellied sunbirds in captivity have been reported to generally show the most constant food intake rate during the first half of the day (Fleming et al. 2004a), and have longer feeding durations and lower feeding frequency in the early morning (Köhler et al. 2006), hence the choice of experimental period. Experimental diets were randomized and feeders switched at 10.00 h (after 3 h) to compensate for possible side biases (Jackson et al. 1998). To further compensate for possible side biases, the initial feeder position was also reversed at the start of the second test day. The maintenance diet was provided at the end of the experimental period each day so that the birds could replace any mass lost as a result of feeding on sugar-only solutions (Nicolson et al. 2005). Spilt food was collected beneath feeders in containers with liquid paraffin, to prevent evaporation, and corrections were made to the consumption values (this averaged 0.089 ± 0.014 SE g per 6 h experiment, *n* = 10).

Based on the results of the broad scale concentration preferences (see Results), discrimination ability was examined at a finer scale following the same experimental procedures. The difference between pairs around concentrations of 0.25, 0.50 and 1 M was reduced to 0.03 M for sucrose and 0.05 M for equicaloric glucose and fructose mixtures (Table 1).

Table 1: Concentration pairs differing by 0.03 M for sucrose and 0.05 M for hexose mixtures used in discrimination tests (representing differences of 1 and 2 % w/w respectively).

Sucrose (M)	**Glucose: Fructose (M)**
0.27 vs. 0.3	0.25 vs. 0.3
0.47 vs. 0.5	0.45 vs. 0.5
0.97 vs. 1	0.95 vs. 1

Data were collected as mass (g) of each solution consumed (mean ± SE). This was then converted to volumetric consumption (ml) based on the density of sugar solutions at each concentration (from Weast 1980), and to sugar (energy) consumption (in grams). Hexoses and sucrose have the same energy value per unit mass, 16.48 x 10^3 kJ/g (Nicolson & Fleming 2003b). Sugar consumption data were analyzed using the STATISTICA 7.1 (StatSoft Inc. ©, Tulsa, OK, USA, 2006) software package. Total consumption is defined as the sum of the sugar consumed for both feeders of paired diets for each sugar type per test day. The preference index is defined as the sugar consumed at the higher concentration divided by the total consumption.

Statistical Analysis

For initial analysis of both broad and fine scale data, an overall repeated-measures ANOVA (RM-ANOVA) was performed for all of the consumption data to determine where differences occurred. The overall RM-ANOVA comprised three within-effects, nested as follows: "*DIET*(*CONC*(*TESTDAY*))" where *TESTDAY* (the difference between the two days of each preference test) was nested within *CONC* (the difference between the two concentrations), which was nested within *DIET* (the difference between the five (broad scale) or three (fine scale) different concentration pairs). Following the overall RM-ANOVA, consumption values were tested separately for each concentration pair by separate RM-ANOVA. The structure of these RM-ANOVAs therefore comprised two within-effects nested as follows: "*CONC(TESTDAY)*". Differences between sugar types were compared by separate RM-ANOVA comprising two within-effects nested as follows: *"SUGAR(TESTDAY)"* where *SUGAR* is the difference between sucrose and the hexose mixture. Post-hoc analyses for each RM-ANOVA were conducted using Tukey's Honest

Significant Difference (HSD) test for equal sample sizes. Data are presented as means ± 1 SE.

For broad scale concentration preferences, preference indices were further analysed to compare concentration preferences for each diet to a value of 0.5 (no preference) by Wilcoxon's test for non-parametric data (Sokal & Rohlf 1995).

Results

Broad Scale Concentration Preferences

The overall RM-ANOVA revealed that there were significant differences between concentration pairs (diets) and the concentrations of each concentration pair for the hexose mixtures (Table 2). There was a highly significant interaction between diet and concentration for both sugar types. The interaction between concentration and test day was significant for sucrose, which suggests that birds varied their feeding preferences between the two test days. Post-hoc analysis (Tukey's HSD) revealed that these significant effects were due to the mean total consumption of each concentration pair increasing as concentrations increased, and to variation in consumption between concentration pairs and test days.

Based on the above differences, data were then separated according to concentration pairs, and consumption patterns for both sugar types were analysed by separate RM-ANOVA. These results are summarized for both sugar types in Table 3 and the values for sugar consumption are illustrated in Figure 1.

Table 2: Results of the overall RM-ANOVA for sugar consumption (g) of sucrose and the equicaloric hexose mixture (glucose and fructose) by *Cinnyris talatala*. The RM-ANOVA comprised three within-effects structured as follows: "*DIET(CONC(TESTDAY))*". Significant effects after Tukey's HSD are indicated by asterisks (**P*<0.05, ****P*<0.001); *n* = 10 in all cases.

		Sucrose		**Glucose: Fructose**	
Effect	**d.f.**	***F***	***P***	***F***	***P***
DIET	4	0.924	0.461	7.688	0.000***
Error (DIET)	36				
CONC	1	3.697	0.086	6.327	0.033*
Error (CONC)	9				
TESTDAY	1	0.125	0.732	0.586	0.463
Error (TESTDAY)	9				
DIET*CONC	4	14.435	0.000***	7.158	0.000***
Error (DIET*CONC)	36				
DIET*TESTDAY	4	0.483	0.748	1.821	0.146
Error (DIET*TESTDAY)	36				
CONC*TESTDAY	1	7.827	0.021*	0.327	0.581
Error (CONC*TESTDAY)	9				
DIET*CONC*TESTDAY	4	0.613	0.656	0.633	0.642
Error (DIET*CONC*TESTDAY)	36				

Table 3: Difference in sugar consumption (g) between concentrations (*CONC*), days (*TESTDAY*), and their interaction (*CONC x TESTDAY*) for sucrose and the hexose mixture (1:1 equicaloric glucose and fructose) for *Cinnyris talatala*. Values are F-values from RM-ANOVA (within-effects: *CONC(TESTDAY)*), with significant effects after Tukey's HSD indicated by asterisks (**P*<0.05, ***P*<0.01, ****P*<0.001); *n* = 10 and d.f. = 1 in all cases.

	Sucrose			**Glucose: Fructose**		
Concentration Pairs (M)	**CONC**	**TESTDAY**	**CONC x TESTDAY**	**CONC**	**TESTDAY**	**CONC x TESTDAY**
0.25 vs. 0.5	73.21***	0.67	4.98	36.79***	1.86	0.79
0.5 vs. 1	10.44*	0.54	2.38	13.5**	3.52	1.25
1 vs. 1.5	1.846	0.069	0.008	0.185	0.211	0.487
1.5 vs. 2	5.187*	0.011	0.558	0.134	0.038	0.032
2 vs. 2.5	1.859	0.878	0.718	2.302	2.998	0.183

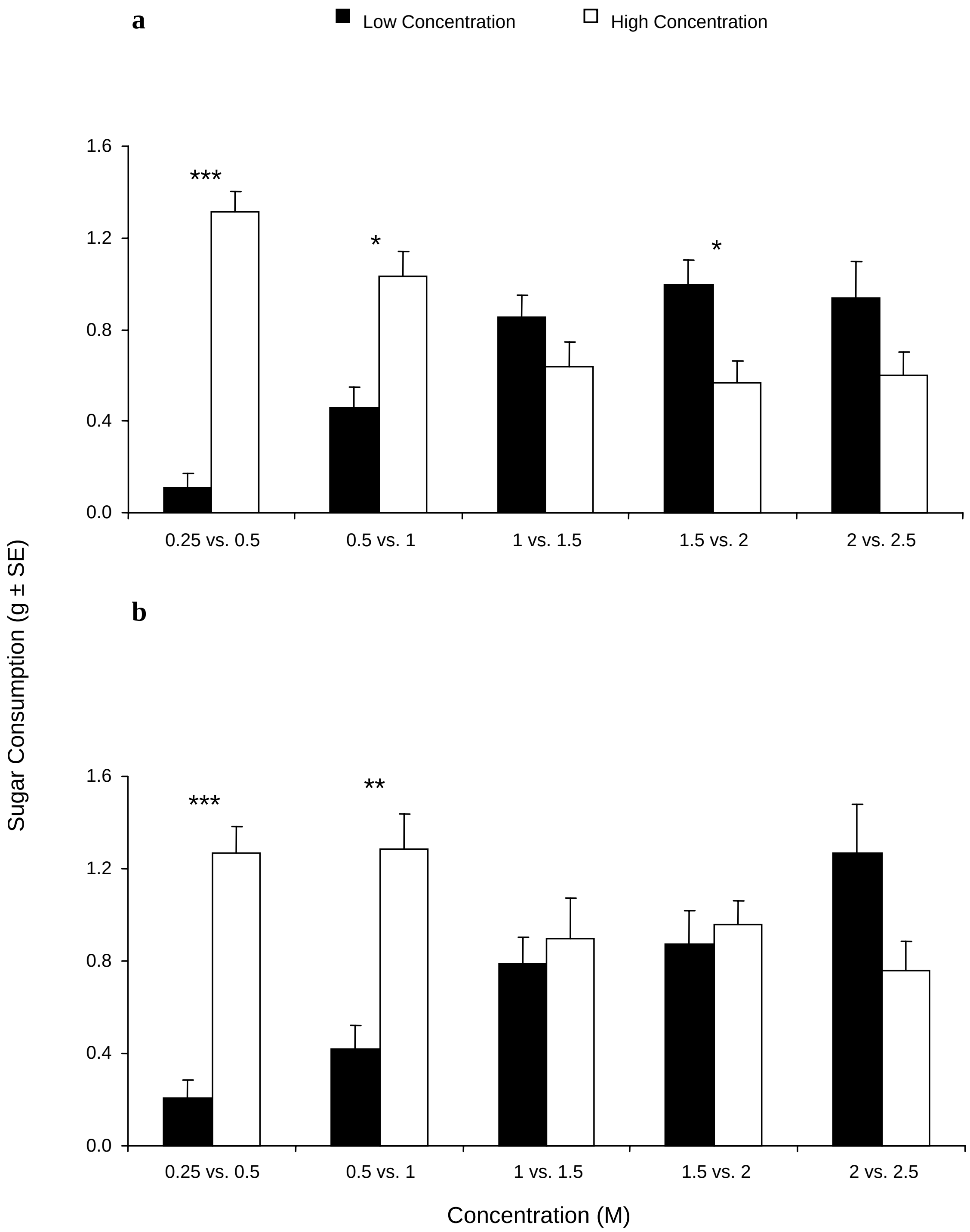

Figure 1: Sugar consumption (averaged over 2 days) by *Cinnyris talatala* of sucrose (a) and hexose (equicaloric 1:1 glucose and fructose) (b) solutions during paired preference tests (*n* = 10). Asterisks (*) indicate significant differences in consumption between solutions of a pair (* *P*<0.05; ** *P*<0.01; *** *P*<0.001).

There is a significant difference between concentrations for the two lowest concentration pairs in both sugar types, because more sugar was consumed at the higher concentration, and also for 1.5 vs. 2 M sucrose, where more sugar was consumed at the lower concentration. Post-hoc analysis (Tukey's HSD) showed that neither consumption between the different test days (*TESTDAY*, $P>0.05$ for all cases), nor the interaction between concentrations and test days (*CONC x TESTDAY*, $P>0.05$ for all cases) was significant.

Preference indices are shown in Figure 2. Wilcoxon's test was used to test the median of the preference index per concentration pair against 0.5 (indicating no preference). On both sucrose and hexose the sunbirds showed a strong preference for the higher concentration in the lowest concentration pairs ($Z_9 = 2.80$, median = 0.925, $P<0.01$, 0.25 vs. 0.5 M sucrose, and $Z_9 = 2.29$, median = 0.708, $P<0.05$, 0.5 vs. 1 M sucrose; $Z_9 = 2.80$, median = 9.59, $P<0.01$, 0.25 vs. 0.5 M hexose, and $Z_9 = 2.59$, median = 0.765, $P<0.01$, 0.5 vs. 1 M hexose). This result supports the findings of the RM-ANOVA. However, the preference for 1.5 vs. 2 M sucrose was not significant ($Z_9 = 1.78$, median = 0.359, $P=0.07$). This is due to the sensitivity of the Wilcoxon test, which is more powerful than the RM-ANOVA (Sokal & Rohlf 1995).

An additional RM-ANOVA and post-hoc analysis was performed to compare total consumption between sugars (see Table 4). There was a significant difference between sugar types for 1 vs. 1.5 M ($F_{1,9} = 9.045$, $P<0.05$), 1.5 vs. 2 M ($F_{1,9} = 10.11$, $P<0.05$) and 2 vs. 2.5 M ($F_{1,9} = 25.72$, $P<0.001$).

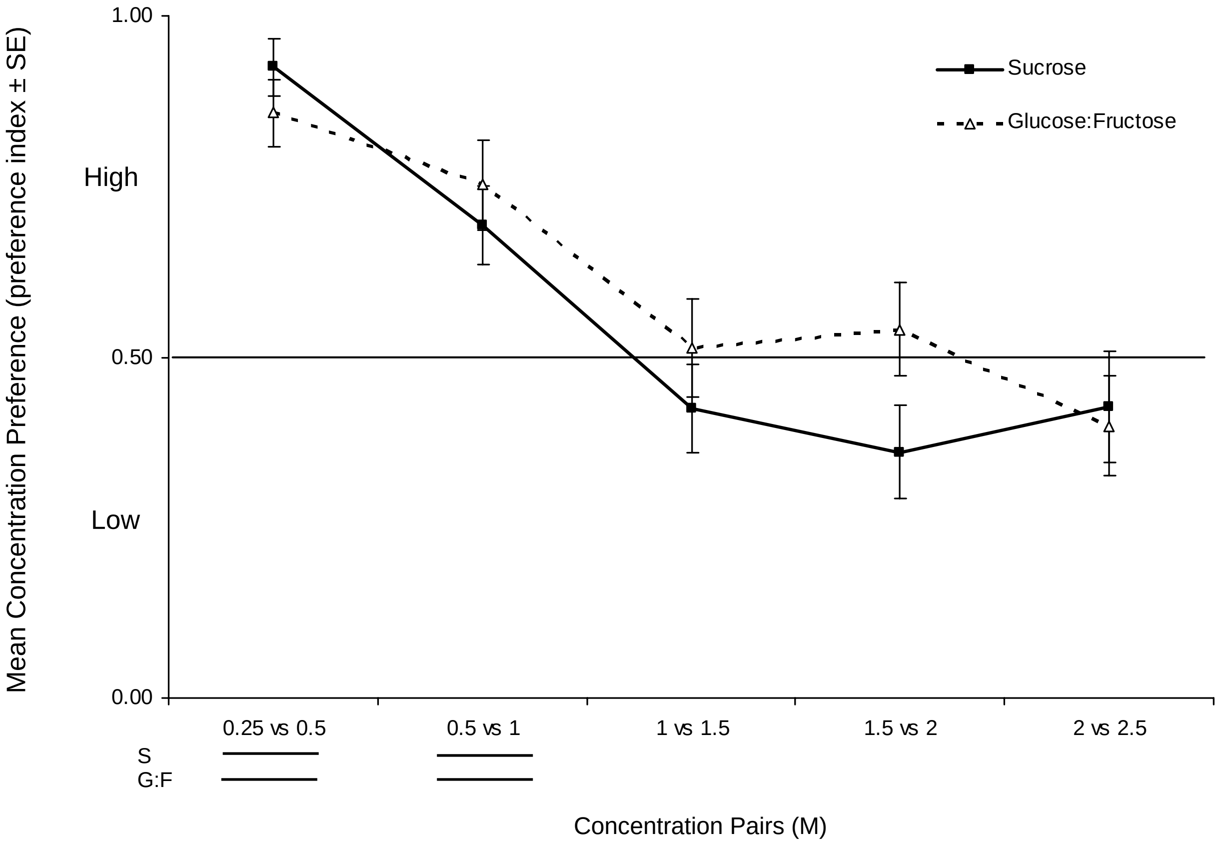

Figure 2: Mean concentration preference (preference index ± SE, *n* = 10, averaged over 2 days for each bird) for solutions of sucrose and hexose (equicaloric 1:1 glucose and fructose) by *Cinnyris talatala*. The preference index is defined as the grams of sugar consumed at the higher concentration divided by the total grams of sugar consumed. Bold lines indicate significant differences in consumption between concentration pairs (sucrose (S) and hexose (G:F); Wilcoxon's test, *P*<0.05).

Table 4: Mean sugar (g ± SE) intake on all paired diets for *Cinnyris talatala* (defined as the sum of sugar consumed for both feeders of paired diets, $n = 10$). See Figure 1. Significant differences between sugar types from RM-ANOVA (within-effects: *SUGAR(TESTDAY)*), after Tukey's HSD, are indicated by asterisks (*$P<0.05$, **$P<0.01$) as for Table 3; $n = 10$ and d.f. = 1 in all cases.

	Sugar (g ± SE)		
Concentration Pairs (M)	**Sucrose**		**Glucose:Fructose**
0.25 vs. 0.5	1.42 ± 0.08		1.55 ± 0.08
0.5 vs. 1	1.49 ± 0.10		1.79 ± 0.14
1 vs. 1.5	1.49 ± 0.10	*	1.77 ± 0.15
1.5 vs. 2	1.56 ± 0.10	*	1.93 ± 0.13
2 vs. 2.5	1.53 ± 0.13	**	2.10 ± 0.18

Fine Scale Concentration Preferences

The analysis of this experiment followed the same procedure as for the broad scale concentration preferences. The overall RM-ANOVA for sugar consumption of both sugar types revealed that there were significant differences between concentration pairs for both sucrose and hexose (Table 5). There was also a significant difference between test days for hexoses. Some interactions between effects were also significant for both sugar types. After post-hoc analysis (Tukey's HSD), these significant effects were due to the mean consumption of each concentration pair increasing as concentrations increased.

Table 5: Results of the overall RM-ANOVA for sugar consumption (g) of sucrose and the equicaloric hexose mixture (glucose and fructose) by *Cinnyris talatala*. The RM-ANOVA comprised three within-effects structured as follows: "*DIET(CONC(TESTDAY))*". Significant effects after Tukey's HSD are indicated by asterisks (**P*<0.05, ***P*<0.01, ****P*<0.001); *n* = 10 in all cases.

		Sucrose		**Hexose**	
Effect	**d.f.**	***F***	***P***	***F***	***P***
DIET	2	4.134	0.033*	60.626	0.000***
Error (DIET)	18				
CONC	1	57.462	0.000***	25.636	0.000***
Error (CONC)	9				
TESTDAY	1	0.215	0.654	17.474	0.002**
Error (TESTDAY)	9				
DIET*CONC	2	31.853	0.000***	8.712	0.002**
Error (DIET*CONC)	18				
DIET*TESTDAY	2	5.954	0.010*	7.902	0.003**
Error (DIET*TESTDAY)	18				
CONC*TESTDAY	1	0.007	0.935	4.718	0.058
Error (CONC*TESTDAY)	9				
DIET*CONC*TESTDAY	2	0.519	0.604	2.068	0.155
Error (DIET*CONC*TESTDAY)	18				

Based on the above mentioned differences, data was separated as for broad scale preferences for further analysis. These results are summarized in Table 6 and shown in Figure 3. Concentrations were significantly different for all experimental diets of sucrose and hexose except for the mid-range diets of both sugars. The difference in concentration for 0.45 vs. 0.5 M hexose was almost significant, but this is because three birds showed a slight preference for the lower concentration; differences between the concentrations for all other individual birds were small.

Table 6: Differences in sugar consumption (g) between concentrations (*CONC*), test days (*TESTDAY*), and their interaction (*CONC x TESTDAY*) for sucrose and the hexose mixture (1:1 equicaloric glucose and fructose) for mature white-bellied sunbirds, *Cinnyris talatala*. Values are F-values from RM-ANOVA (within effects: *CONC(TESTDAY)*), with significant effects after Tukey's HSD indicated by asterisks (*$P<0.05$, **$P<0.01$, ***$P<0.001$); $n = 10$ and d.f. = 1 in all cases.

	Sucrose			**Glucose: Fructose**		
Concentration Pairs (M)	**CONC**	**TESTDAY**	**CONC x TESTDAY**	**CONC**	**TESTDAY**	**CONC x TESTDAY**
0.27 vs. 0.3	6.104*	0.006	0.414	-	-	-
0.47 vs. 0.5	0.786	1.142	0.114	-	-	-
0.97 vs. 1	7.61*	31.64***	0.39	-	-	-
0.25 vs. 0.3	-	-	-	175.7***	1.6	1.5
0.45 vs. 0.5	-	-	-	3.064	0.007	0.016
0.95 vs. 1	-	-	-	30.36***	10.89**	1.5

These results reveal that sunbirds could discriminate differences in sucrose solutions of 0.03 M (representing 1% w/w) and differences in equicaloric hexose solutions of 0.05 M (representing 2% w/w). Post-hoc analysis (Tukey's HSD) showed that consumption differences between the two test days were significant for 0.97 vs. 1 M sucrose and 0.95 vs. 1 M hexose. This may be attributable to individual variation and possible side biases in this experiment. From the raw data, it is evident that these effects were significant due to the fact that birds (in each case three different birds) consumed different combined amounts between days (individual variation) or consumed more from one feeder position on both days, attributable to side bias (Jackson et al. 1998).

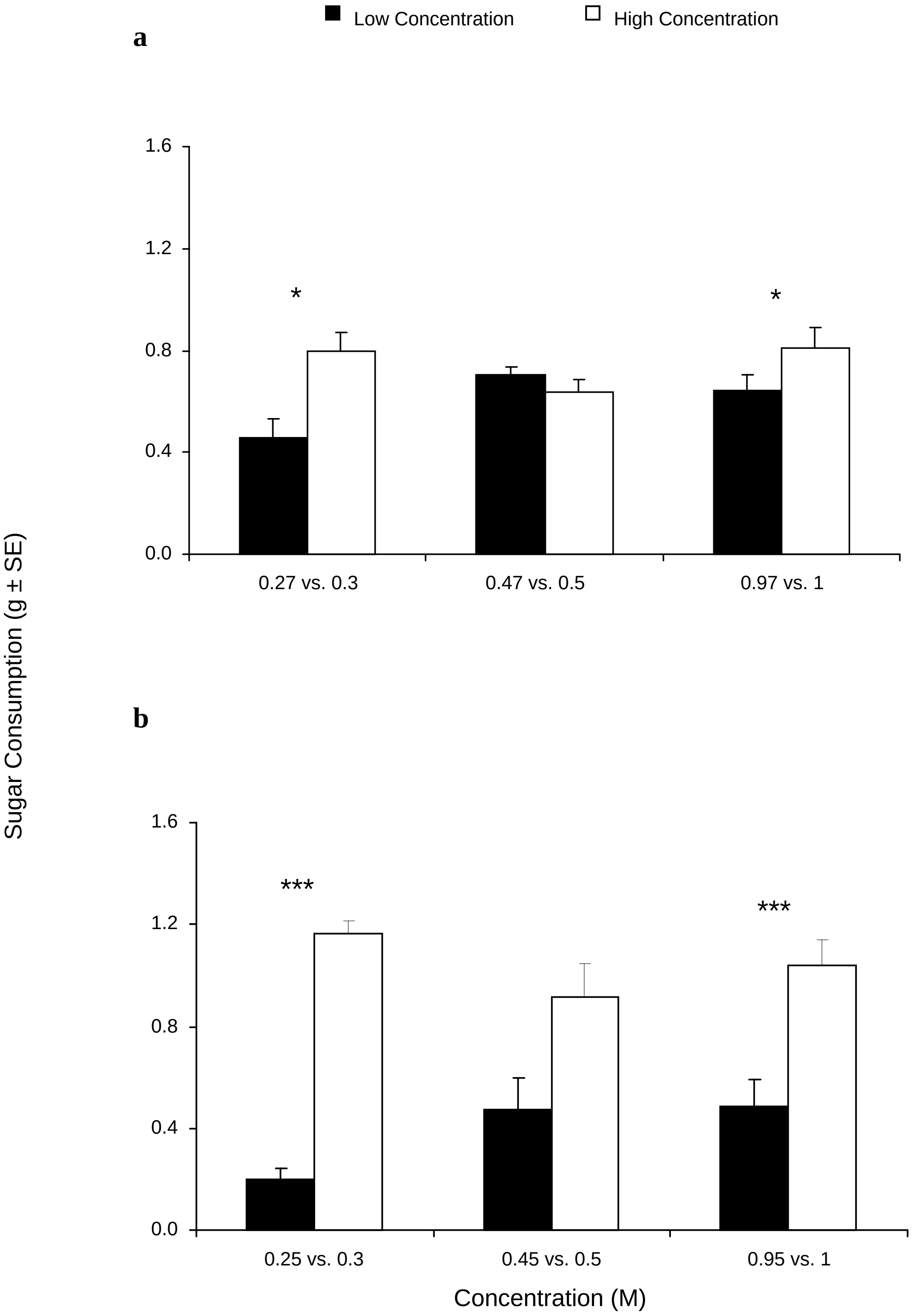

Figure 3: Sugar consumption (g, averaged over 2 days) by *Cinnyris talatala* of sucrose (a) and hexose (equicaloric 1:1 glucose and fructose) (b) solutions during paired preference tests (*n* = 10). Asterisks (*) indicate significant differences in consumption between solutions of a pair (**P*<0.05; ****P*<0.001).

Discussion

White-bellied sunbirds show concentration preferences within a sugar type at both broad and fine scales. Contrary to expectations, sunbirds showed similar preferences in concentration over broad scale differences for both sucrose and equicaloric glucose: fructose solutions. The general preference found was for concentrations around 1 M for both sugar types, and this confirms an earlier finding that white-bellied sunbirds can effectively dilute higher concentrations of artificial nectar to approximately 1.05 M by consuming supplementary water (Nicolson & Fleming 2003b). This concentration (1 M) is higher than that of most natural nectars for sunbirds (Nicolson 2002; Johnson & Nicolson 2008), and the result concurs with previous laboratory findings of other birds preferring concentrations higher than those common for natural nectars (Stiles 1976; Tamm & Gass 1986; Blem et al. 2000; Martínez del Rio et al. 2001). The reasons for this are unclear. It may be that birds can more easily maintain energy and water balance when consuming higher concentrations than those commonly found in floral nectars, through decreased foraging time and reduced water loading. Based on the results of the present study, and the dilution of concentrated nectars by consumption of supplementary water (Nicolson & Fleming 2003b), it appears that sunbirds may better maintain energy and water requirements at a concentration of 1 M sugar. Bypassing of water absorption in the kidneys occurs with dilute nectars in Palestine sunbirds, *Nectarinia osea* (McWhorter et al. 2003). This ability may allow other species of sunbirds to cope with very dilute nectars, more so than hummingbirds (Nicolson & Fleming 2003b). This water shunting requires rapid absorption of sugars and other solutes from the ingested nectar (Nicolson 2006). Activities such as foraging and physiological processes incur energetic costs which may be reduced with nectar of 1 M sugar concentration. On 1 M sucrose the sunbirds have the lowest feeding frequency, take the longest breaks between feeding events and have the lowest total feeding duration from 9.00 to 14.00 (Chapter 2, Figures 6, 8 and 9). This implies that nectar of 1 M sucrose, and likely the same for equicaloric hexose, the birds can meet their energy and water demands while having more spare time to devote to other activities.

Since sunbirds show perfect compensatory feeding in the range of 0.25 to 2.5 M sucrose (Nicolson & Fleming 2003b), their energy intake was expected to be fairly constant across all concentrations tested. White-bellied sunbirds have been previously found to average 2.77 ± 0.42 g of daily (13 h light) energy intake over the same range of concentrations (average body mass 9.27 ± 0.34 g; Nicolson & Fleming 2003b). This is approximately 0.21 g h^{-1}. Over the same concentration range in this study, the sunbirds (average body mass 8.91 ± 0.25 g) had high energy consumption gaining an average of 1.49 ± 0.10 g of sucrose over 6 h, or 0.25 g h^{-1}. The mean body mass of the two groups of sunbirds was very similar, and therefore sugar (energy) intakes are comparable. White-bellied sunbirds have a higher energy intake (Nicolson et al. 2005) and total feeding duration (Köhler et al. 2006) in the early morning under laboratory conditions. Since the 6 h experimental period was from the early morning until early afternoon (7.00 to 13.00 h), it appears that the birds can easily gain the bulk of their energy requirements during the early hours of the day.

Sunbirds consumed greater volumes of the hexose mixture than sucrose solutions at the highest concentrations (1.5 to 2.5 M), and therefore gained more grams of sugar (energy) from hexoses. This is difficult to explain, as both sugar types are assimilated with high efficiency (Lotz & Nicolson 1996; Jackson et al. 1998; McWhorter & Martínez del Rio 2000) and sucrose and equicaloric hexose solutions are equally accepted by sunbirds (Lotz & Nicolson 1996; Fleming & Nicolson 2004b). Given the high assimilation efficiency, sunbirds likely have intestinal sucrase activity, though this has not been measured to date (Nicolson & Fleming 2003a). The hydrolysis of sucrose to glucose and fructose may however still be a rate limiting step for sunbirds, leading to a preference for hexoses as they can be actively and passively absorbed. Passive uptake of glucose has been shown for several bird species. For example, northern bobwhite quail show almost complete passive absorption of L-glucose (92%, Levey & Cipollini 1996). The yellow-rumped warbler (*Dendroica coronata*) shows high D-glucose (90%) and L-glucose (91%) passive uptake efficiency (Afik et al. 1997). House sparrows (*Passer domesticus*) have been found to have >70% passive glucose absorption (Chang & Karasov 2004). In nectarivorous rainbow lorikeets, passive glucose absorption has also been found to be high (80%, Karasov & Cork 1994). Rufous and Anna’s hummingbirds have also been found to have a high passive permeability to glucose, and rely partially on passive uptake to meet energy demands (McWhorter et al. 2005). It is possible that passive absorption in sunbirds may be more heavily relied on to meet energy demands than active intestinal uptake of sugars (see Martínez del Rio and Karasov 1990; Levey & Martínez del Rio 2001), and in a recent study it has been shown that white-bellied sunbirds have extensive paracellular (passive) glucose absorption (Napier et al. 2008). Aside from possible physiological reasons, preingestional limitations caused by preingestional food processing (interference such as corolla length and other possible mechanical influences or constraints (Kingsolver & Daniel 1983)) may be affecting intake rates. Viscosity increases dramatically at the high end of the concentration range used in this study. From 1.5 to 2.5 M, the viscosity of sucrose solutions is approximately double that of equicaloric glucose: fructose solutions (Chapter 2, Figure 2), and this may impose limitations on volumetric ingestion rates (see McWhorter 2005), which in turn would influence energetic intake. These physiological and preingestional factors may help explain why the sunbirds ingested more sugar on hexose solutions.

At a finer scale of preference, I hypothesized that sunbirds, like hummingbirds and some other passerines (Levey 1987; Lloyd 1989; Blem et al. 2000; Schaefer et al. 2003) should be able to discriminate between small differences in sugar concentrations. Sunbirds were found to discriminate between diets differing by as little as 0.03 M (1% w/w) for sucrose and 0.05 M (2% w/w) for equicaloric hexose mixtures, at both low and high nectar concentrations. However, they did not show this ability at mid-range nectar concentrations. Lloyd's (1989) results for the fine (2%) discrimination between sucrose solutions at low concentrations (8, 10 and 12%) for two African sunbirds (*Cinnyris afer* and *Nectarinia famosa*, the southern double-collared and malachite sunbirds) were based on a small sample size (n = 3). The results shown here confirm that sunbirds do possess fine discrimination abilities for sucrose at low concentrations. Sunbirds are specialized nectarivorous passerines, and my results indicate that they can discriminate between diets as well as both a hummingbird and other passerines. White-bellied sunbirds respond to high concentrations like hummingbirds, which can discriminate small differences (as little as 1% w/v) around 20% w/v solutions in choice tests in the field (Blem et al. 2000). At low concentrations, white-bellied sunbirds respond like other sunbirds and tanagers, which can discriminate small differences (2%) around 0.25 to 1 M on liquid diets (sunbirds, Lloyd 1989), and 1% differences around 6 and 8% w/w on solid diets (banana mash with agar and sucrose mix; Levey 1987; mixed ingredients; Schaefer et al. 2003).

Since sugars and concentrations in nectar vary naturally over wide ranges (see Pyke & Waser 1981; Johnson & Nicolson 2008), the ability to discriminate among foods plays a vital role in foraging decisions. It must be said that the results shown here for fine scale concentration preference are to be interpreted with caution, as there was much individual variation and side bias. Some birds could discriminate "better" between diets (their selection of one diet over the other was almost exclusive) than others. Other birds simply liked feeding from one feeder regardless of the diet, and this may be attributable to side bias (Jackson et al. 1998) which could have developed due to time spent in captivity (see Appendix). Overall, the results indicate that sunbirds do have the ability to discriminate between concentrations within a sugar type. This ability, at both a broad and a fine scale, may facilitate their ability to employ foraging strategies. By selecting concentrations that will allow them to gain sufficient energy and water, and allow them more free time to allocate to other behaviours like territory defence and mate choice, they may improve their fitness.

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Chapter 3

The viscosity of artificial nectar: effect on the feeding behaviour of white-bellied sunbirds, *Cinnyris talatala* (Nectariniidae)


Carolina Leseigneur

ABSTRACT

Nectars of plants pollinated by birds, and by passerines in particular, have been widely reported to be dilute in terms of their sugar concentration. It has been proposed that the low viscosity of dilute nectars makes drinking easier for birds. Does the viscosity of nectar represent a preingestional limitation to nectarivorous birds? How does it affect their feeding behaviour? The present study represents the first analysis of the effect of viscosity on the feeding response of a bird, separate from the effects of sugar concentration. A specialized nectarivorous passerine, the white-bellied sunbird (*Cinnyris talatala*), was exposed to three different test series of sucrose solutions (control series, pure sucrose from 0.25 to 2.5 M - CS, constant viscosity series, 0.25 to 0.7 M – CVS, and constant concentration series, 1 M - CCS). Viscosities were artificially altered with Tylose ®. On dilute sucrose concentrations with increased viscosity (CVS - all equivalent to the viscosity of a pure 1 M solution), the sunbirds reduced intake rates and gained less energy compared to pure solutions of the same concentrations and pure 1 M (CS). On sucrose solutions of 1 M but with increased viscosity (CCS - equivalent to 1.5, 2 and 2.5 M solutions respectively), the birds consumed solutions at similar rates to those on pure 1.5, 2 and 2.5 M solutions (CS), but gained less energy as these intake rates are lower than the intake rate on pure 1 M sucrose. These reduced intake rates when feeding on more viscous artificial nectars occurred because sunbirds did not alter their feeding behaviour (feeding frequency, feeding duration, total feeding duration and feeding interval) in any significant way. These results suggest that sunbirds suffer a pre-ingestional limitation when consuming nectars with viscosities higher than those due to sugar concentration alone, leading to an energy deficit. On dilute solutions sunbirds appear to regulate their feeding behaviour according to viscosity, while on the mid-range (1 M) solutions they regulate feeding behaviour according to concentration.

Introduction

The question of why bird nectars, particularly those of passerine-pollinated flowers, are so dilute has been addressed in many studies (Johnson & Nicolson 2008). Of the many hypotheses proposed (for a review, see Nicolson 2002; Johnson & Nicolson 2008), the importance of low viscosity for drinking by birds was first proposed by Baker (1975). Nectarivorous birds imbibe nectar by capillarity, which is inversely proportional to viscosity (Heyneman 1983). Capillarity is defined as a physical phenomenon caused by surface tension that results in the surface of a liquid rising or falling in contact with a solid (Bourne 1982; Vogel 1994). Thus high sugar concentrations could impose constraints on the feeding behaviour and efficiency of these birds. Feeding in nectarivorous birds, especially hummingbirds, has been extensively studied, but the effect of viscosity on feeding behaviour has not been explicitly examined separately from sugar concentration for any bird species (but see Stromberg & Johnsen 1990).

Dynamic viscosity can be defined as the internal friction of a fluid which resists forces that cause change in its form (Weast 1980; Bourne 1982; Vogel 1994) and is measured in mPa.s. Sugar solutions are Newtonian fluids (Bourne 1982). A Newtonian fluid is a fluid that continues to display fluid properties regardless of the speed at which it is deformed, its stress versus rate of strain is linear. The viscosity of a Newtonian fluid depends only on temperature, pressure and its chemical composition (Vogel 1994). The viscosity of sucrose solutions increases exponentially as a function of concentration while the energy content increases linearly, and volumetric flow rate (capillarity) decreases with concentration (Weast 1980; Heyneman 1983; Gass & Roberts 1992; Josens & Farina 2001; Nicolson & Thornburg 2007). Based on these variables, it is expected that maximum energy intake rates are a compromise between energy density and viscosity. This implies that the most efficient energy intake by nectarivorous birds (and other pollinators) could fall at intermediate levels of sugar concentration (Baker 1975; Pyke & Waser 1981). Based on empirical measurements of sucrose intake rates, this has been shown to be true for many different pollinators across various taxa, including bird species (Nicolson 2007b).

Ambient temperature and nectar solutes affect the viscosity of nectar (Heyneman 1983; Bourne 1982; Mathlouthi & Génotelle 1995). Salts are mostly present in nectars as charged ions such as sodium (Na^+), potassium (K^+) and chloride (Cl^-) (Hiebert and Calder 1983). Ions can affect the viscosities of solutions by forming new chemical bonds or altering existing bonds (Bourne 1982; Vogel 1994). Amino acids can also alter the viscosity of solutions, the effect depending on concentration and the molecular mass of the amino acids (Bourne 1982; Nicolson 2007a). Low molecular weight amino acids (both D- and L-enantiomers) are always sweet to humans, but spatial barriers (their effective size in water or solutions) according to apparent specific volumes of amino acids are reported to account for the exclusion of larger L-amino acids from sweet receptors, hence they are perceived as bitter (Birch & Kemp 1989).

Sunbirds and hummingbirds imbibe nectar by licking it from flowers, and it flows via capillarity along grooves on their long tongues (Skead 1967; Schlamowitz et al. 1976; Kingsolver & Daniel 1983). Heyneman (1983) modelled feeding in hummingbirds and proposed that for large nectar volumes, a 22 to 26% w/w sucrose concentration would be optimal as feeding costs are high beyond such concentrations, assuming a steady-state continuous nectar flow. In contrast, Kingsolver and Daniel (1983) proposed that nectar flow is not a steady-state if it is induced by capillarity; they state that flow will be discontinuous, and they predicted that optimal concentrations should be 40 to 45% for high volume nectar pools that require several licks, and 20 to 25% for small volumes that require single licks for hummingbirds. However, temporal scale must also be considered. Gass and Roberts (1992) modelled hummingbird visits to flowers based on three temporal scales: the time it takes to complete tongue loading, a licking cycle (inserting the bill, inserting and withdrawing the tongue, and withdrawing the bill) and entire visits to flowers. Small volumes of nectar can be loaded onto the tongue in a single lick via capillarity, and because of fluid flow due to capillarity they require no unloading phase. The unloading phase is when the tongue is actively retracted by the bird by pulling the tongue back and up against the upper bill with the hyoid muscles, causing a negative pressure which sucks the nectar from the tongue grooves into the back tongue cavity for swallowing (Skead 1967). However for larger volumes that require several licks, fluid loading and unloading are required and higher concentrations maximize extraction efficiency in terms of energy content (Gass & Roberts 1992). This provides support for Kingsolver and Daniel's (1983) model.

Studies on certain insects provide some insight into the effects of viscosity on feeding behaviour. In the following studies, the effects of viscosity and concentration were separated by adding Tylose H 10000 YP2 ®, an inert polysaccharide, to sugar solutions. In honeybees, trophallactic food transfer by donor bees increases with sucrose concentration over a range of concentrations (10-30%). However, trophallaxis is impeded at higher concentrations by the effect of viscosity, either because it is not energetically worthwhile for the donor or because of a direct physical limitation (Tezze & Farina 1999). In a nectarivorous ant (*Camponotus mus*), foraging behaviour is affected by body size in conjunction with viscosity (Medan & Josens 2005). When given sucrose solutions with artificially increased viscosity, only large workers increased feeding time. When given solutions of constant viscosity with different concentration, large ants were indifferent in their feeding time, but smaller workers increased their feeding time on the more concentrated solution. Variations observed between different sized workers in their response to different characteristics of their food source may be a foraging specialization based on worker size. Josens and Farina (2001) analysed intake rates in response to artificially increased viscosity of sucrose solutions in the hovering hawk moth (*Macroglossum stellatarum*). Hawk moths were presented with different solutions at single feeders, and ingestion volumes and feeding times were recorded. The first series consisted of 5 to 60% sucrose solutions (base series, BS). The second comprised a series of 10 to 50% sucrose solutions mixed with Tylose ® to keep the viscosity constant at a value corresponding to that of a 50% sucrose solution (constant viscosity series, CVS). The third series kept concentration constant at 30% sucrose, but viscosity was increased by adding Tylose ® to correspond with sucrose solutions up to 60% (constant concentration series, CCS). It was found that both viscosity and concentration of sucrose in a solution influence the nectar intake rate.

The only study to date on nectarivorous birds is that of Stromberg & Johnsen (1990) who examined the independent effects of artificial sweeteners, sucrose solutions and viscosity (equal to that of sucrose solutions, altered artificially with sodium carboxymethylcellulose (CMC)) in field experiments on the feeding preferences of black-chinned hummingbirds (*Archilochus alexanderi*). The authors concluded that a sweet stimulus, rather than the mechanical effect of high viscosity, is required to induce feeding in black-chinned hummingbirds. However, these results are tentative as CMC is anionic, and ions can alter viscosity (Bourne 1982; Vogel 1994). In addition, many artificial sweeteners, like aspartame, are comprised primarily of amino acids. Aside from the chemistry of amino acids in solutions, it has been found that amino acids in artificial nectar affect the preferences of both hummingbirds and sunbirds (Hainsworth & Wolf 1976; Leseigneur et al. 2007).

Considering Baker's (1975) hypothesis, does viscosity impose a feeding limitation on nectarivorous birds, and if so, how does it affect their feeding behaviour? Preingestional limitations caused by preingestional food processing (interference caused by factors such as corolla length and other mechanical influences or constraints (Kingsolver & Daniel 1983)) may be affecting intake rates. In this study I assessed the effects of viscosity separately from those of concentration on a specialized nectarivorous passerine, the white-bellied sunbird (*Cinnyris talatala*). Sucrose solutions were prepared using molar concentrations, ranging from 0.25 to 2.5 M: below 0.25 M the birds cannot maintain their energy balance at 20ºC (Nicolson & Fleming 2003b). The birds were exposed to three different treatment series as per Josens & Farina (2001) – control series (CS, pure sucrose solutions), constant viscosity series (CVS, dilute solutions with increased viscosity equivalent to that of 1 M sucrose), and constant concentration series (CCS, 1 M solutions with variably increased viscosity). CVS and CCS sucrose solutions were altered with Tylose ® to artificially increase viscosity. To maintain energy consumption at required levels, it was expected that sunbirds would consume solutions of low concentration with high viscosities at higher rates by increasing their feeding frequency, feeding duration per feeding event, total feeding duration and by decreasing the feeding interval between feeding events. This is based on the fact that white-bellied sunbirds employ compensatory feeding over the range of sucrose concentrations used in this study (Nicolson & Fleming 2003b), and the assumption that increased viscosity will limit the amount of food ingested per unit volume. Alternatively, if viscosity is the only physical limitation, solutions of equal viscosity should be ingested at the same rates.

Materials & Methods

Birds and Maintenance

White-bellied sunbirds were captured by mist-netting at Jan Cilliers Park in Pretoria, South Africa. Six males and five females (8.41 ± 0.43 g), all with full adult plumage, were captured during April 2007. Birds were maintained in an outdoor aviary (8 x 2 x 5 m), at the University of Pretoria's Experimental Farm. They were fed a maintenance diet of 0.6 M sucrose (20% w/w) mixed with a nutritional supplement (Ensure$^{®}$, Abbott Laboratories, Johannesburg, South Africa). This diet and supplementary water were available to the birds *ad libitum* in inverted stoppered syringes (feeders), and water baths were also provided.

During the laboratory trials, the birds were maintained individually in cages of 40 x 42 x 28 cm in a climate-controlled room at 20 ± 2 ºC and 45% RH on a 12:12 h light: dark cycle (lights on at 07.00 h) as in previous studies on white-bellied sunbirds (for example, Nicolson & Fleming 2003b; Fleming et al. 2004a and b). Temperature and humidity were maintained by automated Siemens RLU 222 and 210 temperature and humidity regulators and an indoor air conditioner (Johnson Controls cr 722 CAREL). The birds were allowed to acclimatize to laboratory conditions for one week prior to experiments. Dawn and dusk were simulated with 0.5 h of dimmed light at the beginning and the end of the photoperiod. The same maintenance diet was provided during the acclimatization period. Birds were released at the end of the experiments.

Experiments

Viscosity measurements

The dynamic viscosities (mPa.s) of sugar solutions were determined with an Anton Paar (GmbH) Rheometer (Model Physica MCR 301, Cup – C-PTD200, Spindle – CC27, Advanced Laboratory Solutions, Johannesburg), with the RHEOPLUS/32 v.3.00 software package at 20°C and a shear rate 60.8 s^{-1} (automatically maintained by the system). The cup and spindle were chosen based on the nature (Newtonian fluid) and density (low density solutions) of the sugar solutions. The rheometer was calibrated with pure water as the standard (1.002 mPa.s at 20°C). Concentrations measured were sucrose and equicaloric 1:1 glucose and fructose mixtures at 0.25, 0.5, 0.7, 1, 1.5, 2, and 2.5 M. Additionally, a 0.7 M sucrose solution mixed with asparagine, serine and glutamine at 10 mM each, and 0.7 M sucrose solutions each containing either 10 or 20 mM NaCl were also measured.

The dynamic viscosity of sucrose solutions with added Tylose H 10 000 YP2 ® (SE Tylose GmbH & Co. KG, Wiesbaden, Germany - hereafter Tylose ®) was also measured. Tylose ® is a hydroxyethylcellulose ether (a polysaccharide, molecular weight 10 000 Da) used as a binder or thickener in various substances, including industrial paints and cement, make-up materials and food substances. It is non-ionic and does not alter the nutritional value or density of foods. Because it is a polysaccharide, solutions containing it will still be Newtonian fluids (Bourne 1982; Vogel 1994). Tylose ® amounts added to sucrose solutions were estimated by extrapolating from Josens & Farina’s (2001) data of kinematic viscosity of sugar solutions, using a regression equation. Sucrose solutions containing Tylose ® were then measured with the rheometer. This was necessary to determine whether the added amounts of Tylose ® increased viscosities of sucrose solutions to the desired levels. These values are tabulated in Table 1.

Table 1: Quantities of Tylose ® (following Josens & Farina 2001) added to sucrose solutions to alter viscosities for CVS and CCS series. Dynamic viscosities were measured with the rheometer at 20°C, 60.8 s^{-1} shear rate.

Series	**Sucrose Concentration**		**Desired Viscosity**		**Tylose ® added**	**Dynamic Viscosity (mPa.s)**	
	M	**Label**	**M**	**mPa.s**	**% w/w**	**Pure Sucrose**	**with Tylose ®**
CVS	0.25	0.25+T1	1	3.23	0.160	1.37	3.21
CVS	0.5	0.5+T1	1	3.23	0.108	1.79	3.23
CVS	0.7	0.7+T1	1	3.23	0.076	2.27	3.25
CCS	1	1+T1.5	1.5	8.08	0.100	3.23	8.08
CCS	1	1+T2	2	70.3	0.185	3.23	70.7
CCS	1	1+T2.5	2.5	162	0.230	3.23	161

Feeding experiments

Each bird was tested with the three different test series to differentiate between the effects of viscosity and concentration on feeding behaviour. Sunbirds were exposed to the test series in individual cages fitted with infra-red photo-detection LED systems on the feeders for automated recordings of feeding frequency, feeding duration, total feeding duration and feeding intervals (Figure 1). Sunbirds were trained to feed from these feeders during the acclimation period prior to the experiments. The birds took approximately 3 h to learn to feed from the novel food source after being initially fed by hand, except for one male that took 3 days to learn.

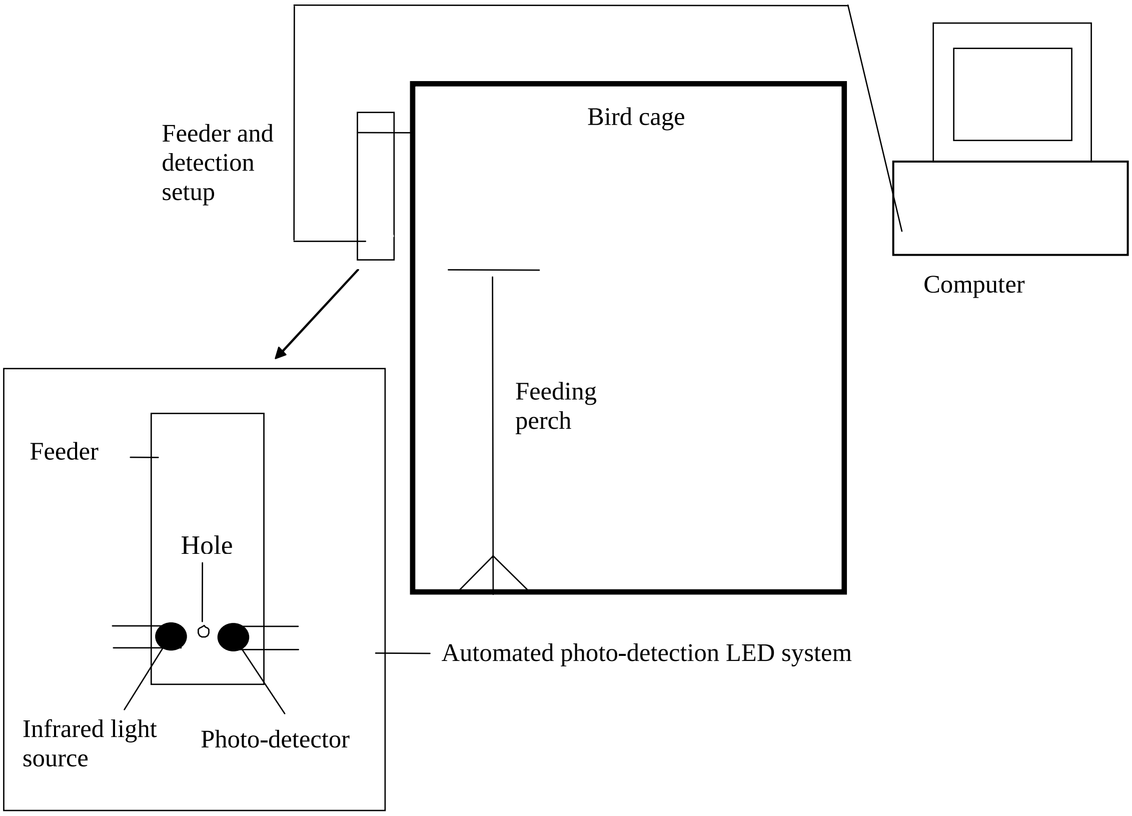


Figure 1: Schematic representation of the experimental setup and infra-red photo-detection LED system for automated recordings. Feeding frequency, feeding duration, feeding interval and total feeding duration by white-bellied sunbirds (*Cinnyris talatala*) were simultaneously recorded using custom software (L Verburgt) during test series.

The control series (CS) consisted of pure sucrose solutions of 0.25, 0.5, 0.7, 1, 1.5, 2 and 2.5 M. Feeding responses were determined by presenting single solutions in random order to all sunbirds (*n* = 11). This was conducted from 9.00 to 14.00 each day as white-bellied sunbirds in captivity have longer feeding durations (in s h^{-1}) and lower feeding frequencies in the early morning (Köhler et al. 2006), and show the most constant food intake rate during the first half of the day (Fleming et al. 2004). The maintenance diet was provided at the end of the experimental period each day so that the birds could replace any mass lost as a result of feeding on sugar-only solutions (Nicolson et al. 2005). The birds were given a rest day between test series and were also provided with the maintenance diet and water.

In the second series (constant viscosity series, CVS), the birds underwent the same procedure but were presented with dilute sucrose solutions with the viscosity of each solution held equivalent to that of a 1 M sucrose solution (Table 1). In the third series (constant concentration series, CCS), the birds again followed the same procedure but were presented with 1 M sucrose solutions with viscosities altered to be equivalent to that of 1.5, 2 and 2.5 M sucrose solutions respectively (Table1). A 1 M control solution was included in both the CVS and the CCS.

The feeder mass was measured at the start and end of the experimental period to determine food intake and thereby sugar (energy) consumption (± 0.01g, Mettler Toledo PB-602S, Microsep Ltd., Johannesburg). Containers with liquid paraffin were placed beneath feeders to collect any potential drips. These were also weighed together with the feeders, and corrections to the consumption data were made where necessary. For all test series, food consumption in grams was converted to millilitres based on the density of sugar solutions (Weast 1980), and grams of sugar (energy) consumed per solution was calculated (g ± SE). Hexoses and sucrose have the same energy value per unit gram, 16.48 x 10^3 kJ/g (Nicolson & Fleming 2003b). Feeding frequency (FF), feeding duration (FD), feeding interval (FI) and total feeding duration (Tot FD) was automatically recorded for all birds simultaneously on all solutions (resolution = 1 ms). The sugar (energy) intake rate (hereafter intake rate) was then calculated and averaged (μmol s^{-1} ± SE) by dividing sugar (energy) consumption (converted to μmol sugar consumed) by the total feeding duration (Tot FD) recorded.

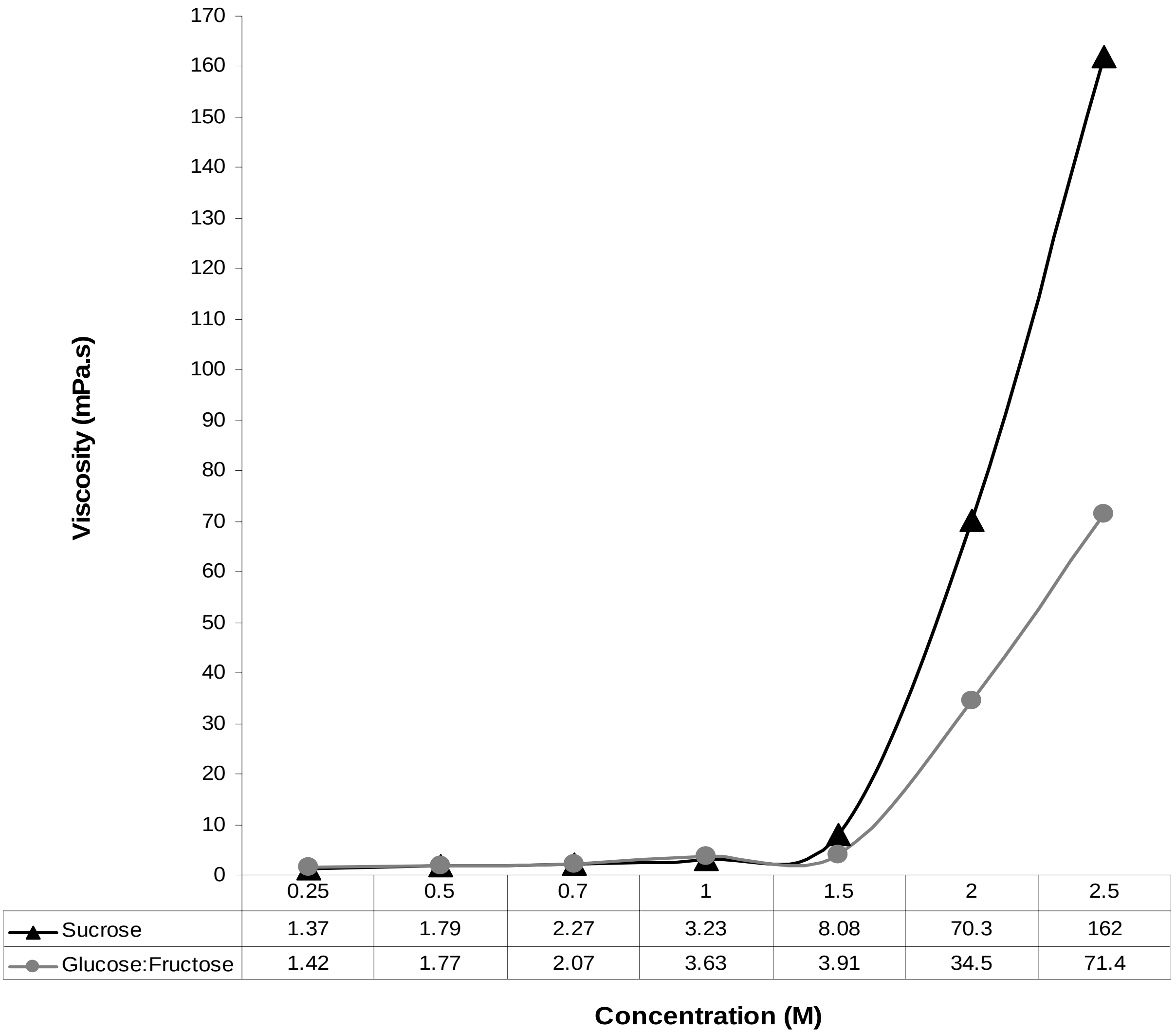
Statistical analysis

After normality tests, all data were log-transformed for analyses. For sugar (energy) consumption (g), intake rates (μmol s^{-1}) and feeding behaviour data (FF, FD, FI and Tot FD), separate overall repeated-measures ANOVA (RM-ANOVA) were performed for CS, CS vs. CVS and CS vs. CCS. The RM-ANOVAs comparing the test series comprised two within-effects, nested as follows: "*SERIES(CONC)*" where *CONC* (the different concentrations) was nested within *SERIES* (the test series). Post-hoc analyses for each RM-ANOVA were conducted using Tukey's Honest Significant Difference (HSD) test for equal sample sizes, followed by sequential Bonferroni corrections for multiple comparisons (Rice 1989). Repeatability ± SE was also calculated for the 1 M solutions of all three test series.

Results

Viscosity measurements

Sugar solutions from 0.25 to 2.5 M showed the expected exponential increase in dynamic viscosity with increasing concentration (Figure 2). Viscosity is similar for the sucrose and equicaloric hexose solutions up to 1 M. For higher concentrations, the viscosity of sucrose solutions is approximately twice that of equicaloric hexose solutions. The slight difference in viscosity at 1 M sucrose (3.23 mPa.s) and hexose (3.63 mPa.s) was unexpected and can be attributed to the natural range in viscosity of the fluid when measured (L Moitsheki pers. comm.).



	0.25	0.5	0.7	1	1.5	2	2.5
▲ Sucrose	1.37	1.79	2.27	3.23	8.08	70.3	162
● Glucose:Fructose	1.42	1.77	2.07	3.63	3.91	34.5	71.4

Figure 2: Dynamic viscosity (data table, mPa.s) of sucrose and equicaloric hexose (glucose and fructose, 1:1) solutions at 0.25, 0.5, 0.7, 1, 1.5, 2 and 2.5 M. All solutions were measured at 20°C and 60.8 s^{-1} shear rate with an Anton Paar (GmbH) rheometer.

For 0.7 M sucrose solutions containing salt (NaCl at 10 and 20 mM) or an amino acid mixture (asparagine, serine and glutamine, each added at 10 mM), the difference in viscosity was small compared to a pure 0.7 M sucrose solution (Figure 3). Viscosity was increased most by the inclusion of 20 mM NaCl.

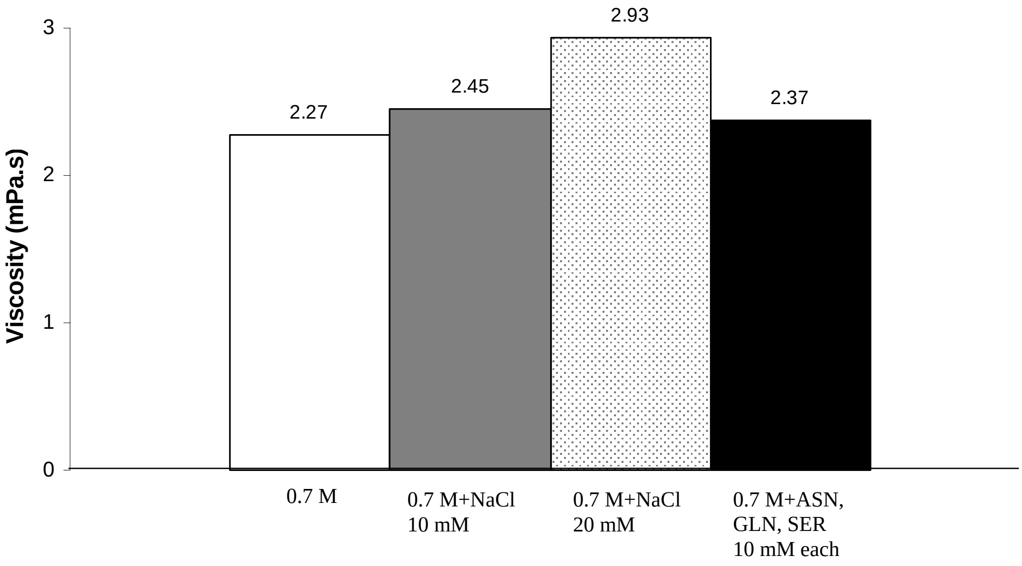


Figure 3: Dynamic viscosity (mPa.s) of 0.7 M sucrose solutions: pure sucrose; with added salt (NaCl) at 10 and 20 mM respectively; with a combination of three amino acids – asparagine (ASN), glutamine (GLN) and serine (SER) - each added at 10 mM. All solutions were measured at 20°C and 60.8 s^{-1} shear rate with an Anton Paar (GmbH) rheometer.

Energy consumption

There were no differences in energy consumption between the CS concentrations except for 0.25 M which was significantly different to all the other concentrations (not shown, but see Figure 4; $F_{6,60} = 11.4$, $P<0.01$). The general trend in consumption across concentrations from 0.5 to 2.5 M is attributable to compensatory feeding (Nicolson & Fleming 2003b).

Energy consumption on CS solutions was significantly different to the CVS solutions ($F_{3,30} = 13.233$, $P<0.01$, Figure 4). The sunbirds ingested less sugar (gained less energy) on all CVS solutions with artificially increased viscosities than on the pure CS solutions of the same concentrations. For example, on the 0.25 M CS solution, the birds ingested 1.16 ± 0.06 g sugar over the experimental period (5 hours), while on the 0.25+T1 CCS solution the birds ingested on average 0.47 ± 0.04 g sugar. Energy consumption of all CVS solutions was also significantly less than the 1 M CVS control solution.

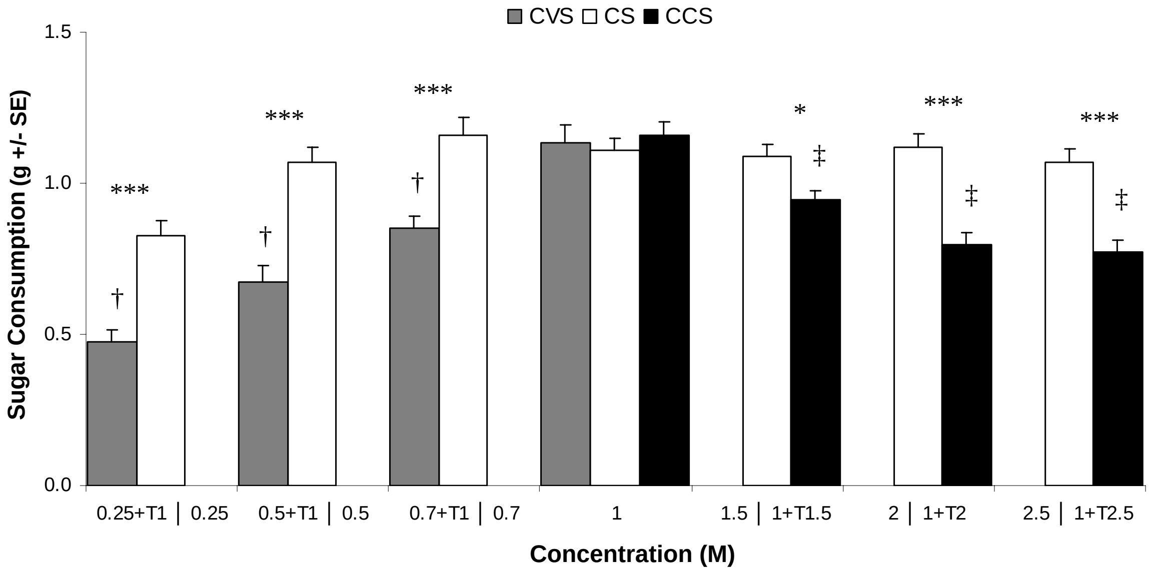


Figure 4: Average sugar consumption (g ± SE) over 5 hours by *Cinnyris talatala* (*n* = 11) for three different test series: pure sucrose solutions (CS, 0.25 – 2.5 M), dilute sucrose solutions with artificially increased viscosity (CVS, concentration+T1; viscosity increased with Tylose ® to that of 1 M sucrose), and 1 M sucrose solutions with increasing viscosity (CCS, 1+T1.5, 2 or 2.5 - viscosities equivalent to the stated molar concentrations respectively). After RM-ANOVA and post-hoc analyses, significant differences between test series are indicated by asterisks (*$P<0.05$, ***$P<0.001$). All CVS (except the CVS 1 M control) are significantly different to 1 M CS (†$P<0.01$), and all CCS are significantly different to the CCS 1 M control (‡$P<0.001$).

At higher concentration and viscosities, energy consumption on all of the CCS solutions was significantly less than on the 1 M CCS control solution ($F_{3,30} = 22.525$, $P<0.001$, Figure 4). Birds ingested less sugar (energy) on the CCS solutions. Sugar consumption of the CCS solutions was also significantly less than that of the 1.5, 2 and 2.5 M CS solutions respectively ($P<0.01$). Energy consumption on the 1 M solutions of all three test series did not differ ($F_{2,20} = 0.560$, $P=0.579$) and was highly repeatable (0.66 ± 0.15 SE).

Energy intake rates

Significant differences were found between the intake rates of the different CS concentrations (not shown but see Figure 5, F $_{6,60}$ = 12.599, P<0.001). The intake rates of the 0.25 and 2.5 M CS solutions were significantly different to the intake rates of 0.5 to 1.5 M and of 0.5 to 2 M CS solutions respectively (P<0.001 and P<0.05 in all cases respectively). From 0.5 to 2 M CS, the intake rates differed, increasing with concentration up to 1 M then decreasing with concentration up to 2.5 M.

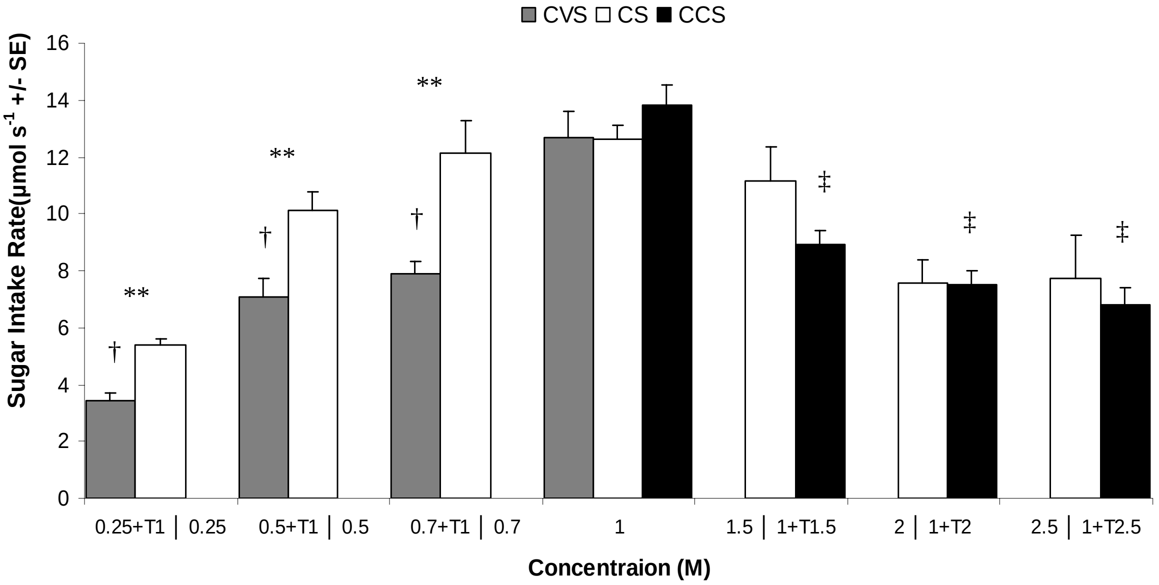


Figure 5: Average sugar intake rates (µmol s^{-1} ± SE) over 5 hours by *Cinnyris talatala* (n = 11) for three different test series (for description of series, see Figure 4). The intake rates were significantly different between CS and CVS (**P<0.01), and all CVS intake rates were significantly different to the 1 M control intake rate (†P<0.01). The intake rates of CCS solutions were only significantly different to the 1 M CCS control rate (‡P<0.01).

Between test series there were marked differences in intake rates at concentrations below 1 M. All the CVS solutions were consumed at significantly lower intake rates than the CS solutions of the same concentrations (F $_{3,30}$ = 4.824, P<0.01 in all cases), and were also consumed at significantly lower rates than the 1 M CVS control solution (P<0.01).

Intake rates of the CVS solutions followed an increasing trend similar to that of the CS solutions, i.e. intake rate increased with increasing concentration up to 1 M.

At higher concentrations and viscosities, the intake rates of all the CCS solutions were significantly lower than the 1 M CCS control solution ($F_{3,30} = 21.025$, $P<0.01$, Figure 5). Interestingly, the intake rates of 1+T2 and 1+T2.5 CCS solutions were not different to pure 2 and 2.5 M CS solutions respectively. There was no significant difference between the 1 M solutions of the CS, CCS control and the CVS control ($F_{2,20} = 1.167$, $P=0.332$), which again indicates that feeding by the birds remained regular between test series. However, intake rate was not highly repeatable (0.29 ± 0.21 SE), and this may be attributed to individual variation between birds.

Feeding behaviour

The results for the automatically recorded data revealed very few differences in feeding behaviour for the different test series (Table 2, Figures 6 - 9). The results are discussed separately for each feeding behaviour measured.

Feeding frequency (FF)

There were differences between the feeding frequencies of the CS solutions (not shown, but see Figure 6): the feeding frequency on 0.25 M was significantly higher than on 1 and 1.5 M, the 2 M feeding frequency was higher than on 1 M, and the 2.5 M feeding frequency was higher than on 0.5, 0.7, 1 and 1.5 M ($F_{6,60} = 17.159$, $P<0.01$ in all cases). The general trend for the CS solutions was a decrease in feeding frequency with increasing concentration up to 1 M, followed by an increase with increasing concentration up to 2.5 M. The high feeding frequency measured on pure 0.25 M CS is attributable to the need to ingest large volumes.

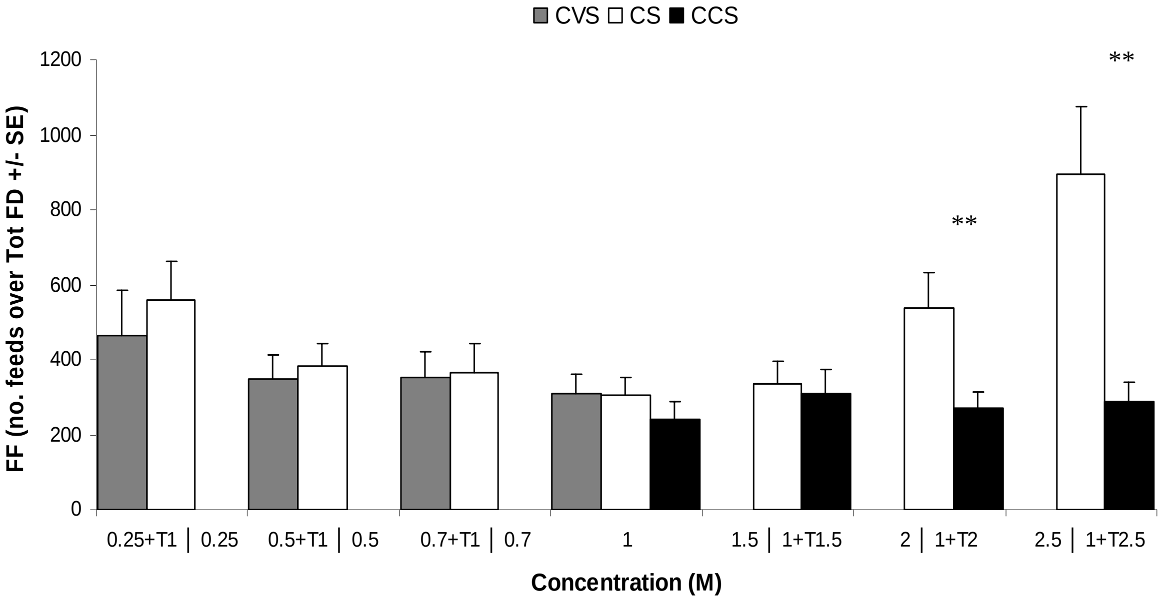


Figure 6: Feeding frequency (FF) on sucrose solutions by *Cinnyris talatala* (*n* = 11) over a 5 hour experimental period on three different test series (for description of series see Figure 4). Significant differences after RM-ANOVA and post-hoc analyses are indicated by asterisks (***P*<0.01).

Feeding frequency was highest for the two highest concentrations, 2 and 2.5 M CS. This higher feeding frequency however does not necessarily mean that the birds ate more at these concentrations, as is demonstrated by the sugar (energy) consumed and sugar intake rate (Figures 4 and 5).

Table 2: Comparison of feeding behaviours of *Cinnyris talatala* (*n* = 11) feeding on various diets of pure sucrose solutions (CS) and sucrose solutions with added Tylose ® (T) (CVS and CCS – viscosities altered to be equivalent to that of the concentration shown, "Tconc."). FF = feeding frequency, FD = feeding duration per feeding event, FI = feeding interval per feeding event, Tot FD = total feeding duration. Significant differences after RM-ANOVA and post-hoc analyses are indicated by asterisks (**P*<0.05, ***P*<0.01, ns = not significant).

Series & Concentration (M)	**FF**		**Mean FD (s)**		**Mean FI (s)**		**Tot FD (s)**	
CS 0.25	557.4 ± 104.2	ns	1.05 ± 0.20	ns	41.5 ± 25.6	ns	456.9 ± 28.2	Ns
CVS 0.25+T1	465.0 ± 119.7		1.19 ± 0.36		68.2 ± 39.5		427.8 ± 48.8	
CVS 1 control	308.8 ± 51.9	ns	1.21 ± 0.51	ns	77.1 ± 50.4	ns	288.7 ± 42.6	ns
CS 0.5	382.4 ± 61.6	ns	1.07 ± 0.21	ns	59.3 ± 34.7	ns	319.0 ± 20.2	ns
CVS 0.5+T1	348.4 ± 64.2		1.16 ± 0.31		71.3 ± 42.1		298.7 ± 25.5	
CVS 1 control	308.8 ± 51.9	ns	1.21 ± 0.51	ns	77.1 ± 50.4	ns	288.7 ± 42.6	ns
CS 0.7	364.9 ± 78.3	ns	1.08 ± 0.24	ns	66.0 ± 35.4	ns	291.0 ± 18.6	ns
CVS 0.7+T1	353.4 ± 66.0		1.18 ± 0.34		65.4 ± 41.5		321.1 ± 21.3	
CVS 1 control	308.8 ± 51.9	ns	1.21 ± 0.51	ns	77.1 ± 50.4	ns	288.7 ± 42.6	ns
CVS 1 control	308.8 ± 51.9	ns	1.21 ± 0.51	ns	77.1 ± 50.4	ns	288.7 ± 42.6	ns
CS 1	304.2 ± 46.9		1.09 ± 0.24		69.9 ± 39.7		261.7 ± 15.3	
CCS 1 control	242.6 ± 44.0	ns	1.55 ± 0.91	ns	93.9 ± 54.4	ns	249.8 ± 15.0	ns
CS 1.5	334.3 ± 63.2	ns	1.28 ± 0.46	ns	68.6 ± 42.3	ns	321.7 ± 41.0	ns
CCS 1+T1.5	309.6 ± 63.1		1.44 ± 0.35		80.1 ± 48.1		317.3 ± 21.2	
CCS 1 control	242.6 ± 44.0	ns	1.55 ± 0.91	ns	93.9 ± 54.4	ns	249.8 ± 15.0	ns
CS 2	535.7 ± 97.5	**	1.29 ± 0.38	ns	41.3 ± 35.2	**	483.2 ± 51.6	ns
CCS 1+T2	270.5 ± 45.4		1.55 ± 0.49		84.7 ± 51.6		324.7 ± 24.8	
CCS 1 control	242.6 ± 44.0	ns	1.55 ± 0.91	ns	93.9 ± 54.4	ns	249.8 ± 15.0	ns

Table 2 continued.

Series & Concentration (M)	FF		Mean FD (s)		Mean FI (s)		Tot FD (s)	
CS 2.5	894.1 ± 0.18	**	1.02 ± 0.36	**	27.1 ± 32.8	**	587.9 ± 11.8	ns
CCS 1+T2.5	288.4 ± 0.49		1.98 ± 0.78		82.8 ± 52.1		357.5 ± 38.1	
CCS 1 control	242.6 ± 44.0	ns	1.55 ± 0.91	ns	93.9 ± 54.4	ns	249.8 ± 15.0	ns

When comparing test series, I expected to see significant differences between the consumption of pure (CS) and increased viscosity (CVS) solutions. There were in fact few differences (Table 2, Figure 6). Feeding frequency remained similar to the 1 M CVS control solution across all concentrations of the CVS solutions. The small decrease in feeding frequency for the CVS solutions compared to the CS solutions of the same concentrations suggests a weak negative effect of viscosity. However, the similarity between the feeding frequencies of the CVS solutions suggests that birds were treating all solutions as being a pure 1 M sucrose concentration. For comparisons between the CS and CCS solutions I had the same expectations as for CS versus CVS solutions. Feeding frequency was significantly lower for the 1+T2 and 1+T2.5 CCS solutions compared to that for pure 2 and 2.5 M CS solutions respectively, and this is contrary to expectations. Feeding frequency was similar for both 1+T2 and 1+T2.5 CCS solutions to the 1 M CCS control solution. The feeding frequency of the 1+T1.5 CCS solution was also similar to that of the 1 M CCS control solution. The fact that feeding frequency was similar for all the CCS solutions implies that birds were responding to CCS solutions as if they were pure 1 M sucrose solutions, regardless of viscosity. For the feeding frequencies on 1 M CS, CVS and CCS solutions, repeatability was very high (0.87 ± 0.06 SE). But there were significant differences between the series ($F_{2,20} = 7.788$, $P<0.01$). Post-hoc analysis showed that this was due to the feeding frequency of the 1 M CCS solution being significantly lower to those on both the 1 M CS and CVS solutions ($P<0.05$ both cases).

Feeding duration (FD)

There were no significant differences between any of the CS concentrations for mean feeding duration per feeding event (not shown, but see Figure 7). Feeding duration was similar for all concentrations between 0.25 and 2.5 M CS.

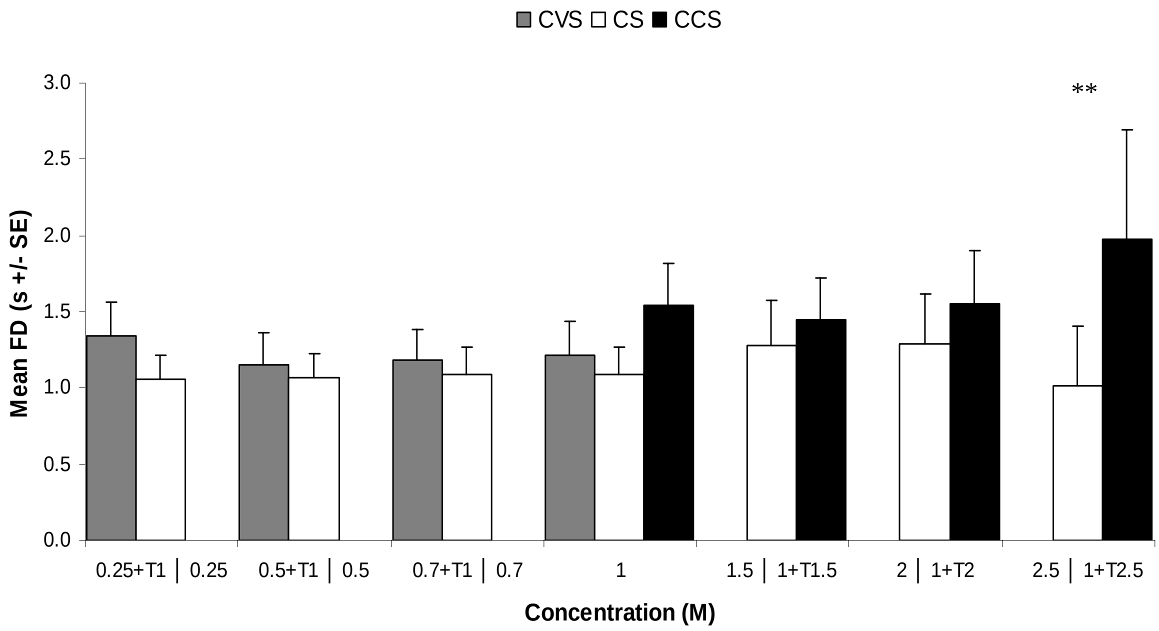


Figure 7: Mean feeding duration (FD) per feeding event on sucrose solutions by *Cinnyris talatala* (*n* = 11), over a 5 hour experimental period on three different test series (for description of series see Figure 4). Significant differences after RM-ANOVA and post-hoc analyses are indicated by asterisks (***P*<0.01).

Between test series (Table 2, Figure 7), the birds had higher feeding durations on the CVS solutions compared to the CS solutions of the same concentrations. All the CCS solutions also showed higher mean feeding durations compared to the CS solutions, but this was significant only for the 2.5 M CS solution compared to the 1+T2.5 CCS solution. All the CCS solutions were similar to the pure 1 M CCS control solution, suggesting a response to concentration alone. For the birds' feeding durations on 1 M CS, CVS and CCS solutions, repeatability was also very high (0.77 ± 0.11 SE), though again there were significant differences across the series for 1 M solutions ($F_{2,20}$ = 7.495, *P*<0.01). Post-hoc analysis again showed that feeding duration on 1 M CCS was significantly higher than on both 1 M CS and CVS solutions ($P<0.05$ both cases).

Feeding interval (FI)

The feeding interval is inversely related to feeding frequency. For the CS solutions, there was an increase in mean feeding interval up to 1 M, and thereafter a decrease with increasing concentration, opposite to the pattern of feeding frequency. Significant differences in mean feeding intervals were the same as for feeding frequency ($F_{6,60} = 17.173$, $P<0.01$ in all cases, not shown but see Figure 8).

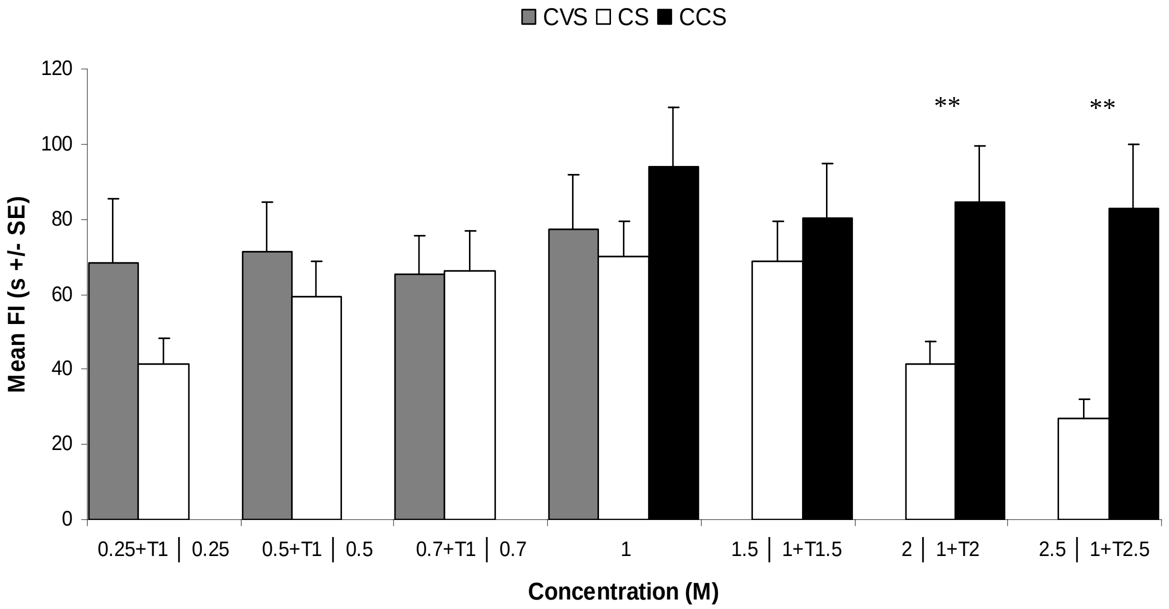


Figure 8: Mean feeding interval (FI) between feeding events of sucrose solutions by *Cinnyris talatala* ($n = 11$) over a 5 hour experimental period on three different test series (for description of series see Figure 4). Significant differences after RM-ANOVA and post-hoc analyses are indicated by asterisks (**$P<0.01$).

When feeding on 0.25 M CS, birds had a lower (i.e. shorter) mean feeding interval than most other CS solutions. This is because the birds have to feed more often on this dilute solution to meet energy demands. Fewer feeding intervals were also recorded for 2 and 2.5

M CS solutions. This can be linked to the birds' high feeding frequencies at these high concentrations, implying that the birds had to feed more often, and further suggests that they may not necessarily have been getting food on every feeding attempt, as a result of high viscosity.

Comparisons between test series revealed small differences in mean feeding intervals (Table 2, Figure 8). There were no significant differences in mean feeding interval between the CS and CVS solutions, though feeding intervals of the CVS solutions were longer, especially for the 0.25+T1 CVS solution. The increase in feeding intervals of the CVS solutions overall may be explained by the response of the birds to CVS solutions as if they were pure 1 M sucrose solutions. Mean feeding interval of the CVS solutions were also all similar to the 1 M CVS control solution. Mean feeding interval for the CCS solutions was longer than for the CS solutions. This was significant for 1+T2 and 1+T2.5 CCS solutions compared to the 2 and 2.5 M CS solutions respectively ($F_{3,30} = 20.730$, $P<0.01$ in both cases). Feeding intervals of the CCS solutions were very similar, indicating that birds may have responded to the CCS solutions as pure 1 M sucrose solutions (i.e. a weak response to viscosity). Mean feeding intervals of 1 M CS, CVS and CCS solutions had very low repeatability (0.22 ± 0.21 SE). The feeding intervals on 1 M CS, CVS and CCS solutions were significantly different ($F=_{2,20} = 6.956$, $P<0.01$). Post-hoc tests showed this was due to the feeding interval on 1 M CCS being significantly higher than on both 1 M CS and CVS ($P<0.05$ both cases).

Total feeding duration (Tot FD)

The total feeding duration was significantly higher for 2 and 2.5 M CS solutions against the 1 M CS solution ($F_{6,60} = 6.947$, $P<0.01$ in both cases, not shown but see Figure 9). The total feeding duration of 0.25 M CS was higher (not significantly) than other CS solutions up to 1.5 M CS, which can also be attributed to compensatory feeding. The differences seen in the most dilute (0.25 M) and the two most concentrated (2 and 2.5 M) CS solutions suggest that the birds 'struggle' to meet energy demands at the two extremes of the concentration range.

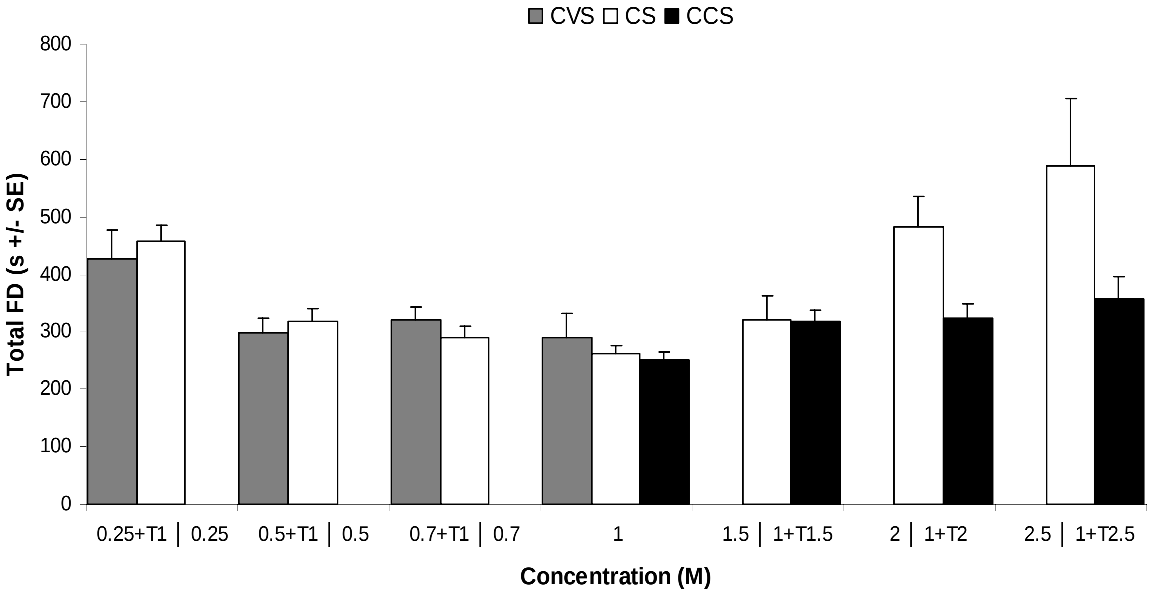


Figure 9: Total feeding duration (Tot FD) of sucrose solutions by *Cinnyris talatala* (*n* = 11) over a 5 hour experimental period on three different test series (for description of series see Figure 4). There were no significant differences after RM-ANOVA and post-hoc analyses.

Between test series, no significant differences were found in total feeding duration (Table 2, Figure 9). Total feeding duration of the CVS solutions was very similar to the CS solutions of the same concentrations, and also largely comparable to the 1 M CVS control solution. The 0.25+T1 CVS was slightly higher in total feeding duration than all other CVS solutions, which compares well to the total feeding duration of the pure 0.25 M CS.

There is a small increase in total feeding duration across all the CCS solutions compared to the pure 1 M CCS control solution, but all CCS solutions were broadly similar to each other. However, the CCS total feeding durations were still lower than those of pure 2 and 2.5 M CS solutions. Total feeding duration between the 1 M solutions of the CS, CVS and CCS were not different ($F_{2,20}$ = 0.67, *P*=0.52). For the total feeding durations of 1 M CS, CVS and CCS solutions, repeatability was low (0.43 ± 0.19 SE), and this contributed to the low repeatability in energy intake rates.

Discussion

There are consequences for nectarivorous birds feeding on dilute nectar which have led to several different adaptations (Nicolson & Fleming 2003a; Nicolson 2007b). However, the effect of the viscosity of nectar on the feeding behaviour of nectarivorous birds has received little attention. Baker (1975) first suggested that the high viscosity of concentrated nectars limited the energy intake rates of avian nectar consumers and may explain the occurrence of dilute nectars. The results of the present study show that for dilute and mid-range sucrose concentrations with artificially increased viscosities, sunbirds have lower energy intake rates which lead to decreased energy consumption. This indicates that viscosity is determining the feeding behaviour of the sunbird and suggests a preingestional limitation that may lead to energy deficits in these birds.

It was expected that the sunbirds would alter their feeding behaviour in response to artificial nectars with increased viscosities in a compensatory manner. However when feeding on more viscous artificial nectars sunbirds did not alter their feeding behaviour in any significant way to compensate for the decrease in energy intake rates and energy consumption. The high feeding frequency and total feeding duration observed on 0.25 M CS, and the similarity in mean feeding duration across all the concentrations of the CS are comparable to results obtained by Köhler et al. (2006). They looked at short-term feeding patterns in white-bellied sunbirds using 10, 20 and 30% w/w sucrose solutions (0.3, 0.7 and 1 M respectively). Significantly more feeding events and a longer total feeding duration were recorded on 10% sucrose, and there were no differences in feeding duration between the concentrations. The only behaviour that showed a weak response to viscosity was mean feeding duration per feeding event, which increased slightly on all concentrations with artificially increased viscosities. This is similar to the response seen in large workers of nectarivorous ants, which increased feeding duration on 30% w/w sucrose solutions with a viscosity equivalent to that of a 60% solution (Medan & Josens 2005). This lack of change in feeding behaviour with higher viscosities led to the lower energy intake rates and energy consumptions observed. In terms of feeding behaviour, sunbirds broadly responded to the CVS and CCS solutions as if they were pure 1 M sucrose solutions. For CVS solutions, this implies that birds responded to viscosity as a feeding stimulus, while for CCS solutions, it implies that birds responded to concentration as a feeding stimulus.

The overall trend in energy intake rates, with a peak at 1 M sucrose, is loosely comparable to the data of Josens & Farina (2001): intake rates in hovering hawk moths increased with increasing concentration to 20%, and decreased beyond this concentration as viscosity increased. Low intake rates were recorded for the sunbirds on the most dilute (0.25 M) and two most concentrated (2 and 2.5 M) concentrations. According to Baker’s (1975) hypothesis, energy intake rate is limited by the low energy content at low concentrations – this is evident by the fact that sunbirds had very high feeding frequencies, total feeding duration and short feeding intervals between feeding events on 0.25 M sucrose. At high sugar concentrations, energy intake rate is limited by the mechanical effect of high viscosity – this is also evident from the high feeding frequency, total feeding duration and short feeding intervals recorded for sunbirds on 2 and 2.5 M sucrose. The birds "struggle" to feed at the two extremes of the concentration range, at the dilute end because of energy content, at the high concentration end because of higher viscosity. The mechanical effect of viscosity on tongue loading may explain this phenomenon. The tongues of sunbirds ranging from 6 to 10 g in body mass are long bifurcated "tubes" (Skead 1967; Cheke et al. 2001), approximately 0.2 mm in diameter and 0.46 µl in volume (Schlamowitz et al. 1976; Downs 2004). Nectar is loaded on the tongue by capillarity (Schlamowitz et al. 1976; Skead 1976; Downs 2004) and high sugar concentrations with high viscosities could impose constraints on the feeding efficiency of birds. If sunbirds were not fully loading their tongues on 2 and 2.5 M sucrose because the solutions were too viscous for efficient capillarity, it would explain the need for higher feeding frequencies, total feeding durations and shorter feeding intervals between feeding events observed.

When viscosity was kept constant (CVS), hovering hawk moths had higher intake rates for lower concentrations than they did for pure 50% sucrose solution (Josens & Farina 2001). This is in contrast to sunbirds, which decreased intake rates when viscosity was kept constant (CVS) at lower sugar concentrations. However, intake rates did not differ between CCS and pure solutions of the same viscosities in the hovering hawk moth (Josens & Farina 2001). The sunbirds also showed no difference in intake rates between CCS and CS (pure) solutions of the same viscosity, which indicates that viscosity is the only physical limitation to feeding behaviour if solutions of different viscosity are presented in an equal manner (such as similar flower morphology).

The results lead to another question - why do white-bellied sunbirds not respond in a compensatory manner to a viscosity higher than that caused by sugar concentration in nectar? One possibility is that sugar concentration alone may be the driving force behind the feeding behaviour of nectarivorous birds, regardless of viscosity. The results for the CCS solutions imply that the birds were responding to concentration as the feeding stimulus. This provides some support for the conclusion of Stromberg & Johnsen (1990) regarding black-chinned hummingbirds, though there are reservations about this work due to the use of CMC (sodium carboxymethylcellulose) and amino acid containing artificial sweeteners. The viscosity of sucrose solutions (10, 20, 30 and 40% w/v) and solutions containing artificial sweeteners (Equal ®, pure aspartame and saccharin, mixed to mimic the sweetness of 20% sucrose) were measured with a capillary viscometer at the ambient temperatures of the study site. Artificial sweetener solutions were increased to sucrose equivalent viscosities with CMC. These solutions were supplied to the birds together with solutions containing sucrose-only and CMC-only (sucrose equivalent viscosities) in three-way tests. Artificial sweeteners alone were first found to be ignored by the hummingbirds, and increasing their viscosity made no difference to their acceptance. 20% sucrose was significantly preferred over a CMC solution of equal viscosity to 20% sucrose. Thus the authors concluded that sweet stimuli rather than viscosity were driving feeding responses in hummingbirds.

Alternatively, or concurrently, it may be that viscosities of natural nectars never increase beyond the viscosities caused by sugar concentration alone, and may therefore be negligible for sunbirds. As the 0.7 M sucrose solutions with added salt or amino acids show, other solutes common in floral nectars may not greatly affect the viscosity. 0.7 M is a typical concentration for floral nectars of plants pollinated by passerines (Nicolson 2002, Johnson & Nicolson 2008). Amino acids typically occur at low concentrations in floral nectars (Nicolson 2007a, Nicolson & Thornburg 2007). Salt concentrations in the nectar of 19 species of hummingbird-pollinated flowers averaged 24.7 mM for potassium, 3.4 mM for sodium and 9.9 mM for chloride (Hiebert & Calder 1983). In some southern African plant species, potassium can occur from 4.2 to 17 mM and sodium from 3.3 to 18 mM in nectar (Nicolson & Thornburg 2007). The impact of other solutes and fallen pollen on nectar viscosity is not known, and small quantities of certain solutes may have a large effect on viscosity. High molecular weight polymers cause a jelly-like consistency in the nectar of vertebrate-pollinated flowers (Johnson et al. 2001; Sazima et al. 2001).

Sunbirds were clearly limited in sugar intake rates and sugar (energy) consumption by viscosity. But why did the sunbirds' feeding behaviour on CVS solutions resemble that on pure 1 M sucrose solutions? In food science, it is generally understood that increasing viscosity through the addition of a thickener results in a decrease in the perceived sensitivity of volatile and non-volatile components in humans, especially reducing sweetness perception (Hollowood et al. 2002). At low concentrations (<0.5g/100g) of hydroxyl propyl methylcellulose (HPMC), the perception of sweetness (sucrose solutions) was unaffected, but at higher concentrations (>0.6g/100g) perceived sweetness decreased. However, if this was the case for sunbirds, they should have shown a response to the perceived decrease in sweetness as a "lower concentration", and should have fed at higher frequencies and longer feeding durations, due to the employment of compensatory feeding. This was not the case.

In Chapter 1 I reported that concentration preferences within a sugar type peak at 1 M for both sucrose and equicaloric hexoses. Data on the feeding behaviour presented here support this finding. White-bellied sunbirds had the lowest feeding frequency and total feeding duration, as well as the highest feeding interval between feeding events when offered 1 M sucrose. This gives the birds more "free time" to devote to other behaviours, such as territorial defence and courtship, and would imply an advantage to selecting nectar of 1 M sugar concentration. But this does not really explain the results on the dilute-high viscosity solutions (CVS), unless the birds base their feeding preferences of dilute (less than 1 M sugar) concentrations on viscosity. This is possible, as the viscosities of sucrose solutions from 0.25 to 1 M are very similar. Most natural nectars occur in this range and concentrations above 1 M are generally not common (Pyke & Waser 1981; Nicolson 2002; Nicolson & Fleming 2003a; Johnson & Nicolson 2008). In a natural context, the comparison of sucrose and equicaloric hexose mixtures is not important for viscosity – the effect of viscosity will occur regardless of sugar type. However, effects may be somewhat different between sugar types: nectars have complex compositions, and sugars are never pure, though some nectars are dominated by sucrose or by hexoses (Nicolson & Thornburg 2007; Johnson & Nicolson 2008). The viscosity of equicaloric hexoses is approximately half that of sucrose from 1.5 to 2.5 M, and suggest an advantage to feeding on hexose-dominated nectars at high concentrations.

Despite these possible explanations for the lack of response in feeding behaviour by sunbirds, it is clear that viscosities of artificial nectar higher than those caused by sugar concentration alone do impede energy intake rates, and therefore energy consumption. The results imply that this limitation is mechanical as the birds did not alter their behaviour to compensate for higher viscosities. In other words, the sunbirds are experiencing reduced sugar intake rates potentially leading to energy deficits because the higher viscosity of their food may be reducing the amount of nectar consumed per lick of the tongue. This is possible, because the birds appear to regulate their feeding behaviour according to viscosity on dilute solutions (less than 1 M), while on the mid-range solution (1 M) they appear to regulate their feeding behaviour according to concentration.

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Chapter 4

Conclusion

Sunbirds are specialized nectarivorous passerines and important pollinators of many plant species (Skead 1967; Cheke et al. 2001). They rely heavily on nectar as their primary food source and have highly energetic lifestyles. The white-bellied sunbird *Cinnyris talatala*, in particular, habituates well to captivity and has been the subject of much research concerning floral nectars and energy management, and the physiological adaptations of the birds to a simple, watery diet (for example, Nicolson & Fleming 2003; Fleming et al. 2004; Köhler et al. 2006). For this reason, the white-bellied sunbird was chosen for the present study to address specific aspects of their feeding preferences and behaviour.

Though research into feeding behaviour, physiology and plant-pollinator relationships of nectarivorous birds is extensive (for some reviews see Martinéz del Rio et al. 2001; Nicolson 2002; Lotz & Schondube 2006; Johnson & Nicolson 2008), there is a lack of research with regards to the concentration preferences of nectarivorous birds within a sugar type, and nothing clear is known about the effects of viscosity on feeding behaviour. The present study included the analysis of concentration preferences by the white-bellied sunbird within sugar types, and the first analysis of the effect of viscosity separate from sugar concentration on the feeding behaviour of a nectarivorous bird.

The results from the concentration preference experiments revealed that sunbirds do prefer certain concentrations over others within a sugar type. The peak preference occurred at 1 M for both sucrose and equicaloric hexose solutions. This is significant in terms of the birds' physiology. White-bellied sunbirds effectively dilute higher concentrations to near 1 M by consuming supplementary water (Nicolson & Fleming 2003). This indicates that a 1 M solution not only meets energy demands, but also meets water requirements perhaps without the necessity of shunting excess water past the kidneys (McWhorter et al 2003; Nicolson & Fleming 2003; Nicolson 2006). Palestine sunbirds (*Necatrinia osea*) can bypass the kidneys by not absorbing up to two thirds of ingested water when feeding on dilute diets, passing the water to be expelled (McWhorter et al. 2003). It has also been found that fractional water reabsorption in the kidney is sensitive to water status (McWhorter et al. 2004). The results are also of ecological significance. Birds that can discriminate between concentrations and choose those that maximize their free time for other activities, such as territory defence and mate choice, may more effectively increase their fitness. Fine scale discrimination between concentrations was also observed for sunbirds, as in hummingbirds and other nectarivorous passerines (Levey 1987; Lloyd 1989, Blem et al. 2000; Schaefer et al. 2003). However, this is stated with caution, as there was high individual variation and side bias in this experiment and the fact that it was not repeatable (see Appendix) casts some doubt on the certainty of the result.

The present study also revealed that viscosity appears to be of great importance as a limiting factor in energy intake rates and energy consumption of white-bellied sunbirds. Given the concentration preferences, and the expected mechanical effect of viscosity on feeding by affecting tongue loading, sunbirds were expected to alter their feeding behaviour as viscosity of sugar solutions changed. However, the results showed that sunbirds do not alter their feeding behaviour to compensate for higher viscosity, but they did respond in an unexpected way. They responded in their feeding behaviour to dilute solutions whose viscosity was equivalent to a pure 1 M sucrose as if they were pure 1 M solutions, and they treated 1 M sucrose solutions with variedly increased viscosities as pure 1 M solutions too. In other words, viscosity appears to be the primary factor in driving foraging choices on dilute solutions, while concentration becomes the primary factor from mid-range concentrations. This is a highly significant result as it agrees with the findings of concentration preference, and the results provide support for Baker's (1975) hypothesis: the viscosity of nectar increases exponentially with sugar concentration, and low concentrations are necessary for efficient extraction of nectar from flowers.

Of great significance in the present study was the fact that sunbirds consumed greater volumes of equicaloric hexose than sucrose solutions at the highest concentrations (1.5 – 2.5 M). This suggests an advantage to feeding on hexose dominated nectars for white-bellied sunbirds, as the viscosity of hexose solutions at these high concentrations is approximately half that of sucrose solutions. It is important to note that these concentrations (1.5 - 2.5 M) are not common in nature (Pyke & Waser 1981; Nicolson 2002; Nicolson & Fleming 2003a; Johnson & Nicolson 2008). In a natural context, the comparison of sucrose and equicaloric hexose mixtures is not important – the effect of viscosity will occur regardless of sugar type. However, effects may be somewhat different between sugar types due to the difference in viscosity. Nectars have complex compositions and sugars are never pure, though some are dominated by either sucrose or by hexoses (Nicolson & Thornburg 2007; Johnson & Nicolson 2008).

In conclusion, concentration drives foraging in the white-bellied sunbird, but viscosity impedes normal intake rates and determines choices of dilute solutions. It is however important to state that the viscosity of artificial nectars comprising solutes typical of natural nectars (at typical concentrations) do not increase viscosity as much as was done in this study. But other solutes may more adversely affect viscosity, especially trace amounts of certain carbohydrates. For example, high molecular weight polymers cause a jelly-like consistency in the nectar of an African lily and a species of Combretum, which are vertebrate-pollinated flowers (Johnson et al. 2001; Sazima et al. 2001).

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Appendix

Further experiments on fine scale concentration preferences of white-bellied sunbirds

Summary

In Chapter 1, fine scale concentration preferences of white-bellied sunbirds were examined. The results demonstrated that the birds could distinguish between concentrations differing by as little as 0.03 M (1% w/w) for sucrose and 0.05 M (2% w/w) for equicaloric hexose solutions. These resembled some previous findings in hummingbirds and other nectarivorous passerines (see Chapter 1 for results and discussion). However, since there was much individual variation between birds and side bias in the first fine scale concentration preference experiment, and a discrepancy between sucrose and hexose differences (1 and 2 % w/w differences), I opted to repeat the experiment. The experiment was repeated twice: once more on the same set of birds (after one year in captivity – the original experiment (Chapter 1) was after six months in captivity) and again on a new set of relatively young birds (after one month in captivity). Both groups of sunbirds however showed no fine scale preferences except for the second group which showed a preference for the highest concentration available – but this result is tentative as there was high individual variation between these birds.

Repetition of this experiment on fine scale concentration preferences has produced results that conflict with those in the original experiment. This can not be explained by the time in captivity, since similar data were obtained with the original group of birds (now older) and the new group of sunbirds. It may be that there is a factor of experience missing for the new group of sunbirds as several were relatively young, though their ages are not defined and this is speculative. The reasons for these conflicting results are unclear and warrant further investigation.

Materials & Methods

Birds and maintenance

The same mature birds used for experiments in Chapter 1 were used for the first fine scale concentration preference experiment reported here (set 1, captured in July 2006), (n = 8, 8.51 ± 0.18 g). The second set of sunbirds for the second experiment reported here (set 2), three males and four females (7.7 ± 0.69 g), were captured in April 2007 at Jan Cilliers Park. Maintenance and housing of the birds followed the procedure described in Chapter 1.

Fine scale concentration preference experiments were repeated as described in Chapter 1 for both sets of sunbirds. The differences between concentrations in pairwise tests in the new experiments were 0.03 M (1% w/w) for both sucrose and equicaloric hexose solutions (0.27 vs. 0.3 M, 0.47 vs. 0.5 M and 0.97 vs. 1 M). Data were arranged and analysed as described in Chapter 1: data were collected as mass (g) of each solution consumed (mean ± SE), then converted to volumetric consumption (ml) based on the density of sugar solutions at each concentration, and to sugar consumption (g). Sugar consumption data were analysed using the STATISTICA 7.1 (StatSoft Inc. ©, Tulsa, OK, USA, 2006) software package.

For initial analysis, an overall repeated-measures ANOVA (RM-ANOVA) was performed for all of the consumption data to determine where differences occurred. The overall RM-ANOVA comprised three within-effects, nested as follows: "*DIET*(*CONC(TESTDAY))*" where *TESTDAY* (the difference between the two days of each preference test) was nested within *CONC* (the difference between the two concentrations), which was nested within *DIET* (the difference between the three different concentration pairs). Following the overall RM-ANOVA, consumption values were tested separately for each concentration pair by separate RM-ANOVA. The structure of these RM-ANOVA therefore comprised two within-effects nested as follows: "*CONC(TESTDAY)*". Post-hoc analyses for each RM-ANOVA were conducted using Tukey's Honest Significant Difference (HSD) test for equal sample sizes.

Results

Fine scale concentration preferences – set 1

The first set of sunbirds showed no preferences between concentrations differing by 1% w/w (Figure 1). The overall RM-ANOVA for sugar consumption for both sugar types show that there were also significant differences between diets for sucrose and hexose mixtures (sucrose $F_{2,14} = 112.8$, $P<0.001$, hexose $F_{2,14} = 27.226$, $P<0.001$), but there were no significant differences between concentrations for either sugar type (sucrose $F_{1,7} = 1.409$, $P=0.273$, hexose $F_{1,7} = 0.063$, $P=0.809$). Post-hoc analysis (Tukey's HSD) revealed that these significant effects were, as for broad scale preferences (Chapter 1), due to the mean consumption of each concentration pair increasing as concentrations increased, and some birds also showed side biases. Also, consumption varied more between test days than during the first fine scale preference experiment (Chapter 1).

Data were separated by diet for further analysis. No significant differences were found between any concentration pairs for either sugar type (Table 1 and Figure 1). The difference between test days for sucrose diets was due to individual variation and side biases.

Table 1: Sugar consumption (g) differences between concentrations (*CONC*), days (*TESTDAY*), and their interaction (*CONCxTESTDAY*) for sucrose and the hexose mixture (1:1 equicaloric glucose and fructose) for white-bellied sunbirds, *Cinnyris talatala* (set 1). Values are F-values from RM-ANOVA, with significant effects after Tukey's HSD indicated by asterisks (*$P<0.05$); $n = 8$ and d.f. = 1 in all cases.

	Sucrose			**Glucose: Fructose**		
Concentration Pairs (M)	**CONC**	**TESTDAY**	**CONC x TESTDAY**	**CONC**	**TESTDAY**	**CONC x TESTDAY**
0.27 vs. 0.3	0.3072	10.1396*	6.8416	0.0867	2.8729	0.0572
0.57 vs. 0.6	0.9650	6.2815*	4.4634	0.0115	0.6129	0.0005
0.97 vs. 1	3.0637	0.225	0.2831	0.154	0.882	0.002

Fine scale concentration preferences – set 2

The same experiment was conducted on a second set of sunbirds. Sunbirds failed to show preferences between concentrations differing by 0.03 M (1% w/w) except at the highest concentrations for sucrose (Figure 2). The overall RM-ANOVA for sugar consumption showed significant differences between experimental diets for sucrose and hexose mixtures (sucrose $F_{2,14} = 46.391$, $P<0.001$, hexose $F_{2,14} = 52.601$, $P<0.001$), and between concentrations for sucrose ($F_{1,7} = 6.714$, $P<0.05$). Post-hoc analysis (Tukey's HSD) revealed that significant effects were due to the mean consumption of each concentration pair increasing as concentrations increased.

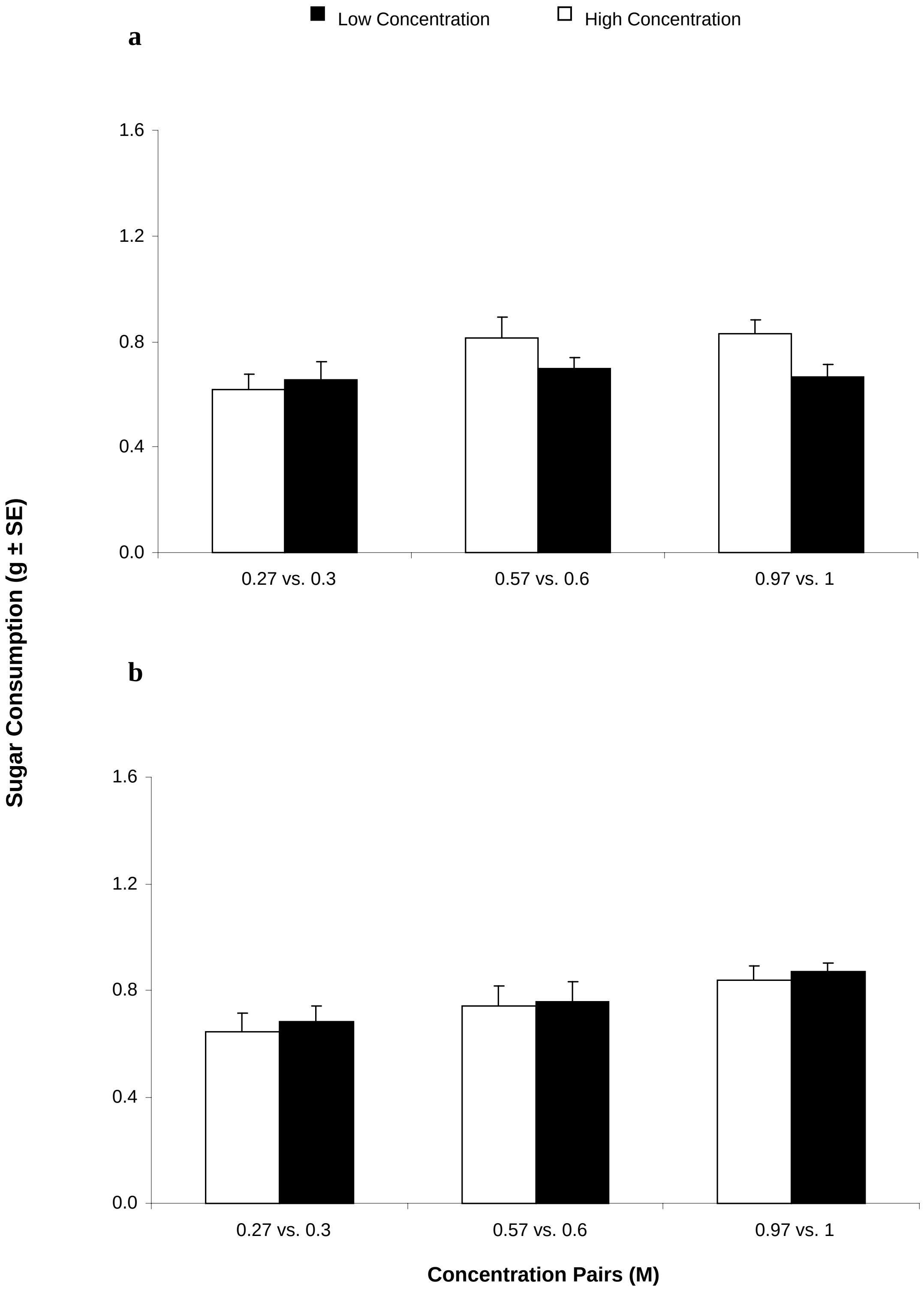

Figure 1: Sugar consumption (g) by *Cinnyris talatala* (set 1) of sucrose (a) and hexose (equicaloric 1:1 glucose and fructose) (b) solutions from paired preference tests (*n* = 8). No significant differences in consumption between pairs were found after Tukey's HSD (RM-ANOVA Table 1).

Data were again separated by diet for further analysis. There was a significant difference in concentration between 0.97 and 1 M sucrose, indicating a preference by young birds for 0.97 M (Table 2 and Figure 2). However this result is uncertain. On day one of the preference test all birds were indifferent to either concentration, and on day two all but two of the birds consumed the 0.97 M sucrose solution almost exclusively over the 1 M solution. This caused a significant difference between test days (Table 2), and this was possibly due to experience gained from the first test day.

Table 2: Sugar consumption (g) differences between concentrations (*CONC*), between days (*TESTDAY*), and their interaction (*CONCxTESTDAY*) for sucrose and the hexose mixture (1:1 equicaloric glucose and fructose) for white-bellied sunbirds, *Cinnyris talatala* (set 2). Values are F-values from RM-ANOVA, with significant effects after Tukey's HSD indicated by asterisks (**P*<0.05); *n* = 8 and d.f. = 1 for all effects.

	Sucrose			**Glucose: Fructose**		
Concentration Pairs (M)	**CONC**	**TESTDAY**	**CONC x TESTDAY**	**CONC**	**TESTDAY**	**CONC x TESTDAY**
0.27 vs. 0.3	3.9682	0.1998	1.0921	0.647	2.354	0.394
0.57 vs. 0.6	0.5543	0.0005	0.9102	0.095	0.196	0.514
0.97 vs. 1	8.3522*	6.0473*	5.1215	1.031	0.691	0.276

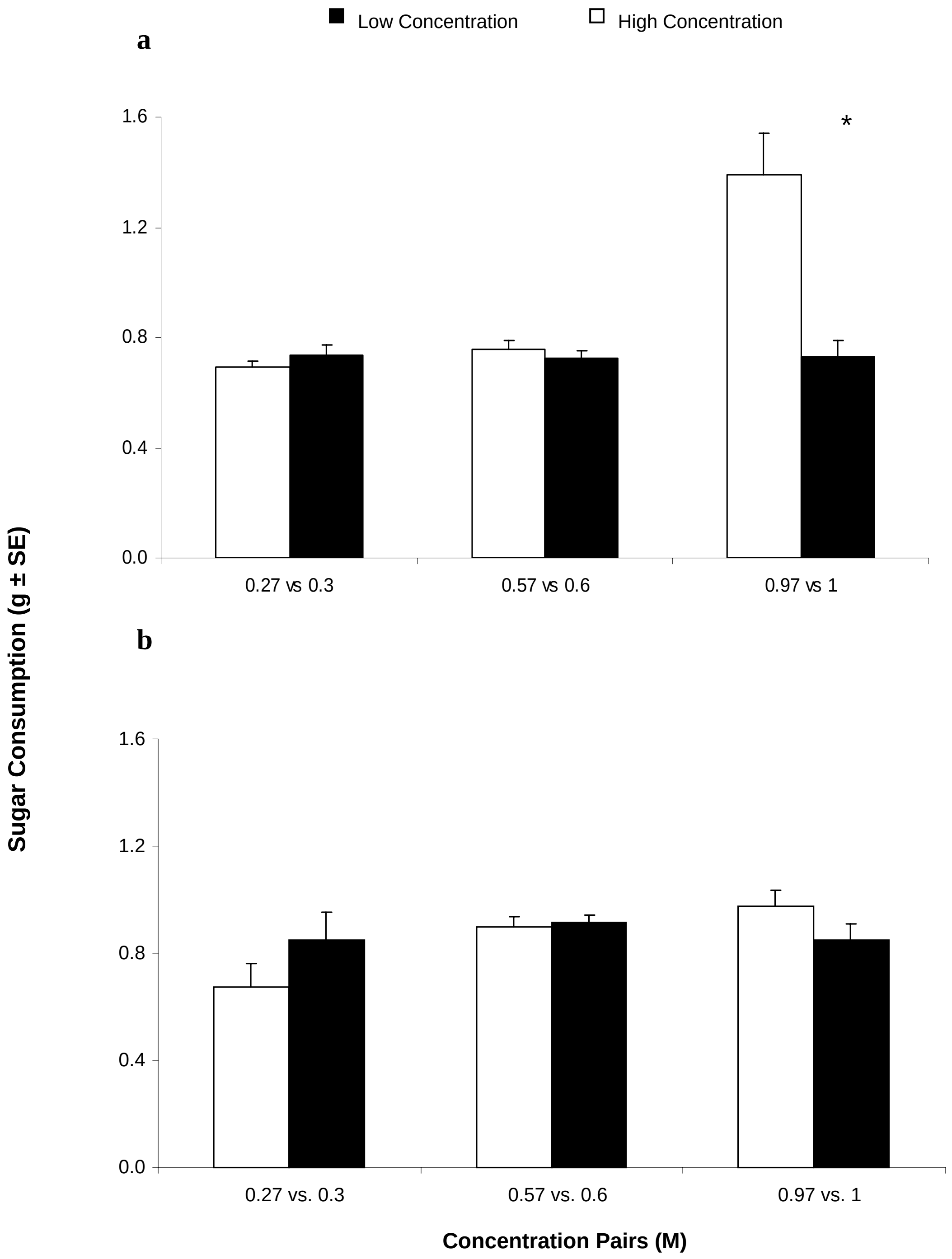

Figure 2: Sugar consumption (g) by *Cinnyris talatala* (set 2) of sucrose (a) and hexose (equicaloric 1:1 glucose and fructose) (b) solutions from paired preference tests (*n* = 8). Asterisks (*) indicate significant differences in consumption between pairs after Tukey's HSD (RM-ANOVA Table 2; CONC: *$P < 0.05$).

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