CHAPTER 1

FOOD INTAKE OF WHITEBELLIED SUNBIRDS (*CINNYRIS TALATALA*): CAN MEAL SIZE BE INFERRED FROM FEEDING DURATION?

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Abstract

A positive relationship between feeding duration and meal size of avian nectarivores has often been assumed in earlier studies. We investigated whether feeding duration can be used as a surrogate for the amount of sugar solution ingested by whitebellied sunbirds, *Cinnyris* (*Nectarinia*) *talatala*. Feeding durations of sunbirds consuming three sucrose concentrations (10, 20 and 40% w/w) were measured using an infrared photodetection system, and the amounts consumed were recorded simultaneously by weighing the feeder throughout the experiment. For all three diet concentrations, a positive relationship was found between the time spent feeding per 30 min and the mass consumed. Therefore, feeding duration is demonstrated to be an index of the amount ingested on a particular sugar concentration. The rate of ingestion, however, depended on the sugar concentration, being highest at the lowest concentration of 10% and lowest at 40%. Total time spent feeding was lower on 20% than on 10% sucrose, but increased on the 40% diet due to viscosity effects. There appeared to be a weak relationship between feeding patterns and sex, but this was not significant, probably due to inter-individual variation.

Introduction

Food intake as a fundamental physiological function of animals has been extensively investigated. Feeding patterns are examined by measuring characteristic parameters such as meal size and feeding duration. Meal size can be recorded by weighing the food continuously, for example by placing food containers on scales as in experiments with rats (Blanton et al. 1998; Kahler et al. 1998). In nectar-feeding insects, meal size has been measured by weighing the insect before and after the meal (Hainsworth et al. 1991; Paul and Roces 2003). For avian nectarivores, however, food intake is commonly determined on a daily or hourly basis only. Nectar intake has been measured by weighing the feeder (Nicolson and Fleming 2003; Nicolson et al. 2005) or by reading changes in fluid meniscus levels in glass or plastic feeders, as done for honeyeaters (Collins and Clow 1978), sunbirds and sugarbirds (Jackson et al. 1998) and hummingbirds (López-Calleja and Bozinovic 2003). Feeding durations of nectar-feeding birds, on the other hand, have been recorded with a stopwatch (Collins and Clow 1978) or a video camera (Mitchell and Paton 1990). Feeding durations have also been measured using a photodetection system, connected to a timer, with a photo beam being broken when the bird feeds (Gass 1978; Garrison and Gass 1999; Köhler et al. 2006).

The relationship between feeding duration and meal size has often been assumed in previous studies on nectar-feeding birds. Mitchell and Paton (1990), for instance, recorded honeyeaters' handling times for artificial flowers containing nectar of known volume and concentration. They calculated sugar intake rate on the assumption that handling time is positively related to the volume consumed. However, this relationship has not been sufficiently investigated in avian nectarivores. To our knowledge, only one study, on western spinebills (*Acanthorhynchus superciliosis*), has demonstrated that longer feeding durations are reflected in larger volumes of nectar ingested (Collins and Clow 1978).

Previously we have used an infrared photodetection system to investigate short-term feeding patterns of whitebellied sunbirds, *Cinnyris* (*Nectarinia*) *talatala*, fed on artificial nectar of various sugar concentrations (Köhler et al. 2006). However, the relationship between feeding duration of the sunbirds and their meal size could not be determined directly. We used feeding duration as an estimate of meal size, based on the assumption that food intake rates should be constant for birds feeding on a single sugar concentration. The present study aimed to determine whether feeding duration of whitebellied sunbirds can in fact be used as a surrogate for the amount of sugar solution ingested. Here we defined meal size as the amount of sugar solution ingested in 30 min, since the minute amounts of solution ingested in a single feeding event could not be detected. We also compared males and females to determine whether there is a sex-specific feeding pattern in this species.

Materials and methods

Study animals and their maintenance

Ten non-reproductive whitebellied sunbirds were captured with mist nets in Jan Cilliers Park, Pretoria. Birds were initially housed together in an outdoor aviary measuring 8 x 5 x 2 m. One week prior to experiments, birds were moved to individual cages of 45 x 45 x 32 cm in a climate-controlled room. They were acclimated to the experimental temperature of 20 ± 2ºC and a light : dark photoperiod of 12:12 h, with lights on at 07h00. The maintenance diet consisted of 20% weight / weight (w/w) sucrose (0.63 M) with a nutritional supplement (Ensure®, Abbott Laboratories, Johannesburg, South Africa) to provide dietary nitrogen (van Tets and Nicolson 2000). The maintenance diet and supplementary water were provided *ad libitum* in inverted, stoppered syringes. Body mass (mean ± SE) of five males and five females was 9.31 ± 0.39 g and 8.08 ± 0.18 g respectively. Our experiments were approved by the Animal Use and Care Committee of the University of Pretoria (AUCC 060515-012).

Experimental design

Each sunbird received 10%, 20% and 40% w/w sucrose solutions (0.32, 0.63 and 1.35 M) in randomized order for a period of 24 h each. The experimental diet was changed every morning before lights on. To prevent mass loss on sugar-only diets (Nicolson and Fleming 2003) all experimental diets contained Ensure®. The amount of Ensure® added was adjusted according to the sugar concentration of the experimental diet, since the volumes ingested are inversely proportional to sugar concentration. This means that the 40% diet contained the largest amount of Ensure® to keep protein intake constant. Even if the carbohydrate component of the added Ensure® were completely digested by sunbirds, it would increase the highest sugar concentration to only 42.3%, and is therefore negligible.

Birds were moved sequentially to an experimental cage (Köhler et al. 2006) and were allowed to acclimate for one day before measurements commenced. The cage (50 x 40 x 45 cm) was constructed from Perspex, with ventilation holes, and contained a feeding perch and a resting perch (Fig. 1). Automated recording of the time spent feeding as well as the number of feeding events was achieved by an infrared photodetection system interfaced to a computer (Köhler et al. 2006). Outside the cage, the feeder with the sucrose solution was mounted on an electronic balance (Mettler Toledo PB-602S, 0.01 g, Microsep Ltd, Johannesburg), interfaced to the same computer. Feeder mass was recorded every 0.2 s to determine the mass consumed by the bird. Occasional drips were collected in a container with liquid paraffin (to avoid evaporative mass loss), which was also placed on the electronic balance (Fig. 1). Thus, the dripping of the solution did not affect the results.

Data processing

For each bird we obtained the start and end times of all feeding events and their durations (± 0.001 s). Separate feeding events could not be defined by a return to the perch after feeding since sunbirds remain perched during feeding events and do not hover to feed like hummingbirds. We therefore defined feeding event duration as the time between insertion of the bill into the sucrose solution and its removal (Köhler et al. 2006). Feeding event durations of less than 0.1 s were excluded from analysis since observations revealed that they were caused by wing movements of the bird or incomplete insertion of the bill into the feeding aperture. Separate feeding events were merged into a single feeding event when the interval between them was less than 0.25 s, since turns of the bill or its incomplete removal from the feeder were recorded as two feeding events (A. Köhler, pers. observation).

The light period of the day was divided into 24 intervals of 30 min each. The first and last of these were excluded because individuals differed in the time of their first and last feeding event of the day (described in detail in Köhler et al. 2006), leaving 22 intervals that were used for analysis. For each individual and each diet concentration, the number of feeding events was calculated for each of the 30 min intervals, defined as feeding frequency. Furthermore, the total time that the bird spent feeding during each 30 min interval, defined as feeding duration, was determined by summing the durations of all feeding events. Although we recorded the feeder mass every 0.2 s, the minute amount of sugar solution consumed in a single feeding event could not be detected, since the resolution of our balance was too coarse (0.01 g). Therefore, we defined the mass consumed per 30 min interval as meal size. Meal size was calculated by subtracting the last feeder mass record of each 30 min interval from the first one. Furthermore, we determined the mass of solution consumed daily by summing the meal size of each interval. Sucrose intake per 30 min interval was calculated for all birds on all diet concentrations.

Statistical procedures

1) Meal size and feeding behaviour

Linear regressions were calculated to determine relationships between (a) feeding duration and meal size and (b) feeding frequency and meal size on all diets. For each relationship (a and b), data were arranged for each individual (n=10) and for each diet concentration (n=3), by 30 min intervals (n=22). This meant that each regression (n=30 for each relationship) was based on 22 data points. This analysis was performed for each individual separately since we demonstrated large differences in feeding pattern between individuals in our previous study (Köhler et al. 2006). The regression slopes obtained from (a) are a measure of food intake rate and are hereafter exclusively referred to as intake rate. Intake rates of each individual were compared between the three diet concentrations by repeated-measures ANOVA (RM-ANOVA).

2) Differences between the sexes and body mass relationships

Data were tested for normality and homogeneity of variance; log transformations were used when data were not normally distributed. Although body mass data were normally distributed (Kolmogorov-Smirnov test: $D>0.21$; $P>0.20$), we used the more conservative non-parametric Mann-Whitney U test to test for differences in body mass between males and females because of the problems associated with distribution estimation from small sample sizes (Zar 1999). RM-ANOVAs were conducted to determine differences between the sexes in feeding duration, feeding frequency and meal size. Data were arranged so that intervals (n=22) were nested within each diet concentration (n=3), with sex being the categorical predictor (between-effect). The intake rates obtained from 1(a) were also subjected to RM-ANOVA to test for differences between the sexes. Intake rates of the 10 sunbirds on all diet concentrations (n=3) were dependent variables, with sex being the categorical predictor. Linear regressions were calculated to determine sex-independent relationships between body mass and feeding duration over the whole day and between body mass and mass of solution consumed daily for all diets. For each relationship, data were arranged for each diet concentration (n=3), by individual (n=10). This meant that each regression (n=3 for each relationship) was based on 10 data points.

3) Daily rhythm, feeding patterns and consumption on the different diets

Data were tested for normality and homogeneity of variance; square root transformations were used when data were heteroscedastic. RM-ANOVA was performed to determine differences in feeding duration and meal size throughout the day (between 30 min intervals) and between diet concentrations. Data were arranged as follows: intervals (n=22) were nested within sucrose concentration (n=3), yielding 66 dependent variables. The daily mass of solution consumed and sucrose intake of the 10 sunbirds were compared between the diet concentrations (n=3) by RM-ANOVA.

Linear regressions describing individual relationships between feeding behaviour and meal size were followed by a sequential Bonferroni correction to avoid Type I errors (Rice 1989). *Post-hoc* comparisons for all RM-ANOVA were conducted with Tukey’s Honest Significant Difference test for equal sample sizes, followed by a Bonferroni correction for multiple comparisons. All data are presented as means ± SE. Prior to Bonferroni corrections the level of significance was $P \leq 0.05$ for all tests.

Results

1) *Meal size and feeding behaviour*

1.1) Feeding duration: The feeding duration of each sunbird was positively related to meal size for all three sucrose concentrations (all $F_{1,20} \geq 4.54$, all $P \leq 0.05$, all $R^2 \geq 0.19$), except for one individual on the 40% diet ($F_{1,20}=2.94$, $P=0.10$, $R^2=0.13$). However, this bird was not excluded from analysis since there was no obvious reason such as poor condition of the bird or equipment malfunction which would justify its exclusion. Figure 2 shows the positive relationship between meal size and feeding duration of all 10 birds for each sucrose concentration (10%: $y = 0.013\ [\pm 0.0004]\ x + 0.125$, $R^2=0.98$; 20%: $y = 0.011\ [\pm 0.0012]\ x + 0.014$, $R^2=0.82$; 40%: $y = 0.005\ [\pm 0.0003]\ x + 0.008$, $R^2=0.90$).

Intake rates for all 10 sunbirds, obtained from the regression analysis of feeding duration and meal size on the three diets, are shown in Figure 3. The intake rate decreased with increasing sucrose concentration ($F_{2,18}=27.86$, $P<0.001$). *Post-hoc* analysis revealed a significant difference in intake rates between 10 and 40% ($P<0.001$) and between 20 and 40% ($P<0.001$). The intake rates on the 10 and 20% sucrose concentrations did not differ ($P=0.67$).

1.2) Feeding frequency: A positive relationship was evident between meal size and feeding frequency, with 24 out of the 30 linear regression analyses of all three sucrose concentrations being significant (all $F_{1,8} \geq 5.63$, all $P \leq 0.03$, all $R^2 \geq 0.22$). However, the regression slopes varied greatly between individuals. Mean values of these slopes (mg per feeding event) ± SE (range) were: 10%: 10.2 ± 1.4 (3.2–16.0); 20%: 8.0 ± 1.5 (1.7–16.2); 40%: 4.2 ± 0.8 (0.9–8.1). Therefore, our analysis focuses exclusively on the relationship between feeding duration and meal size.

2) Differences between the sexes and body mass relationships

A significant sexual body mass dimorphism was evident ($U<0.01$, $P<0.01$), with males being 15.2% heavier than females. No significant difference between the sexes was found for feeding duration (10%: $F_{1,8}=0.61$, $P=0.46$; 20%: $F_{1,8}=0.26$, $P=0.62$; 40%: $F_{1,8}=0.44$, $P=0.53$) or meal size (10%: $F_{1,8}=4.61$, $P=0.06$; 20%: $F_{1,8}=1.17$, $P=0.31$; 40%: $F_{1,8}=3.84$, $P=0.09$). However, there appeared to be a weak relationship between sex and feeding duration or meal size respectively, with males feeding for longer and consuming larger amounts than females on all diets. Feeding duration over the whole day was not related to body mass on any diet concentration (all $F_{1,8}\leq0.49$, all $P\geq0.51$, all $R^2\leq0.06$). There was also no significant relationship between the mass of solution consumed daily and body mass on any of the diet concentrations (all $F_{1,8}\leq1.33$, all $P\geq0.28$, all $R^2\leq0.14$). Feeding frequency did not differ between the sexes (10%: $F_{1,8}=0.87$, $P=0.38$; 20%: $F_{1,8}=2.81$, $P=0.13$; 40%: $F_{1,8}=1.80$, $P=0.22$), but females appeared to have higher feeding frequencies than males on all diets. The intake rates on each diet concentration (Fig. 3) did not differ between the sexes ($F_{1,8}=0.11$, $P=0.75$).

3) Daily rhythm, feeding patterns and consumption on the different diets

Irrespective of diet concentration, birds showed a daily rhythm in their feeding patterns. Both feeding duration ($F_{2,21}=5.02$, $P<0.001$) and meal size ($F_{2,21}=5.37$, $P<0.001$) varied significantly over the course of the day. Both were lower in the last 2.5 h of the experimental period than during the rest of the day (feeding duration: $P\leq0.03$; meal size: $P\leq0.003$). In Fig. 2, these evening intervals lie at the lower end of the regression lines for all three sucrose concentrations.

Feeding duration differed between sucrose concentrations ($F_{2,21}=31.86$, $P<0.001$). Feeding duration was lower on the 20% diet than on 10%, but increased on the 40% diet (Fig. 2). *Post-hoc* analysis showed significant differences in feeding duration between all concentrations (10% and 20%: $P<0.001$; 10% and 40%: $P<0.001$; 20% and 40%: $P=0.01$). Meal size decreased with increasing diet concentration (Fig. 2; $F_{2,21}=424.30$, $P<0.001$). *Post-hoc* analysis revealed a significant difference in meal size between all concentrations ($P<0.001$). Mean masses of solution consumed daily (g) ± SE were: 10%: 19.30 ± 0.75; 20%: 10.26 ± 0.35; 40%: 5.04 ± 0.18 ($F_{2,18}=861.39$, $P<0.0001$). *Post-hoc* analysis showed a significant difference in mass of solution consumed daily between all concentrations ($P<0.001$). The daily sucrose intake did not differ between diet concentrations ($F_{2,18}=2.59$, $P=0.08$; Fig. 4). Sucrose intake was highest during the morning and decreased during the afternoon (Fig. 4).

Discussion

Meal size and feeding duration

Feeding duration of whitebellied sunbirds has been clearly demonstrated to be an index of the meal size on a particular sugar concentration. This evidence of the positive relationship between feeding duration and meal size has major implications for other studies, since it could only be assumed up to now. With the exception of one study on western spinebills, which showed that feeding durations are positively related to volumes of nectar consumed (Collins and Clow 1978), no other evidence exists for nectar-feeding birds. In hovering hawk moths (*Macroglossum stellatarum*) a relationship between feeding duration and the volume of sucrose solution ingested has also been demonstrated (Josens and Farina 2001). A study measuring the food intake of Sprague-Dawley rats (*Rattus norvegicus*), on the other hand, showed that the correlation between meal size and feeding duration is not as strong as would be needed to accurately estimate meal size from duration data (Castonguay et al. 1986). Cameron (1998) reviewed studies that have correlated time spent suckling by mammalian infants with their milk intake. Less than half of the reviewed studies found a significant positive relationship, which is insufficient evidence that time spent suckling provides an index of milk intake (Cameron 1998).

Viscosity effects and compensatory feeding

The food intake rate depended on the sugar concentration of the diet, being highest at the lowest concentration of 10% and lowest at 40%. Increasing viscosity of the solution with increasing concentration might be an explanation for this observation. A study on licking rates of rufous hummingbirds (*Selasphorus rufus*) showed that both licking frequency and volume per lick decreased with increasing sugar concentration (Roberts 1995). In our study, feeding duration on the 40% solution was prolonged due to the increased viscosity, accentuated by the Ensure® which was added in adjusted quantity to the experimental diets (see Materials and methods). As a result, feeding duration was lower on 20% than on 10%, but was found to increase on the 40% diet, although meal size decreased with increasing sugar concentration due to compensatory feeding (Nicolson and Fleming 2003). Thus, birds were able to maintain a constant daily sucrose intake (Fig. 4) by adjusting meal size according to diet concentration.

Daily rhythm in feeding patterns

Irrespective of diet concentration, birds showed a daily rhythm in their feeding patterns. Both feeding duration and meal size were found to be lower in the late afternoon and evening, compared to the rest of the day. Our previous study also showed the tendency for birds to spend less time feeding during the afternoon than in the late morning (Köhler et al. 2006). A rather different pattern was evident when food intake was examined on an hourly basis: whitebellied sunbirds fed steadily during the morning, followed by reduced intake during the afternoon and then an increase in food intake in the end of the day to provide energy stores for the night (Fleming et al. 2004).

Differences between the sexes and individual variation

A sexual body mass dimorphism was evident for whitebellied sunbirds used in this study, which suggests possible differences in food intake between the sexes. Markman et al. (2006) demonstrated sex-specific differences in transit time of Palestine sunbirds (*Cinnyris oseus*), which might affect foraging behaviour. However, the sexual size dimorphism of their birds was exceptionally high, with males being 28% heavier than females (Markman et al. 2006). In our study the sexes did not differ in their feeding patterns, despite males being 15% heavier than females. However, males appeared to feed for longer and have larger meal sizes than females on all diets. This may indicate the possibility of a sex-specific feeding pattern, since there was no sex-independent relationship between body mass and both feeding duration over a whole day and mass of solution consumed daily. Interestingly, females appeared to have higher feeding frequencies than males. Meal size of females might be smaller due to their smaller body size (and thus greater volumetric constraint since sunbirds do not have a crop), and they might compensate by feeding more often.

Sex-specific feeding patterns were probably obscured by individual feeding patterns. Inter-individual differences were also evident in the large variation in the regression slopes obtained from the relationship between meal size and feeding frequency. Our previous work has already highlighted pronounced variation between individual sunbirds, which differ greatly in their feeding frequency and duration of feeding events (Köhler et al. 2006). Nicolson and Fleming (2003) also demonstrated large variation in sucrose intake between individual whitebellied sunbirds. Therefore, we suggest that the number of birds used in further investigations could be increased to avoid the likelihood of sex-specific feeding patterns being obscured by inter-individual variation.

Acknowledgements

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Figure legends

Fig. 1. The experimental cage was constructed from Perspex and contained two perches. An infrared photodetection system, interfaced to a computer, was set up on either side of the feeding aperture in order to record feeding events. Next to the cage, the feeder with sugar solution was mounted on an electronic balance, interfaced to the same computer.

Fig. 2. Meal size (g) as a function of feeding duration (s) for 10 sunbirds each fed three sucrose concentrations (10, 20 and 40% w/w). Each data point (mean ± SE) represents one 30 min interval (n=22 per concentration). Grey lines represent 95% confidence intervals; x-axis error bars were omitted for clarity.

Fig. 3. Intake rates for individual birds (n=10; mean ± SE) for the three sucrose concentrations. The ranges in intake rate ($g \bullet min^{-1}$) for each concentration were as follows: 10%: 0.61–1.15; 20%: 0.51–1.40; 40%: 0.19–0.39. Statistical results derive from the Tukey’s Honest Significant Difference *post-hoc* test that followed comparison of the intake rates on the different diets by RM-ANOVA (n. s. $P>0.05$; ** $P<0.01$).

Fig. 4. Sucrose intake ($mg \bullet g$ body $mass^{-1}$) per 30 min interval of 10 sunbirds (mean ± SE) on each of the three sucrose concentrations (10, 20 and 40% w/w). Birds maintained a constant sugar intake irrespective of sucrose concentration. Error bars have been partly omitted for clarity.

Figures

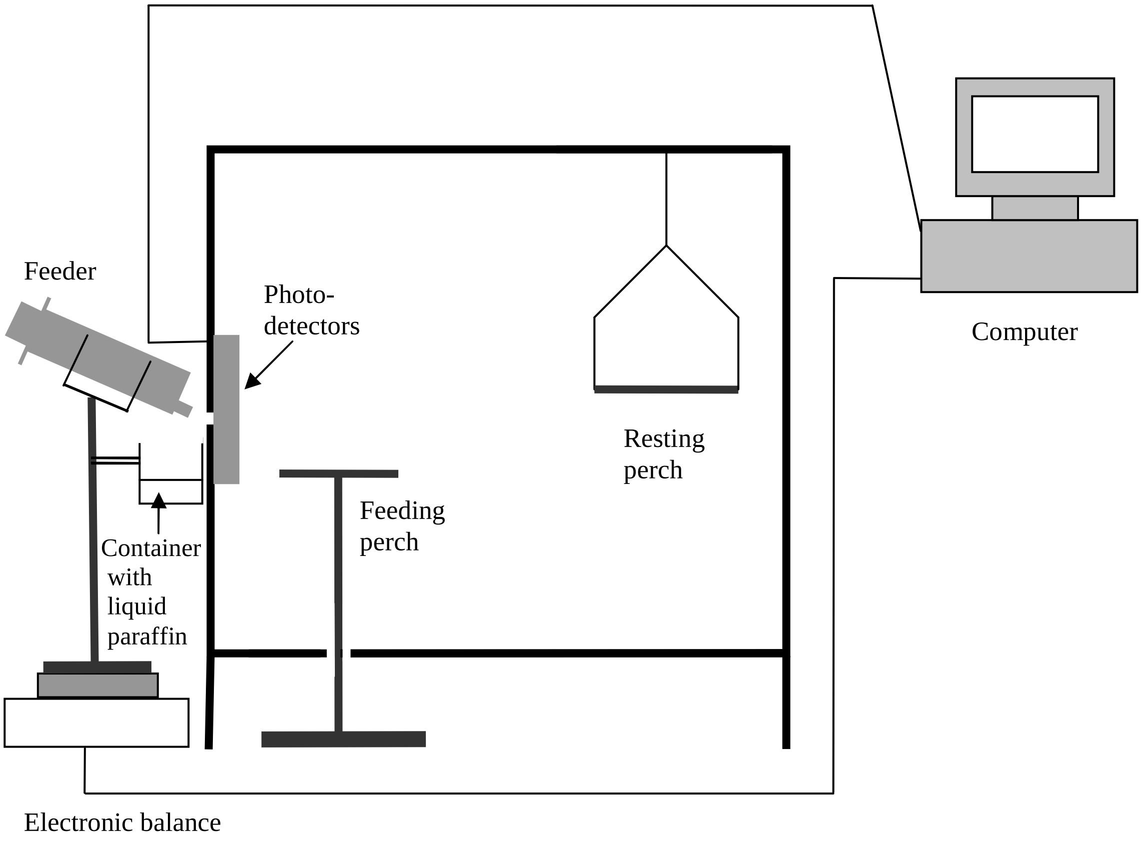

Figure 1.

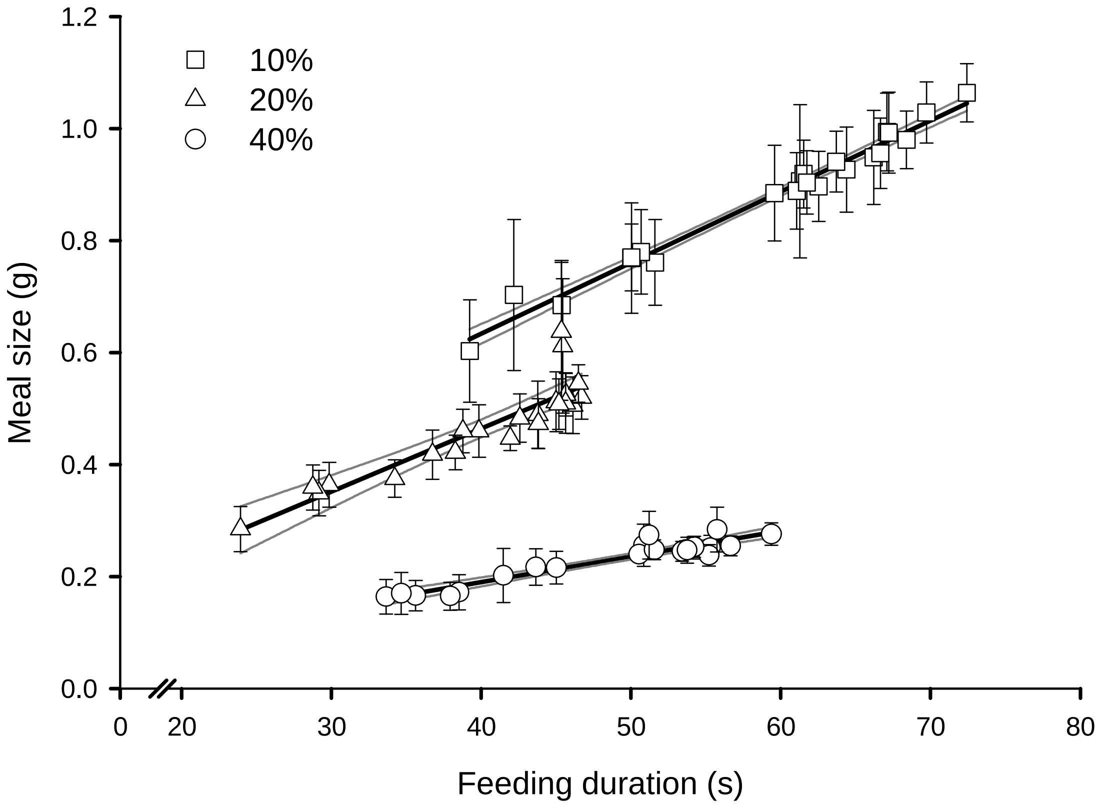
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Meal size (g)
□ 10%
△ 20%
○ 40%
0
20
30
40
50
60
70
80
Feeding duration (s)

Figure 2.

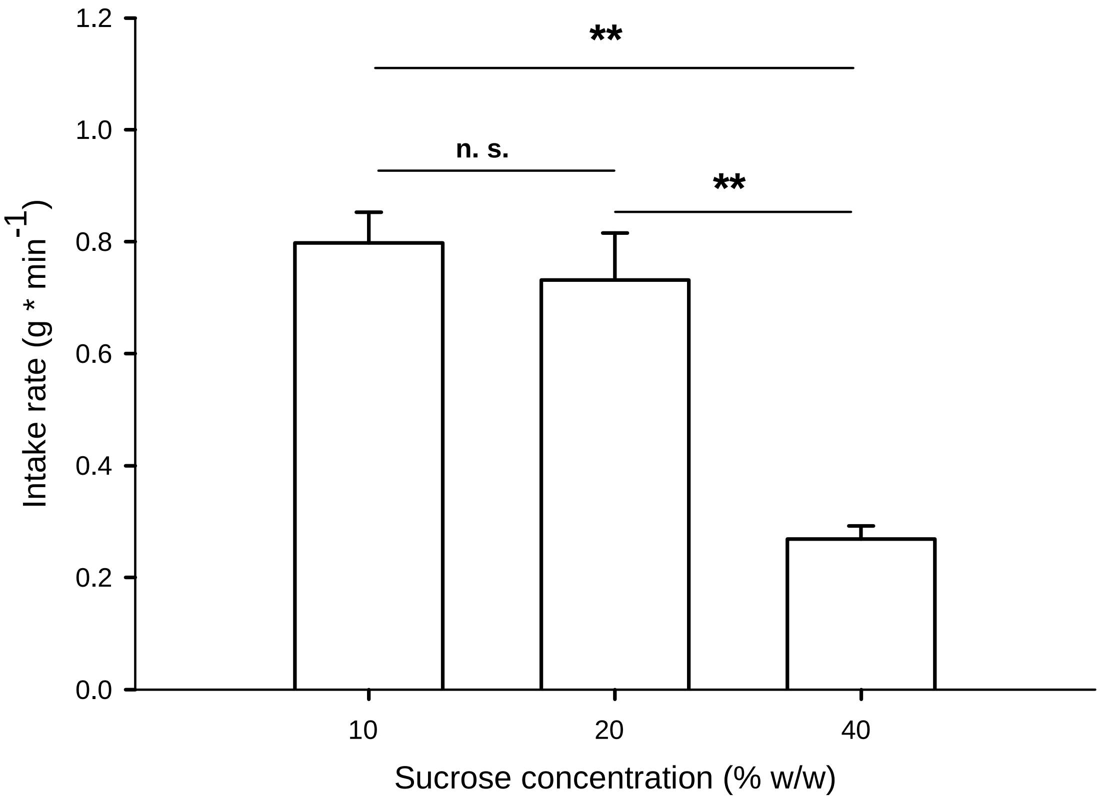
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Intake rate (g * min^{-1})
**
n. s.
**
10
20
40
Sucrose concentration (% w/w)

Figure 3.

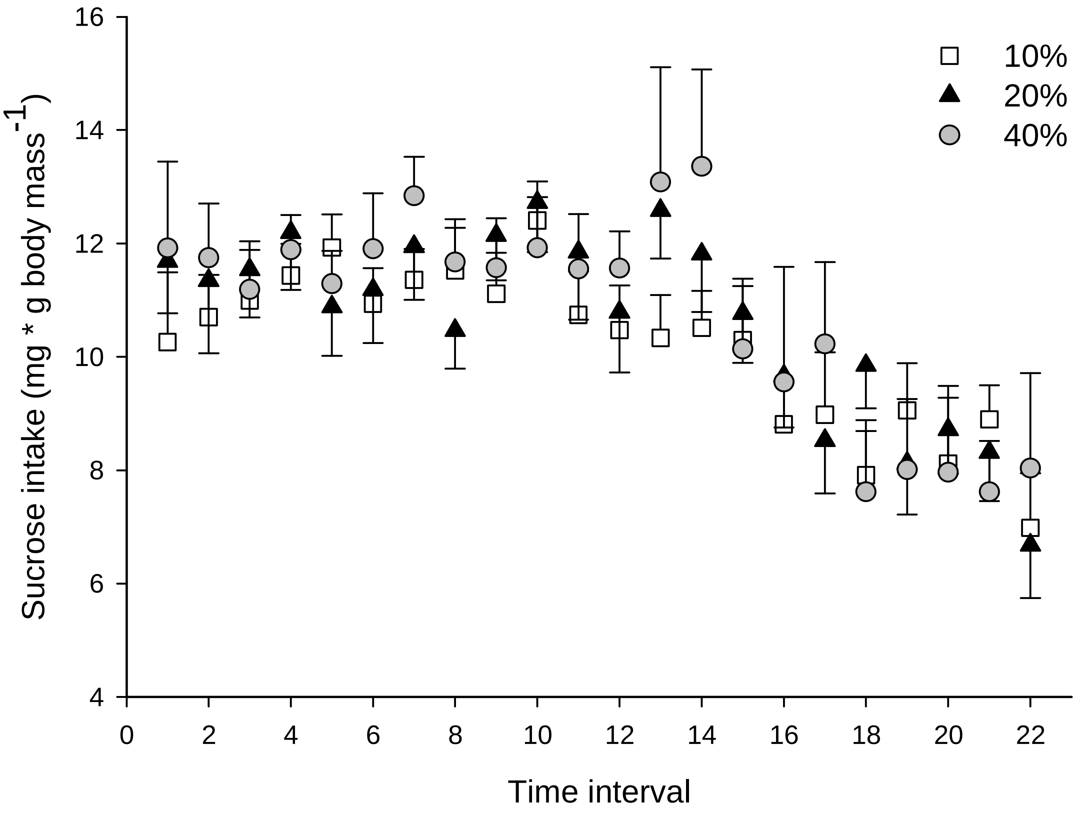
16
14
12
10
8
6
4
0
2
4
6
8
10
12
14
16
18
20
22
Sucrose intake (mg * g body mass^{-1})
Time interval
□ 10%
▲ 20%
● 40%

Figure 4.

CHAPTER 2

CHANGES IN NECTAR CONCENTRATION: HOW QUICKLY DO WHITEBELLIED SUNBIRDS (*CINNYRIS TALATALA*) ADJUST FEEDING PATTERNS AND FOOD INTAKE?

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Abstract

Nectarivorous birds encounter varying nectar concentrations while foraging on different food plants and must adjust their consumption to maintain constant energy intake. We determined how rapidly captive whitebellied sunbirds (*Cinnyris talatala*) adjust their volumetric intake and feeding patterns after changes in diet concentration. On four consecutive days, birds were fed sucrose diets alternating between a standard diet of 16% w/w and test diets of 2.5, 8.5, 16 or 30% w/w respectively for 1.5 h periods. Feeding events were recorded with an infrared photodetection system and food intake and body mass were monitored continuously by electronic balances interfaced to a computer. Generally, birds demonstrated a measurable increase in feeding frequency and food intake within 10 min after a decrease in sucrose concentration. However, individuals responded differently to the most dilute diet (2.5%): while most increased their food intake, others stopped feeding for a short while, appearing to dislike this diet. Furthermore, the number and duration of feeding events increased in the first 5 min after the switch from 2.5% back to 16%, as the birds attempted to compensate for previous reduced sugar intake. Daily sugar intake was lower when birds alternated between 2.5% and 16% diets than on other test days, but birds were able to maintain body mass, presumably through behavioural adjustments.

Key-words: compensatory feeding, feeding duration, feeding frequency, food intake, sugar concentration, sunbird, *Cinnyris talatala*

Introduction

The nectar of bird flowers is characteristically dilute, but still varies widely in sugar concentration (Pyke and Waser 1981; Stiles and Freeman 1993). In bird-pollinated plants of southern Africa, nectar concentrations range from less than 5% up to 55% w/w within and among plant species (Nicolson and Fleming 2003a). As an example, major food plants for three sunbird and one sugarbird species are *Greyia sutherlandii* (11.4% w/w), *Aloe arborescens* (13.2%), *Halleria lucida* (21.3%) and *Leonotis leonurus* (24.4%), which flower at the same time at one site in the Drakensberg region of South Africa (Daniels 1987). Other southern African passerine-pollinated flowers provide more dilute (e.g. *Aloe speciosa*: 6.5%) or more concentrated nectar (e.g. *Leucospermum tottum*: 28.5%; *Liparia splendens*: 36.7%) (Nicolson 2002). Nectarivorous birds may therefore encounter very different sugar concentrations within a short time period while foraging on the nectar of different food plants in the wild.

Although avian nectarivores feed on nectar of varying sugar concentration, and thus varying energy content, they are able to maintain a constant energy intake by adjusting their volumetric intake according to the concentration of nectar; this is commonly known as compensatory feeding (Martínez del Rio et al. 2001). The increase in food intake in response to a decrease in sugar concentration has been shown for hummingbird, honeyeater and sunbird species (Collins and Clow 1978; Collins et al. 1980; Downs 1997; López-Calleja et al. 1997; Lotz and Nicolson 1999; McWhorter and Martínez del Rio 1999; McWhorter et al. 2004). Whitebellied sunbirds, *Cinnyris talatala*, for instance, defend a constant sugar intake on diets from 0.25–2.5 M (8.5–65% w/w) sucrose (Nicolson and Fleming 2003b). As a result of adjusting volumetric intake, nectarivorous birds may need to switch between water conservation and water excretion when they experience changes in nectar concentration (Fleming et al. 2004a; Lotz and Martínez del Rio 2004). On very low sugar concentrations, birds may face physiological constraints, such as handling large amounts of preformed water (Nicolson and Fleming 2003b). Furthermore, sucrose hydrolysis rates and hexose absorption rates in the gut may limit feeding (McWhorter and Martínez del Rio 2000; McWhorter et al. 2006).

The adjustment of food intake according to variation in nectar concentration can take place by altering meal frequency, meal size or both. Data for sunbird, hummingbird and honeyeater species demonstrate that they increase the number of feeding events as the sugar concentration is decreased, while the length of feeding events remains nearly constant (Wolf and Hainsworth 1977; Collins and Clow 1978; Collins and Cary 1980; López-Calleja et al. 1997; Köhler et al. 2006).

In earlier studies, the resolution of feeding data has been too coarse to determine how quickly the birds detect a change in sugar concentration and adjust their nectar intake (e.g. McWhorter and Martínez del Rio 2000; Fleming et al. 2004a). For whitebellied sunbirds, the rate of feeding increases within 1 h of changing from a concentrated to a dilute sucrose diet (Fleming et al. 2004a), but no detailed studies providing finer temporal resolution are available for sunbirds. Only one experiment on a single rufous hummingbird (*Selasphorus rufus*) has demonstrated that nectar intake is altered quickly after a concentration change: the bird responded to an increased concentration within 10 min, while its response to decreased concentration was preceded by a lag (unpublished data in Gass 1978). It is therefore important to collect data on a short-term basis to provide understanding of how quickly sunbirds adjust their feeding patterns and food intake according to the sugar concentration of nectar.

In the present study, we examined how rapidly whitebellied sunbirds adjust their feeding pattern and food intake following an acute change in sugar concentration. The concentration of artificial nectar was changed every 1.5 h and feeding events (frequency and duration), food intake and body mass of the birds were recorded continuously throughout the experiment. We predicted that sunbirds adjust their feeding frequency, and thus their food intake, within minutes after a switch in diet concentration.

Materials and methods

Study animals and their maintenance

Eight non-reproductive whitebellied sunbirds, *Cinnyris* (*Nectarinia*) *talatala* (A. Smith), were mist-netted in Pretoria and were initially housed together in an outdoor aviary measuring 8 x 5 x 2 m. One week prior to experiments, birds were moved to individual cages of 45 x 45 x 32 cm in a climate-controlled room. They were acclimated to the experimental temperature of 20 ± 2ºC and a 11.5:12.5 h L:D photoperiod with lights on at 07h00. The maintenance diet consisted of a 20% w/w sucrose diet (0.63 M) with a nutritional supplement (Ensure®, Abbott Laboratories, Johannesburg, South Africa) to provide dietary nitrogen (Van Tets and Nicolson 2000). All sucrose concentrations used in this study were mixed on a % weight / weight basis. A sucrose diet was used, since sunbirds are indifferent to sucrose vs. hexoses on a 20% diet and both are equally well assimilated (Lotz and Nicolson 1996). The maintenance diet and supplementary water were provided *ad libitum* in inverted, stoppered syringes. Body mass (mean ± SE) of the four males and four females was 9.09 ± 0.31 g and 7.68 ± 0.16 g respectively.

Experimental procedure

The eight sunbirds were tested sequentially, since there was only one experimental cage (50 x 40 x 45 cm). Each sunbird was allowed one acclimation day on a 16% (0.50 M) sucrose diet before the measurements commenced. The experiment consisted of four treatments in randomized order, which lasted one day each. The first and last 0.5 h of the light phase were omitted from analysis because individuals differed in the timing of their first and last feeding event of the day (Köhler et al. 2006). The remaining feeding time (7h30–18h00) was divided into seven periods of 1.5 h each. During each treatment, a standard and test diet were presented for 1.5 h alternately throughout the day, commencing and ending with the standard diet of 16% sucrose. Test diet concentrations were 2.5, 8.5, 16 and 30% (0.08, 0.25, 0.50 and 0.99 M) sucrose (Table 1). Treatments are hereafter referred to by the sucrose concentration of the test diet, e.g. 2.5% treatment.

The 16% treatment served as a control since feeders were changed but sucrose concentration did not vary. This protocol enabled us to analyze the effect of daily patterns in feeding frequency and feeding duration previously recorded for this species (Köhler et al. 2006).

All experimental diets contained Ensure® to prevent mass loss on sugar-only diets (Nicolson and Fleming 2003b). The amount of Ensure® added was adjusted according to the sugar concentration of the diet, since the diet volumes ingested are inversely proportional to sugar concentration. If the carbohydrate component of Ensure® were completely assimilated by sunbirds, it would increase the highest sugar concentration (30%, which contained the most Ensure®) to only 31.7%, and is therefore negligible.

Data collection

The experimental cage (Köhler et al. 2006) was constructed from Perspex. Automated recording of the time spent feeding as well as the number of feeding events was obtained by an infrared photodetection system interfaced to a computer. Feeding duration and frequency were measured simultaneously to show how rapidly birds change their feeding behaviour to compensate for the differences in sugar concentration.

The feeder was mounted on an electronic balance (Mettler Toledo PB-602S, 0.01 g, Microsep Ltd, Johannesburg) interfaced to the computer. The mass of the feeder was recorded every 0.5 s throughout the experiment to determine the mass of food consumed by the bird. Occasional drips were collected in a container with liquid paraffin (to avoid evaporative mass loss), which was also placed on the balance. Feeder dripping therefore did not affect the results.

The cage contained two perches (a feeding perch and a resting perch), both connected to electronic balances (identical to that for the feeder), interfaced to the computer. The body mass of the bird was recorded every 0.5 s. Since the cage was constructed from Perspex the bird was not able to cling to the sides, and flexible plastic bristles discouraged resting on the bottom of the cage. Holes for ventilation were placed below the plastic bristles and were therefore inaccessible to the birds.

Definitions and processing of feeding data

For each bird we obtained the start and end times of every feeding event and calculated feeding event duration (± 0.001 s) as the time between insertion of the bill into the sucrose diet and its removal (Köhler et al. 2006). Each of the 1.5 h periods (n=7) was further divided into sub-periods of 5 min duration each (n=18 for each period). For all periods and sub-periods, we calculated the number of feeding events (feeding frequency), mean feeding duration, and total time spent feeding (total feeding duration) as the sum of the durations of all feeding events in the period or sub-period respectively. Although we recorded the feeder mass every 0.5 s, the minute amount of diet consumed in a single feeding event could not be detected, since the resolution of our balance was too coarse (0.01 g). Therefore, food intake of the bird (i.e. the mass of diet consumed) was calculated by subtracting the last feeder mass record of each period or sub-period from the first one. Daily sucrose intake was calculated from the food intake on the standard and test diets.

Definitions and processing of bird mass data

Body mass recordings contained unreliable dynamic balance readings caused by movements of the bird. Therefore, the mean of at least three consecutive stable mass records was used for analysis (i.e. the bird had to remain stationary for at least 1.5 s). Mean body mass was calculated for each 1.5 h period. The percentage daily body mass increase was calculated as the difference in mean body mass between the first and last period of the day.

As an estimate of flight activity, the number of flights between the two perches in the cage was calculated for each sunbird and each period. A flight was defined and programmatically detected when the difference between the mass readings of the two balances in the cage changed sign (dynamic balance readings were used). Daily flight activity was calculated by summing the number of flights in each period.

Statistical procedures

Data were tested for normality and homogeneity of variance (Levene's test). Log or square root transformations were used when data were heteroscedastic. Data obtained on the control treatment were subjected to repeated-measures ANOVA (RM-ANOVA) to test for a daily rhythm in feeding frequency, mean feeding duration, total feeding duration and food intake. The fact that most parameters varied over the course of the day (feeding frequency: $F_{6,42}$=2.90, P=0.02; mean feeding duration: $F_{6,42}$=5.43, P<0.001; total feeding duration: $F_{6,42}$=1.33, P=0.26; food intake: $F_{6,42}$=3.66, P<0.01) warranted the separate analysis of each of the seven periods. RM-ANOVA was therefore used to determine treatment effects for each parameter by comparing the same period on each of the four treatments. To detect how quickly the birds adjust their feeding behaviour and food intake after a diet change, feeding parameters for 5 min sub-periods of each 1.5 h period were analyzed by RM-ANOVA. RM-ANOVA were also conducted to test for differences in daily sucrose intake, daily body mass increase and daily flight activity between the four treatments.

Post-hoc comparisons for all RM-ANOVA were conducted with Tukey's Honest Significant Difference test for equal sample sizes, followed by a Bonferroni correction for multiple comparisons (Rice 1989). All data are presented as means ± SE. For all tests, the level of significance was $\alpha \leq 0.05$.

Results

Differences between treatments

Summarized RM-ANOVA results, comparing food intake, feeding frequency and mean feeding duration between the same periods on the four treatments are presented in Table 2. We demonstrated previously that food intake may be used as a surrogate for total feeding duration in *C. talatala* (Köhler et al. 2008). In the present study, results for total feeding duration also corresponded with those for food intake over all periods; results for total feeding duration were therefore omitted.

Food intake: The quantity of diet consumed by the birds increased with decreasing sugar concentration. Fig. 1A shows food intake on the four treatments, where the standard diet and a test diet (2.5, 8.5, 16 or 30%) were alternately presented for 1.5 h periods (n=7). Except for periods 2 and 4, when food intake was similar for the 2.5 and 8.5% diets ($P>0.25$), food intake differed significantly between the four test diets ($P<0.01$). When the birds were feeding on the standard diet in periods 1, 3 and 5, food intake did not differ between the treatments (Table 2). In the last period of the day, however, food intake on the standard diet was higher on the 2.5% treatment than on all other treatments ($P<0.03$).

Feeding frequency: There was a significant difference in feeding frequency between the test diets (Table 2; Fig. 1B), with birds having a higher feeding frequency on the 2.5% diet than on all other test diets (periods 2, 4 and 6: $P<0.02$). Feeding frequency tended to decrease with increasing sucrose concentration, but this was not significant between the 8.5, 16 and 30% diets in those periods ($P>0.07$). Birds fed at a lower feeding frequency on the standard diet on the 2.5% treatment than on the 30% treatment (period 3: $P<0.01$) or on the control and 8.5% treatment (period 7: $P<0.04$).

Mean feeding duration: There was no difference in mean feeding duration between the four test diets (periods 2, 4 and 6; Table 2; Fig. 1C). However, mean feeding duration was longer when the birds were offered the standard diet on the 2.5% treatment compared to all other treatments (periods 3, 5 and 7: $P<0.03$).

How fast do birds adjust feeding patterns and food intake?

2.5% treatment: Birds started to increase the rate of feeding within 10 min after receiving the 2.5% diet (as shown for two individuals in Fig. 2A,B). When the diet was switched to 2.5%, birds significantly increased their feeding frequency ($F_{17,119}>2.00$, $P<0.02$) and food intake ($F_{17,119}>2.24$, $P<0.01$). *Post-hoc* analysis revealed that both feeding frequency and food intake were significantly higher after 75 min (period 2: $P<0.01$), after 45 min (period 4: $P<0.03$) and after 20 min (period 6: $P<0.03$) respectively when compared with the first 5 min sub-period after the concentration change. However, individual birds responded differently to the most dilute diet (2.5%): while most increased their food intake immediately, others probed the diet and then stopped feeding for a short while. These birds increased their feeding duration and feeding frequency immediately after the 2.5% diet was replaced by the standard diet and food consumption only decreased after several minutes (Fig. 2C,D). Thus, birds had a significantly higher feeding frequency ($F_{17,119}>2.34$, $P<0.01$) and higher food intake ($F_{17,119}>4.21$, $P<0.001$) in the first 5 min after the standard diet was returned on the 2.5% treatment (periods 3, 5 and 7: $P<0.03$).

8.5% treatment: Birds increased their feeding frequency on the 8.5% diet in period 4 ($F_{17,119}=3.15$, $P<0.01$) after 70 min ($P=0.02$) and therefore food intake was significantly increased ($F_{17,119}=3.21$, $P<0.001$) after 70 min ($P=0.02$) in the same period. When the birds were feeding on the 8.5% diet in periods 2 and 6, however, there was no significant change in number of feeding events ($F_{17,119}<1.28$, $P>0.21$) or food intake ($F_{17,119}<1.35$, $P>0.18$). Mean feeding duration did not change when birds received the 8.5% diet ($F_{17,119}<1.29$, $P>0.21$). Feeding frequency, mean feeding duration and food intake did not change on the standard diet on the 8.5% treatment ($F_{17,119}<1.53$, $P>0.09$).

30% treatment: There was no change in feeding frequency ($F_{17,119}<1.57$, $P>0.08$) or mean feeding duration ($F_{17,119}<1.40$, $P>0.15$) when the birds received the 30% diet. Food intake decreased significantly in period 2 ($F_{17,119}=1.89$, $P=0.03$) after 30 min ($P<0.01$), but did not change significantly when birds received the 30% diet in periods 4 and 6 ($F_{17,119}<1.08$, $P>0.38$). Feeding frequency, mean feeding duration and food intake did not change when birds were offered the standard diet on the 30% treatment ($F_{17,119}<1.62$, $P>0.07$).

Control treatment: On the 16% treatment, birds did not change their feeding frequency ($F_{17,119}<1.45$, $P>0.13$), mean feeding duration ($F_{17,119}<1.08$, $P>0.38$) or food intake ($F_{17,119}<1.53$, $P>0.10$) after a feeder change.

Sucrose intake, body mass and flight activity on the different treatments

Daily sucrose intake differed between treatments ($F_{3,21}=30.90$, $P<0.001$), with birds consuming less sucrose on the 2.5% treatment than on all other treatments ($P<0.001$). Daily sucrose intake of the eight sunbirds (mean ± SE, g) was: 2.5% treatment: 1.38 ± 0.007; 8.5% treatment: 1.83 ± 0.011; control treatment: 1.85 ± 0.004; 30% treatment: 1.85 ± 0.007. Despite the differences in food intake, body mass increase over the day did not differ between treatments ($F_{3,21}=0.56$, $P=0.65$). Birds gained an average of 2.70 ± 0.50% of their initial body mass during the 11.5 h photophase.

Although there was no difference in daily flight activity of the birds between the different treatments ($F_{3,21}=2.17$, $P=0.12$), patterns were revealed when flight activity was assessed by time of day. Flight activity did not differ between the four treatments in the morning (periods 1 to 3: $F_{3,21}<1.15$, $P>0.35$), but did differ significantly in the afternoon and evening (periods 4 to 7: $F_{3,21}>3.59$, $P<0.03$). Birds showed higher flight activity while feeding on the 2.5% diet than on the control treatment (periods 4 and 6: $P<0.04$). Late in the day on the 2.5% treatment, birds flew less when offered the standard diet than on the standard diet of the control treatment (period 5: $P=0.04$). During the last period of the day, flight activity was lower on the 2.5% treatment than on all other treatments (period 7: $P<0.001$).

Discussion

Differences between treatments

Whitebellied sunbirds increase their food intake with decreasing sucrose concentration. Many nectarivorous birds similarly adjust food intake according to diet concentration, as shown for sunbirds (e.g. Lotz and Nicolson 1999), hummingbirds (e.g. López-Calleja et al. 1997) and honeyeaters (e.g. Collins et al. 1980). In our study, however, birds differed in their response to the 2.5% diet: when exposed to it for the first time, some individuals immediately increased their food intake, while others probed the diet and stopped feeding for a short while, appearing to dislike this very dilute diet. This resulted in a smaller food intake on the 2.5% diet compared with 8.5% upon first exposure to this experimental diet (period 2). The first response of some individuals is therefore to try to avoid the dilute diet, but with repeated exposure to the dilute diet they had to increase their food intake to avoid an energy deficit. Furthermore, on the 2.5% treatment, birds tried to compensate for reduced sugar intake on the 2.5% diet by increasing their intake of the standard diet as soon as it became available again.

The adjustment in food intake due to changes in diet concentration was predominantly caused by changes in feeding frequency, with more feeding events on the 2.5% diet than on all other test diets. This confirms earlier findings for whitebellied sunbirds (Köhler et al. 2006) and other avian nectarivores (Wolf and Hainsworth 1977; Gass 1978; López-Calleja et al. 1997). Mean feeding duration did not increase with decreasing sucrose concentration. Blue-throated hummingbirds (*Lampornis clemenciae*), on the other hand, have been shown to increase their meal size when their energy reserves are depleted (Hainsworth et al. 1981). Hummingbirds use the crop to store food but sunbirds do not have a crop and the volumetric intake at each feeding event might be limited (Mbatha et al. 2002). However, the increased food intake immediately after return of the standard diet on the 2.5% treatment was caused by longer mean feeding durations, indicating that the birds are indeed able to ingest relatively large meals and do so in order to maximize their food intake after feeding on a very low sugar concentration. This suggests that meal size of sunbirds in our study was not limited by the absence of a crop.

How fast do birds adjust feeding patterns and food intake?

Following a decrease in sucrose concentration from the standard diet to the 2.5% diet, most birds started to increase their feeding frequency and food intake within 10 min, while others reduced their feeding for a short while. Feeding frequency and food intake gradually increased until a significant increase occurred after 75 min in the morning. The onset of a significant increase in feeding frequency and food intake on the test diet subsequently accelerated over the day (20 min in period 6). Following a decrease in sugar concentration from the standard diet to the 8.5% diet, birds significantly increased their feeding frequency and food intake in one period only, since the difference in sugar content between these two diets is smaller than between the 2.5% and the standard diet.

After an increase in sucrose concentration from the standard diet to the 30% diet, birds started to decrease their food intake within 10 min, although this was only statistically significant for period 2. When the sucrose concentration was increased from the 2.5% diet to the standard diet, birds increased their feeding duration and feeding frequency, and thus food intake, within the first 5 min after the switch. This shows that whitebellied sunbirds are able to adjust their feeding behaviour and food intake according to the sugar concentration of their diet within minutes.

The response of the sunbirds to a decrease in sucrose concentration was delayed compared to their response to an increase in concentration. Our findings correspond with the observation by Gass (1978), where a single rufous hummingbird showed a delayed response to a decrease in sugar concentration, while it adjusted its food intake within 10 min following an increase in concentration. It is of ecological importance for nectarivorous birds to adjust their food intake immediately when encountering high nectar sugar concentrations since this maximizes their energy gain. This is especially important if the birds previously fed on less concentrated nectar or if their energy reserves are depleted, such as when feeding has been interrupted by a storm (Carpenter and Hixon 1988). When birds face low sugar content of nectar, the increase in intake is also ecologically relevant, but birds may increase their food intake slowly at first and search for flowers that provide nectar with higher energy content.

Choice studies show that sunbirds and hummingbirds prefer more concentrated sugar solutions over dilute ones (Lotz and Nicolson 1996; Roberts 1996; Leseigneur 2008). The preference for more concentrated diets clearly had an influence on how fast the birds in our study adjusted their feeding patterns and food intake according to sucrose concentration. The lag in the response to the 2.5% diet, especially at first encounter, was caused by several individuals that disliked the most dilute diet and attempted to avoid it. Consequently, birds ingested more of the standard diet immediately after its return to compensate for their energy deficit. The adjustment of food intake according to sugar concentration and the preference for the moderate diet imply the ability of sunbirds to distinguish between sugar concentrations. To our knowledge, no studies investigating the taste sense of sunbirds are available to date. A study in whitebellied sunbirds, however, has shown that artificially increasing the viscosity of diets of low and moderate sugar concentrations leads to a decrease in energy intake rates (Leseigneur 2008).

Sucrose intake, body mass and flight activity on the different treatments

Sunbirds were able to maintain a constant sugar intake on diets from 8.5–30% sucrose. This perfect compensatory feeding has been shown previously in whitebellied sunbirds (Nicolson and Fleming 2003b): on diets from 8.5–65% w/w sucrose they maintain a constant energy intake by adjusting their volumetric intake. However, when fed 2.5% or 3.5% sucrose over 24 h these sunbirds are not able to meet their energetic requirements and lose body mass (Nicolson and Fleming 2003b). The failure to maintain energy balance on low diet concentrations is also evident in frugivorous cedar waxwings (*Bombycilla cedrorum*), which reduce their volumetric intake on a 3.5% hexose diet (Levey and Martínez del Rio 1999). In our study, daily sucrose intake of the birds was reduced on the 2.5% treatment (even though they increased their food intake on both the 2.5% and standard diets) compared to all other treatments. That our sunbirds did not increase their food intake sufficiently on the 2.5% diet may reflect physiological constraints to nectar digestion. The processing of sucrose diets requires the hydrolysis of sucrose to glucose and fructose by the enzyme sucrase. Sucrose hydrolysis rates have been suggested as limiting (McWhorter and Martínez del Rio 2000). However, food intake has also been shown to be limited on hexose-only diets, indicating constraints to hexose absorption rates (Levey and Martínez del Rio 1999; Fleming et al. 2004b). In addition, the transit time for digesta decreases with decreasing dietary sugar concentration (Markman et al. 2006); thus the time available for sucrose hydrolysis and hexose absorption may be reduced on very dilute diets. Furthermore, birds may face constraints to osmoregulatory processes. When fed dilute diets, birds may be restricted by having to deal with very large volumes of preformed water (Fleming and Nicolson 2003), although Palestine sunbirds (*Cinnyris oseus*) consuming dilute diets are able to reduce the absorption of ingested water in the gut and thus the load on the kidneys (McWhorter et al. 2004). Avian nectarivores consuming dilute diets are also challenged by electrolyte and metabolite losses in the excreted fluid: the excreta produced by hummingbirds and sunbirds on electrolyte-free diets are extremely dilute but the loss of salts is still substantial (Lotz and Nicolson 1999; Fleming and Nicolson 2003; Lotz and Martínez del Rio 2004). Whitebellied sunbirds are able to maintain energy balance on very dilute sucrose solutions only when these diets are supplemented with electrolytes (C. Purchase, S.W. Nicolson and P.A. Fleming, unpublished data). Nectar-feeding birds also incur high energetic costs during the warming of large nectar volumes to body temperature: experiments on rufous hummingbirds have confirmed that the cost of warming nectar increases exponentially as nectar concentration decreases (Lotz et al. 2003).

It might have been favourable for the birds to adjust their behaviour in order to save energy on the 2.5% treatment, instead of ingesting even more of the diet. The number of flights per day did not differ between the 2.5% and control treatments but birds may have reduced hopping, vertical flights and flights with return to the same perch (A. Köhler, pers. observation) in order to save energy on the 2.5% treatment. Since we only calculated the number of flights between the two perches in the cage as a measure of flight activity, we were not able to quantify other activities or measure other energy-saving strategies that may have taken place. Behavioural adjustments to save energy on the 2.5% treatment are suggested since the birds ingested less sucrose but were able to maintain their body mass increase throughout the day. It is important that birds accumulate sufficient energy during the light period to sustain them during the night when they do not feed. Feeding frequency and flight activity on the standard diet on this 2.5% treatment were reduced. Birds may have saved energy by having less frequent but longer meals on the standard diet, instead of repeatedly visiting the feeder to consume smaller meals. The long feeding events immediately after the return of the standard diet led to an increase in body mass, which may have resulted in higher flight costs (DeBenedictis et al. 1978).

Behavioural and physiological adjustments that enable birds to maintain body mass increase over the day have been found in other nectarivorous birds. Honeyeaters and several hummingbird species reduce their flight activity under energetically challenging conditions to conserve energy (Beuchat et al. 1979; Collins and Morellini 1979; Hainsworth et al. 1981; Fernández et al. 2002). Hummingbirds also use torpor to save energy when exposed to energy stress (Hainsworth et al. 1977; Tooze and Gass 1985; Carpenter and Hixon 1988; Calder 1994). Besides avian nectarivores, zebra finches (*Taeniopygia guttata*) reduce energy expenditures when the energy content of their food is low (Wiersma and Verhulst 2005) and Pekin ducklings (*Anas platyrhyncos domesticus*) reduce their metabolic rate to save energy during phases of low food intake (Moe et al. 2005).

We conclude that sunbirds are able to adjust their feeding pattern and food intake immediately after a change in nectar concentration. Birds increased their food intake on a dilute diet and maintained their body mass increase over the day despite the lower daily sugar intake. Behavioural adjustments to conserve energy are therefore likely but could not be quantified in this study. Besides their ability to increase food intake on a dilute diet, sunbirds in the field probably look for flowers providing more concentrated nectar and would ingest very dilute nectar only if they could not find alternatives. The increase in flight activity on the 2.5% diet may reflect an increased number of trips to the feeder caused by the increase in the rate of feeding. Rufous hummingbirds are more active when energy availability is low because they spend more time foraging (Gass et al. 1999). In our study, however, the birds could have stayed on the feeding perch between meals to save energy. The increased flight activity may therefore represent a search for feeders providing more concentrated nectar. However, no equivalent field studies on wild sunbirds are available to date and future investigations are needed.

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Tables

Table 1. Experimental design for subjecting whitebellied sunbirds to acute changes in sucrose concentration. On four consecutive days (treatments) in randomized order, a standard diet (16% w/w) and one test diet (2.5, 8.5, 16 or 30% w/w) were presented alternately for 1.5 h periods at a time. Light:dark phase was 11.5:12.5 h with lights on at 07h00. The first and last 0.5 h of the light phase, with birds feeding on the standard diet, was omitted from analysis (see text for explanation). Starting time of each period is given in parentheses.

	Period						
Treatment	1 (7h30)	2 (9h00)	3 (10h30)	4 (12h00)	5 (13h30)	6 (15h00)	7 (16h30)
2.5%	16%	2.50%	16%	2.50%	16%	2.50%	16%
8.5%	16%	8.50%	16%	8.50%	16%	8.50%	16%
16% (control)	16%	16%	16%	16%	16%	16%	16%
30%	16%	30%	16%	30%	16%	30%	16%

Table 2. Differences in food intake, feeding frequency and mean feeding duration between the four experimental treatments, where birds were presented alternately with a standard diet (16% w/w, grey background) and test diet (2.5, 8.5, 16 or 30% w/w, white background) for 1.5 h periods at a time. RM-ANOVA results for eight birds are given for each period. Degrees of freedom for all *F*-values were 3,21; significant results are highlighted in bold.

	Food intake		Feeding frequency		Mean feeding duration	
Period	*F*	*P*	*F*	*P*	*F*	*P*
1	1.32	0.30	1.98	0.15	2.53	0.09
2	29.08	**<0.001**	11.12	**<0.001**	0.80	0.51
3	0.26	0.85	4.93	**<0.01**	6.63	**<0.01**
4	78.69	**<0.001**	15.22	**<0.001**	0.37	0.77
5	2.77	0.07	3.69	**0.03**	6.64	**<0.01**
6	106.02	**<0.001**	37.73	**<0.001**	2.07	0.14
7	12.02	**<0.001**	4.43	**0.02**	13.29	**<0.001**

Figure legends

Fig. 1. Food intake (g; A), number of feeding events (B) and mean feeding duration (s; C) per 1.5 h period for eight sunbirds (mean ± SE). The birds fed alternately on a standard diet of 16% (grey background) and test diets of 2.5, 8.5, 16 or 30% w/w sucrose (white background) over four consecutive days (treatments; see Table 1). Each feeding parameter was compared by RM-ANOVA for each period separately, followed by *post-hoc* comparison. Significant differences ($P \leq 0.05$) are indicated by different letters; correspondence of at least one letter indicates no significant difference.

Fig. 2. Cumulative feeding duration (s) as a function of time (min) for four individuals. Each graph shows 15 min before and after a concentration change from 16% to 2.5% w/w sucrose (A,B) or from 2.5% to 16% w/w sucrose (C,D). N=the number of feeding events per 15 min. Graphical presentation of the switches from 8.5 to 16% and from 30 to 16% w/w sucrose were omitted since there were no clear changes in cumulative feeding duration.

Figures

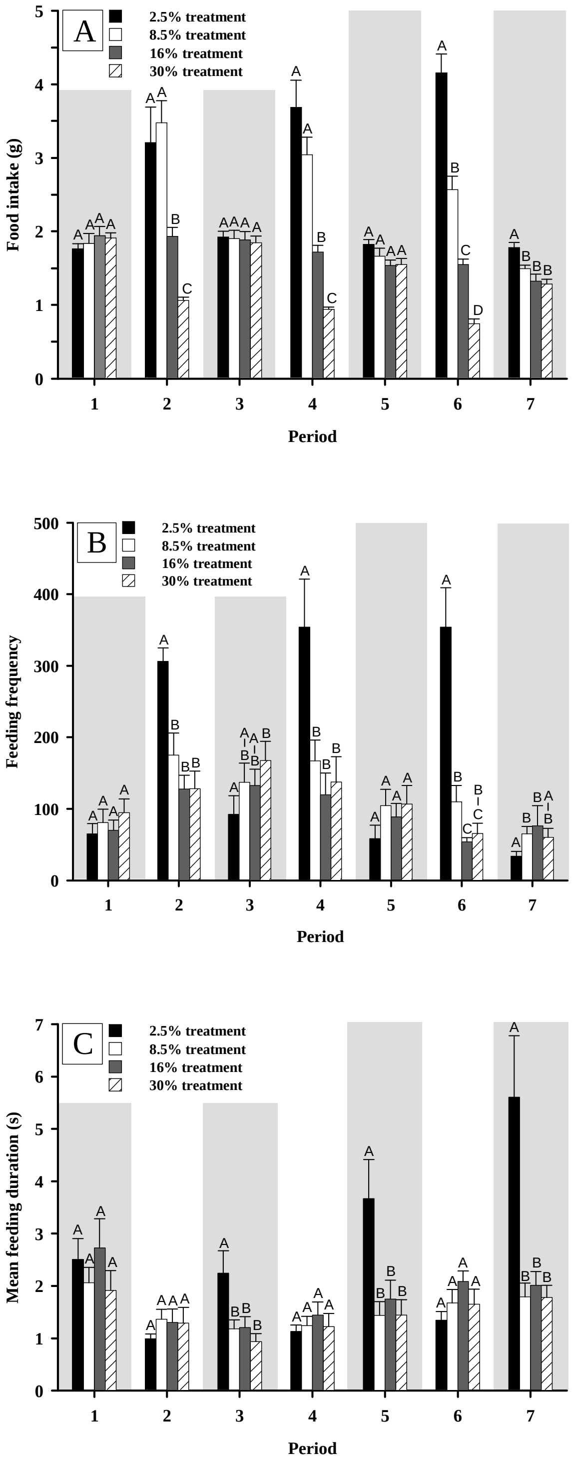

Figure 1.

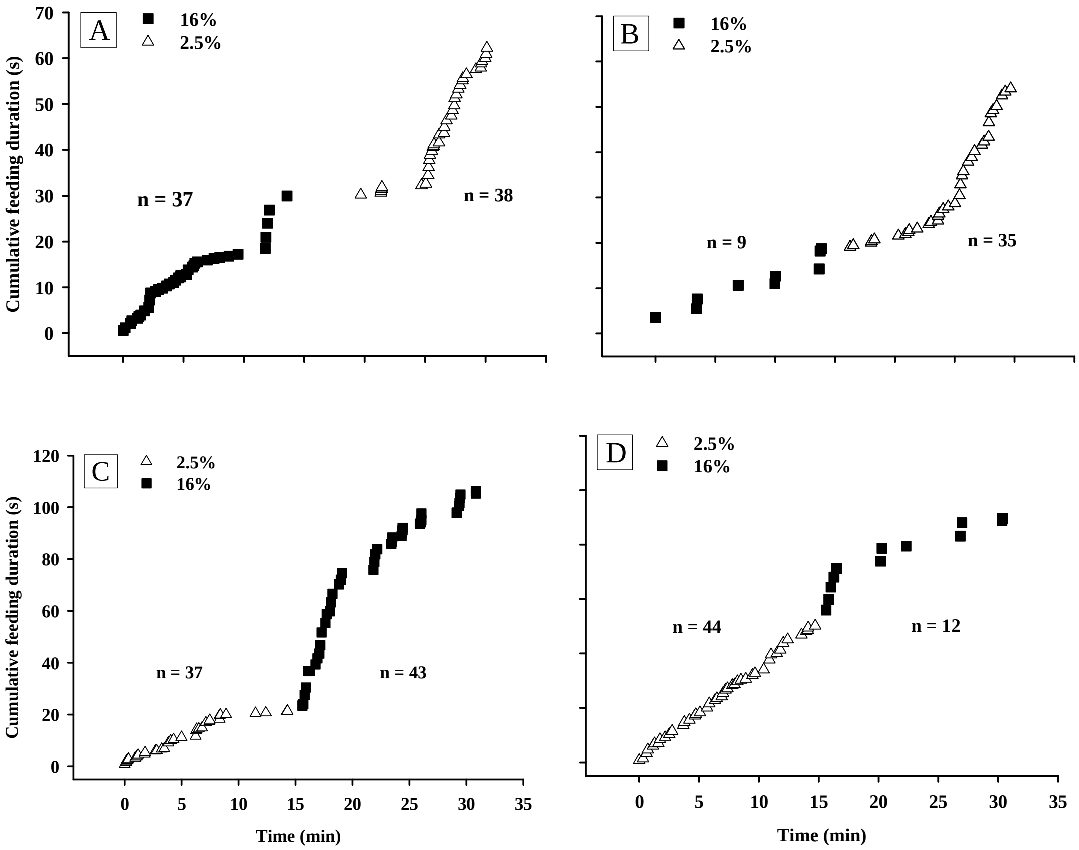
A
■ 16%
△ 2.5%
Cumulative feeding duration (s)
0
10
20
30
40
50
60
70
n = 37
n = 38
B
■ 16%
△ 2.5%
n = 9
n = 35
C
△ 2.5%
■ 16%
Cumulative feeding duration (s)
0
20
40
60
80
100
120
n = 37
n = 43
0
5
10
15
20
25
30
35
Time (min)
D
△ 2.5%
■ 16%
n = 44
n = 12
0
5
10
15
20
25
30
35
Time (min)

Figure 2.