

Osmoregulation in an avian nectarivore, the whitebellied sunbird *Nectarinia talatala*: response to extremes of diet concentration

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Summary

Water intake of nectarivores is intrinsically linked to nectar concentration. Osmoregulation in whitebellied sunbirds *Nectarinia talatala* (body mass 9.3 ± 0.1 g, mean \pm s.d., $N=7$), was examined by feeding them sucrose solutions, equivalent to extreme diet concentrations (0.07 – 2.5 mol l⁻¹ sucrose; 2–65% w/w), with and without supplementary drinking water. Total water gain was 33–515% of body mass daily. Cloacal fluid (CF) volume increased with diet dilution from 0.4% to 309% of body mass while increases in evaporative water loss (obtained by difference) were also recorded. Osmolality of CF demonstrated the largest scope yet recorded for a bird and was significantly correlated with water flux: mean values were 6–460 mosm kg⁻¹ H₂O (minimum 3, maximum 1900 mosm kg⁻¹). When supplementary water was provided, its consumption by birds fed concentrated diets (2.5 mol l⁻¹ sucrose) led to a dramatic reduction in CF osmolality, from 461 ± 253 to 80 ± 119 mosm kg⁻¹ fluid. Sunbirds maintained energy balance on sucrose diets varying tenfold in concentration, from 0.25 to 2.5 mol l⁻¹; however, on extremely dilute diets (0.07 and 0.1 mol l⁻¹

sucrose, lower than natural nectar concentrations) their inability to maintain energy balance was probably due to excess preformed water. Total osmotic excretion and concentrations of Na⁺ and K⁺ increased with high water fluxes, and are a possible physiological constraint for nectarivorous birds on artificial dilute diets devoid of electrolytes. Even low electrolyte levels in nectars may be adequate to replace these losses, but other physiological limitations to the intake of dilute nectars are increased energetic costs of solute recovery, increased heat loss and interference with digestive processes. Sunbirds therefore deal with sugar solutions spanning the range of nectar concentrations by shutting down water excretion on concentrated diets, or, on dilute diets, by producing extremely dilute CF with some of the lowest solute concentrations recorded.

Key words: African sunbird, *Nectarinia talatala*, cloacal fluid, electrolyte balance, nectar concentration, osmolality, urine, water balance.

Introduction

Most studies of avian osmoregulation have focussed on basic patterns of homeostasis, or osmotically challenging ('stressful') situations induced by dehydration, or electrolyte overload or depletion. The focus has also been on domestic birds or on xeric species that survive without drinking (for a review, see Goldstein and Skadhauge, 2000). To date there have been few studies examining the water balance and renal function of birds at the other end of the spectrum – water overload. Nectarivores, for example, must overcome continual digestive and renal challenges. Despite being one of the simplest food resources, nectar shows marked variability in concentration, with average values for plant species ranging from 0.2 to 2.9 mol l⁻¹ sucrose equivalent (or 7–70% w/w; Nicolson, 1998). Nectarivorous birds are therefore faced with a far more extreme range of water load than most birds studied to date.

Of necessity, when fed dilute sugar solutions, avian

nectarivores have to drink up to four or five times their body mass over just 12 h to ingest their requisite energy (Collins, 1981; McWhorter and Martínez del Río, 1999; Nicolson and Fleming, 2003). In addition to the high preformed water load, nectar is low in electrolytes (Hiebert and Calder, 1983). For nectarivores, the problem may be conservation of electrolytes, rather than electrolyte excess (as examined in most avian osmoregulation studies). On the other hand, when only concentrated nectars are available, nectarivores may struggle to maintain water intake sufficient for physiological requirements. Evaporative losses at high ambient temperatures may outstrip water gain on concentrated nectars (Beuchat et al., 1990; Powers, 1992), requiring birds to resort to water conserving strategies such as torpor (Lasiewski, 1964). Only a handful of studies have examined the water flux and osmoregulation of nectarivorous birds (Beuchat et al., 1990; Collins, 1981; Goldstein and Bradshaw, 1998a,b; Lotz and

Nicolson, 1999; McWhorter and Martínez del Rio, 1999; McWhorter et al., 2003). The aim of this study was to examine the osmoregulatory capacity of the whitebellied sunbird *Nectarinia talatala* fed extremes of diet concentration in the presence and absence of supplementary drinking water.

Materials and methods

Seven male whitebellied sunbirds *Nectarinia talatala* (Smith) (9.3 ± 0.1 g, mean \pm S.D.) were mist-netted in Jan Celliers Park, Pretoria, South Africa. Birds were kept in individual cages (27 cm \times 31 cm \times 21 cm) in a constant environment room (maintained at $21 \pm 0.5^\circ\text{C}$ and $45 \pm 3\%$ relative humidity) with a 13 h:11 h L:D photoperiod (light period from 06:30 h to 19:30 h). The maintenance diet consisted of 20% (w/w) sucrose and a nutritional supplement (Ensure[®], Abbott Laboratories, Johannesburg, S. Africa), provided in inverted, stoppered syringes hung on the cage sides, from which the birds could feed *ad libitum*. Birds adjusted to captivity and an artificial diet for at least 4 weeks before diet trials.

Osmoregulation experiments were run over 2 days. Shortly after lights-on on the first day (07:00–07:30 h), birds were weighed (± 0.001 g) and feeders containing maintenance diet were replaced by others containing experimental diets. Feeders of supplementary water were provided where appropriate. Voided cloacal fluid (CF) was collected over the second 24 h (the test day) in trays under the cages; the trays contained liquid paraffin to prevent evaporation of CF. Trays were covered by plastic gauze (vegetable bagging) stretched tightly over the entire tray. Mesh thickness measured 0.13 ± 0.03 mm and mesh size was 6.61 ± 0.57 mm. This prevented birds from touching the paraffin, but was fine enough to offer minimal interference with CF collection. Trays were tipped up and left to stand so that CF droplets coalesced and could be drawn up with Pasteur pipettes. Accuracy of the paraffin collection method was determined by placing collection trays above a larger paraffin-filled tray; waxproof paper was suspended next to the cage sides to direct any CF droplets into the larger tray. Birds were maintained on a dilute diet (0.07 mol l⁻¹ sucrose) for 24 h and then placed in the cage for 1 h without any supply of food. Less than 1% of CF volume was missed with this collection method.

The experimental diets were sucrose solutions of eight concentrations, ranging from 0.07 to 2.5 mol l⁻¹. Osmolalities of these diets ranged from 70 mosm kg⁻¹ H₂O up to an estimated $5,800$ mosm kg⁻¹ (Table 1). For sucrose concentrations ≥ 0.25 mol l⁻¹, separate trials were run with and without supplementary water. Trials at 0.07 and 0.1 mol l⁻¹ were not repeated with and without water since birds lost considerable mass on these diets and very little of the water provided was consumed. Each of the seven birds received every test diet in random order (a total of 14 trials per bird).

Birds and feeders were weighed at the start and end of the test day to assess any change in body mass and consumption of diet and supplementary water. Evaporation from the 1 mm diameter holes through which the birds fed was assumed to be negligible. Dripping was a greater problem, and was controlled

Table 1. *Sucrose diets used in the present study*

| Sucrose concentration | | Osmolality (mosm kg ⁻¹) |
|------------------------|---------|--|
| (mol l ⁻¹) | (% w/w) | |
| 0.07 | 2.7 | 70 |
| 0.1 | 3.6 | 101 |
| 0.25 | 8.3 | 280 |
| 0.5 | 15.8 | 594 |
| 0.75 | 23.1 | 950 |
| 1 | 30.0 | 1376 |
| 1.5 | 43.0 | 2588 |
| 2 | 54.8 | 4052* |
| 2.5 | 65.4 | 5842* |

*Osmolalities of 2 and 2.5 mol l⁻¹ solutions could not be measured directly since they exceeded the range of the osmometer. Values were estimated by extrapolation.

by placing paraffin collection jars directly under the feeders and making appropriate corrections (Nicolson and Fleming, 2003). Water gain (ml day⁻¹) was calculated as the sum of preformed + metabolic + supplementary water volumes. Preformed water was calculated by subtracting the mass of sugar from the mass of solution consumed. Metabolic water was calculated as 198 g water for every mole (342 g) of sucrose consumed (=0.58 ml H₂O for every g sucrose; from the equation of Schmidt-Nielsen, 1997). For this calculation we assumed that sugars ingested were completely assimilated (Jackson et al., 1998; Lotz and Nicolson, 1996), that all sugar assimilated was catabolised (i.e. respiratory quotient=1.0; Collins et al., 1980; Prinzinger et al., 1992) and that birds are approximately in mass balance. Evaporative water loss (EWL, ml day⁻¹) was estimated from the difference between water gain and CF output.

Processing and analysis

After collection of CF from under liquid paraffin, volumes (ml day⁻¹) were measured in a graduated cylinder, and a portion frozen for later analysis of osmolality and Na⁺ and K⁺ concentrations.

Osmolality of CF (mosm kg⁻¹) was measured using a vapour pressure osmometer (Vapro[®] 5520, Wescor Inc., Utah, USA), fitted with a specially selected thermocouple head that gave a range of 0–3200 mosm kg⁻¹. Regular and thorough cleaning ensured that deionised water registered an osmolality of 0 mosm kg⁻¹ with reasonable reliability. Deionised water was processed after approximately every ten samples, and the thermocouple head was cleaned if the reading exceeded 5 mosm kg⁻¹ (approximately every 20 samples). Since the greatest variability was observed between calibration runs, we did not measure osmolality of each sample in sequential triplicate, like some other authors. Rather, samples were sorted into groups of approximately similar expected concentrations, centrifuged and the supernatant measured blind (with no knowledge of the diet during that trial). All samples were thus measured in a random order twice, being refrozen and

centrifuged between measurements. If values differed substantially between the two analyses, a third reading was taken. This method of analysis yielded coefficients of variation of 36, 25 and 16% for dilute (0.07 to 0.25 mol l⁻¹ sucrose), average (0.5 and 1 mol l⁻¹) and concentrated (1.5 to 2.5 mol l⁻¹) sugar solutions, respectively; these equate to osmolality readings differing by an average of 4.9, 13.5 and 32.4 mosm kg⁻¹.

Sodium and potassium ions in CF (mol l⁻¹) were measured by flame photometry (Model 420, Sherwood Scientific Ltd., Cambridge, UK) in random order and in duplicate (or triplicate where values differed substantially). We assumed that solutes were a negligible component of CF volume and that CF approximated water (Lotz and Nicolson, 1999), enabling calculation of total osmotic excretion (osmolality × CF volume; mosm day⁻¹) as well as electrolyte output (ion concentration × CF volume; mmol day⁻¹).

Statistical procedures

Water gain, CF volumes, osmolalities and osmotic excretion, as well as EWL (expressed as volume or proportion of total water gain) were tested for the effects of diet concentration and the provision of supplementary water by repeated-measures analysis of variance (RM-ANOVA). *Post-hoc* comparisons were carried out by Tukey's Honest Significant Difference test. These analyses were conducted for diet concentrations of 0.25–2.5 mol l⁻¹, since these diet trials were performed both with and without supplementary water (experiments using 0.07 and 0.1 mol l⁻¹ sucrose diet were performed with water only). Regression analyses were carried out for CF volume compared with osmolality, as well as osmotic excretion and electrolyte outputs. Regression lines were fitted to data from all individuals on all diet trials.

For all tests, the level of significance was $P \leq 0.05$. Values are means \pm 1 S.D.

Results

Since whitebellied sunbirds maintain a steady energy intake of 2.77 g daily on sucrose solutions ≥ 0.25 mol l⁻¹ (Nicolson and Fleming, 2003), the metabolic water production remains constant at an average of 1.60 \pm 0.23 ml day⁻¹. Preformed water intake, however, changes dramatically with diet concentration. Consequently volumes of CF depended on diet concentration (RM-ANOVA for ≥ 0.25 mol l⁻¹ trials; effect of diet concentration: $F_{5,30}=124.02$, $P < 0.001$) and CF volumes were also significantly higher when supplementary drinking water was available (effect of the provision of water, $F_{1,6}=8.70$, $P=0.026$; diet × water interaction, not significant; NS).

On the lowest sucrose concentrations (0.07 and 0.1 mol l⁻¹), birds were subject to massive water flux and did not maintain energy balance, losing mass (Nicolson and Fleming, 2003). Their mean daily water gain was 47.8 \pm 8.3 ml day⁻¹ (Fig. 1A, Table 2), or 5.15 times body mass. Mean voided CF volumes were 29.6 \pm 7.0 ml day⁻¹ (Fig. 1B, Table 2), or 3.18 times body mass.

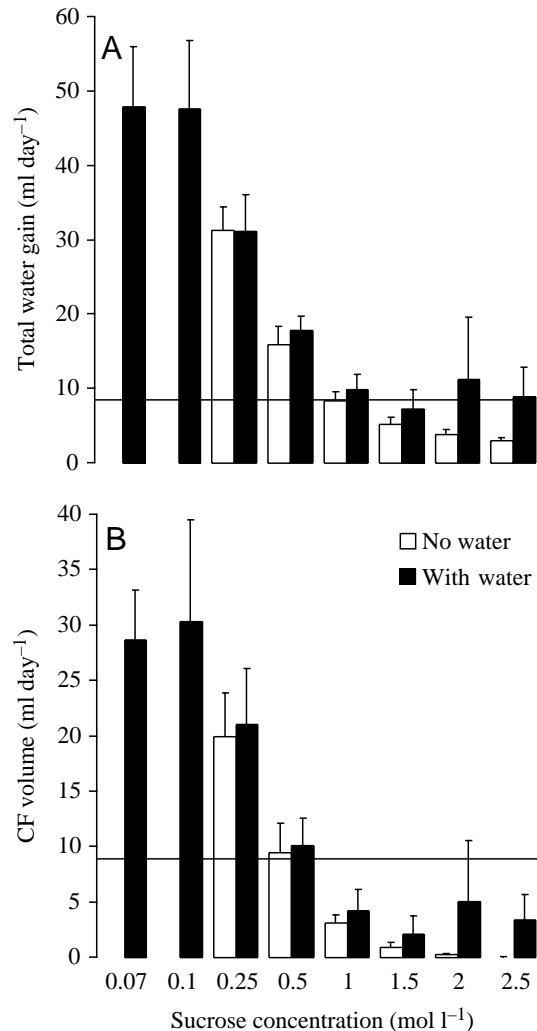


Fig. 1. Daily water gain (A) and cloacal fluid volume (B) were strongly affected by diet concentration (0.07–2.5 mol l⁻¹ sucrose solutions) and the provision of supplementary drinking water (solid bars; open bars indicate no added water). Water gain (ml day⁻¹) was calculated as the sum of metabolic + preformed + free water volumes (for details see text). Horizontal lines indicate mean body mass in g. Values are means \pm 1 S.D. ($N=7$).

In contrast, on the most concentrated sucrose solutions (2 and 2.5 mol l⁻¹) and when no supplementary water was provided, birds ingested only 1.92 \pm 0.50 ml day⁻¹ of preformed water, while metabolic water contributed a further 1.62 \pm 0.22 ml day⁻¹ (total water gain = 3.91 \pm 0.56 and 3.05 \pm 0.37 ml day⁻¹, Fig. 1A). Consequently, volumes of CF fell away sharply with increasing dietary sucrose concentration, so that when birds were not provided with supplementary drinking water, CF volumes were only 0.26 and 0.04 ml day⁻¹ (2 and 2.5 mol l⁻¹, respectively; Fig. 1B). Most waste material was solid for birds on these very concentrated diets.

In addition to altering water excretion through CF, the evaporative component of water loss was affected by diet. A smaller volume of water was lost through evaporation on more concentrated diets, and there were greater evaporative losses

Table 2. Osmoregulatory parameters for whitebellied sunbirds fed sucrose solutions from 0.07 to 2.5 mol l⁻¹, with and without supplementary drinking water

| [Sucrose] (mol l ⁻¹) | Total water gain (ml day ⁻¹) | | CF volume (ml day ⁻¹) | | CF osmolality (mosm kg ⁻¹) | | Total osmotic excretion (mosm day ⁻¹) | | CF Na ⁺ concentration (mmol l ⁻¹) | | CF K ⁺ concentration (mmol l ⁻¹) | |
|-------------------------------------|---|------------|--------------------------------------|------------|---|-------------|--|------------|---|------------|--|-------------|
| | No water | With water | No water | With water | No water | With water | No water | With water | No water | With water | No water | With water |
| 0.07 | * | 47.92±8.05 | * | 28.73±4.44 | * | 14.2±3.1 | * | 0.37±0.04 | * | 1.14±1.84 | * | 0.73±0.37 |
| 0.1 | * | 47.60±9.24 | * | 30.37±9.15 | * | 10.5±3.8 | * | 0.30±0.11 | * | 0.67±0.34 | * | 0.47±0.16 |
| 0.25 | | 31.28±3.17 | | 21.06±5.07 | | 7.1±4.7 | | 0.14±0.10 | | 0.73±0.81 | | 0.37±0.09 |
| 0.5 | | 15.90±2.54 | | 10.16±2.49 | | 30.4±13.4 | | 0.27±0.09 | | 0.38±0.22 | | 0.65±0.33 |
| 1 | | 8.44±1.16 | | 4.27±1.88 | | 58.6±64.8 | | 0.17±0.17 | | 1.01±0.35 | | 1.66±0.78 |
| 1.5 | | 5.18±0.94 | | 2.15±1.64 | | 195.2±129.8 | | 0.18±0.17 | | 5.59±6.71 | | 3.47±1.57 |
| 2 | | 3.91±0.56 | | 5.03±5.53 | | 467.2±654.9 | | 0.13±0.22 | | 8.13±7.83 | | 13.69±12.15 |
| 2.5 | | 3.05±0.37 | | 3.44±2.22 | | 460.9±253.3 | | 0.02±0.01 | | 12.67±8.14 | | 21.79±7.92 |

Values are means ± 1 s.d., N=7.

CF, cloacal fluid.

Total water gain = metabolic+preformed+free.

*Birds fed 0.07 and 0.1 mol l⁻¹ sucrose diets drank minimal supplementary water when available and lost substantial mass over 48 h. For this reason, trials were not repeated without supplementary water.

when the birds were provided with supplementary water (Fig. 2A; diet, $F_{5,30}=23.72$, $P<0.001$; water, $F_{1,6}=10.54$, $P=0.017$; interaction, $F_{5,30}=4.66$, $P=0.003$). As a percentage of water gain, EWL was also significantly affected by diet concentration and the provision of supplementary water (Fig. 2B): a greater proportion of water gain was lost through EWL on trials without water and on more concentrated diets (diet, $F_{5,30}=66.53$, $P<0.001$; water, $F_{1,6}=24.42$, $P=0.003$; interaction, $F_{5,30}=9.41$, $P<0.001$). For example, on the most dilute diets (0.07 and 0.1 mol l⁻¹ sucrose), 18.2±5.3 ml day⁻¹ of water was not accounted for in CF and was presumably lost largely through evaporation (38±9% of water gain), while on 2 and 2.5 mol l⁻¹ sucrose diets without supplementary water, 96±3% of ingested water was unaccounted for. Presumably most of this was lost through evaporation (although the small volumes of voided CF made collection error more likely).

Cloacal fluid osmolality increased significantly with diet

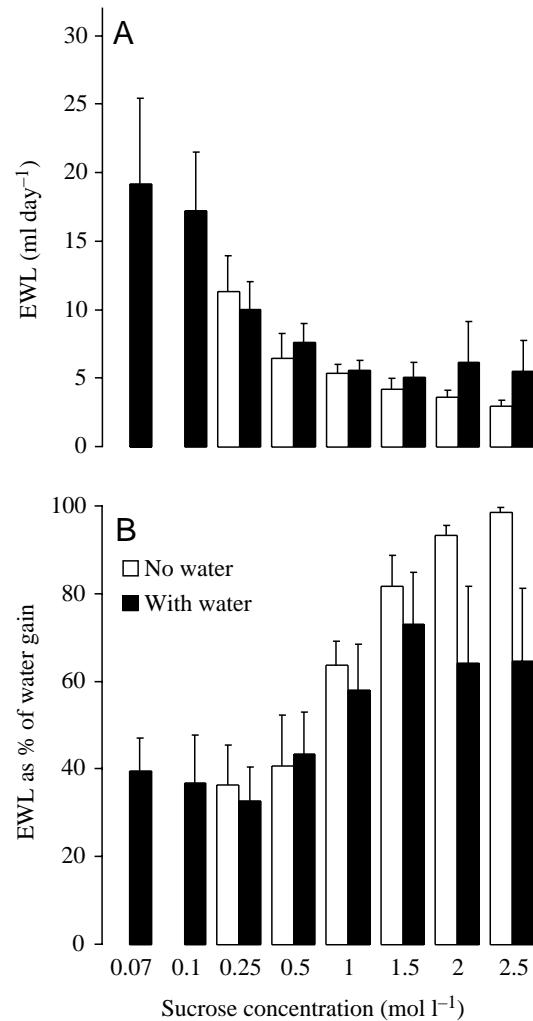


Fig. 2. Estimated evaporative water loss (EWL, i.e. the difference between water gain and cloacal fluid output) for sunbirds fed sucrose solutions of varying concentrations (0.07–2.5 mol l⁻¹), with (solid bars), or without (open bars), supplementary drinking water. EWL is expressed as a volume (A) and as a percentage of total water gain (B). Values are means ± 1 s.d. (N=7).

concentration, even when supplementary water was provided (Fig. 3A, Table 2; diet, $F_{5,30}=4.81$, $P=0.002$; water, $F_{1,6}=6.32$, $P=0.046$; interaction, $F_{5,30}=4.03$, $P=0.006$) and was tightly correlated with CF volume, reflecting water flux of the birds (Fig. 3B, $r^2_{96}=0.762$, $P<0.001$). A remarkably low osmolality was recorded for CF from birds fed 0.25 mol l⁻¹ sucrose with supplementary water (6.2 ± 2.6 mosm kg⁻¹, $N=7$), while the most concentrated CF measured was 461 ± 253 mosm kg⁻¹ (for

birds fed 2.5 mol l⁻¹ sucrose without supplementary water; $N=6$ birds that yielded sufficient volumes to measure accurately). Even though only small volumes of supplementary water were consumed, drinking had a significant effect on CF osmolality (Table 2).

Interestingly, total solute output (osmotic excretion) was also correlated with diet concentration, being highest for the most dilute diets where birds showed the highest water flux

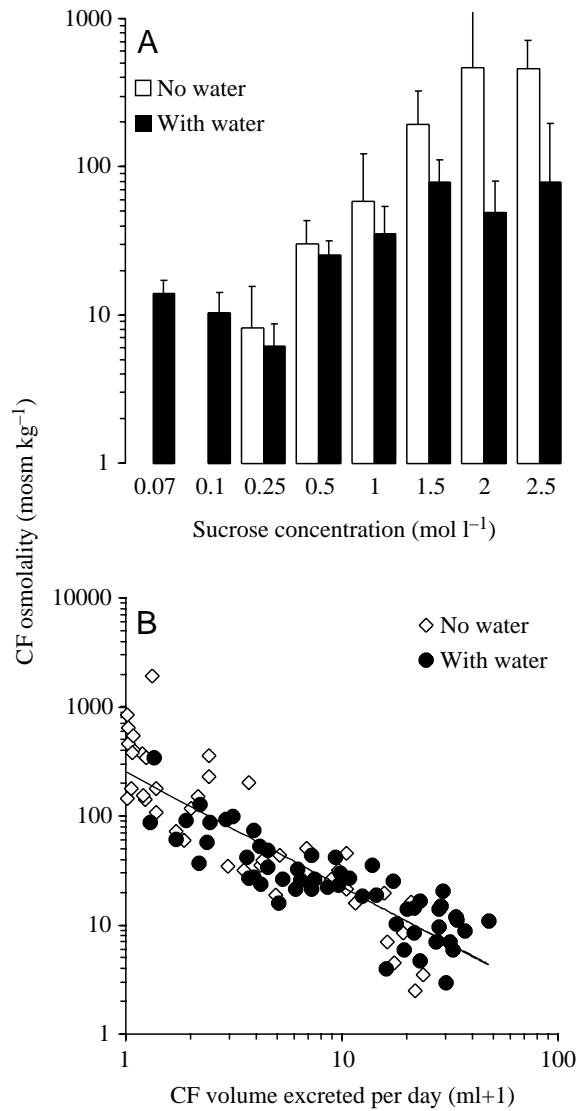


Fig. 3. Cloacal fluid (CF) osmolality (note the logarithmic scale) as a function of water flux. (A) CF osmolality increased with increasing dietary sucrose concentration. Sunbirds were fed sucrose solutions of varying concentrations (0.07 to 2.5 mol l⁻¹), with (solid bars) or without (open bars) supplementary drinking water. Values are means \pm 1 s.d. ($N=7$). (B) CF osmolality was inversely correlated with CF volume (both logarithmic scales). Values are individual data ($N=7$) for the 14 diet trials with (solid circles), or without (open diamonds), supplementary water; the regression line is fitted to data from all diet trials (osmolality = $253.5[\text{CF volume}+1]^{-1.054}$). The volume of CF excreted per day is given as log(ml+1) since the log of values <1 yields negative values.

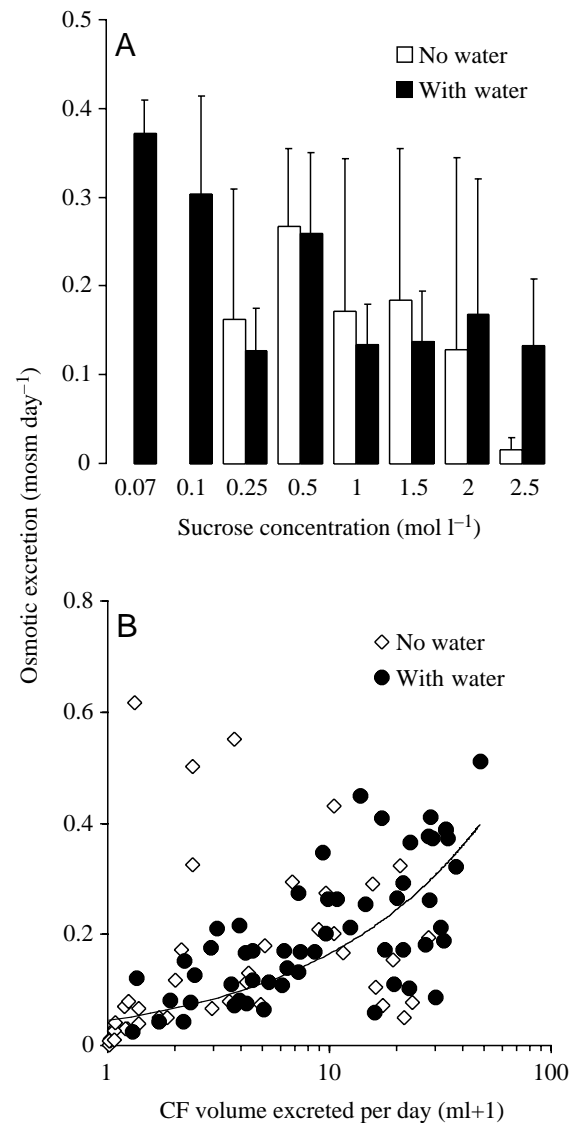


Fig. 4. Total osmotic excretion in cloacal fluid CF (osmolality \times CF volume) as a function of water flux. (A) Total osmotic excretion compared with diet concentration and the provision of supplementary water. Values are means \pm 1 s.d. ($N=7$). (B) Total osmotic excretion was positively correlated with CF volume (note log scale), being highest on more dilute diets, when birds have higher water fluxes. Values are individual data ($N=7$) for the 14 diet trials with (solid circles), or without (open diamonds), supplementary water; the regression line is fitted to data from all diet trials (osmotic excretion = $0.045[\text{CF volume}+1]^{0.563}$). The volume of CF excreted per day is given as log(ml+1) since the log of values <1 yields negative values.

(Fig. 4A, Table 2; diet, $F_{5,30}=5.36$, $P=0.001$; water, $F_{1,6}=0.01$, $P=0.918$; interaction, NS). This was also reflected in a positive correlation between osmotic excretion and CF volume, total osmotic excretion being significantly higher for dilute diets where birds had higher water fluxes (Fig. 4B; $r^2_{96}=0.581$, $P<0.001$).

Diet concentration as well as the provision of drinking water affected electrolyte (Na^+ and K^+) concentrations in CF (Table 2). Minimum electrolyte concentrations were 0.34 ± 0.16 and 0.37 ± 0.09 mmol l^{-1} for Na^+ and K^+ , respectively, on 0.25 mol l^{-1} sucrose diets with supplementary drinking water provided; maximum values were 12.67 ± 8.14 and 21.79 ± 7.92 on 2.5 mol l^{-1} sucrose diets without water. Almost without exception, K^+ excretion exceeded Na^+ excretion. As for total osmotic excretion, electrolyte outputs were highest for the most dilute diets, with high water fluxes, and lowest for concentrated diets without supplementary water provided (Fig. 5A), so that electrolyte output was significantly correlated with volume of CF (Fig. 5B; $r^2_{96}=0.514$, $P<0.001$). Na^+ and K^+ in CF together accounted for $9.1\pm 7.3\%$ of total osmolality over all diets, and reached a maximum of $16.7\pm 10.7\%$ on the 0.25 mol l^{-1} sucrose diet. The composition of the remainder of excreted osmolytes is not known.

Discussion

Sunbirds show remarkable osmoregulatory responses to extremes of diet concentration. They are capable of dealing with both reasonably dilute and concentrated diets, producing some of the lowest and highest values for CF osmolality recorded to date for birds (Table 3). On diets in the tenfold range between 0.25 and 2.5 mol l^{-1} (8–65% w/w), birds show perfect compensatory feeding, increasing intake with diet dilution in order to maintain constant sugar intake (Nicolson and Fleming, 2003). Consumption of 0.25 mol l^{-1} sucrose entailed a total water gain of approximately $3.5\times$ body mass daily, and elimination of water in CF of $2.3\times$ body mass. Only on two very dilute diets (0.07 and 0.1 mol l^{-1} sucrose) are the birds unable to maintain energy balance (Nicolson and Fleming, 2003), and this may be due to the heavy preformed water loads. Excess water intake poses physiological problems connected with thermoregulation and renal function: increased heat loss due to warming ingested food, and possible cooling associated with increased evaporative water loss, energetic expenditure in recovering solutes at the kidneys, and potential electrolyte loss.

How do sunbirds cope with LOW concentration nectars?

Nectar usually includes excess water – even in the desert, hummingbirds flying at ambient temperatures of $23\text{--}37^\circ\text{C}$ produce CF that is still chronically hypo-osmotic to plasma (Hiebert and Calder, 1986). As a consequence, nectarivores may be faced with excess water more often than water deficits. Flowers pollinated by passerine birds tend to produce more dilute nectars than hummingbird-pollinated flowers, a good example being the genus *Aloe* in southern Africa, in which

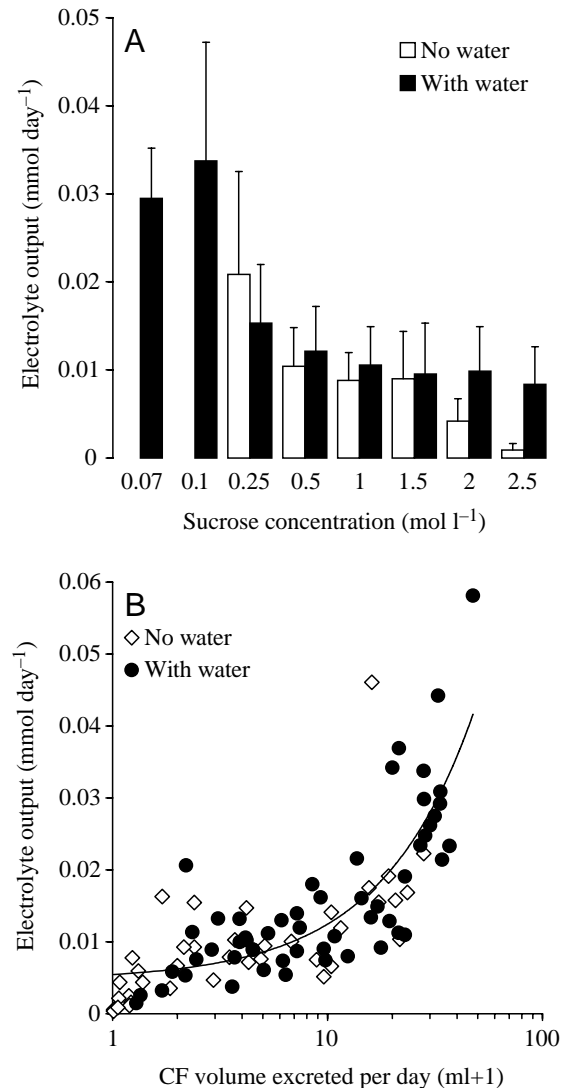


Fig. 5. Electrolyte ($\text{Na}^+ + \text{K}^+$) outputs in the CF (concentration \times CF volume) as a function of water flux. (A) Electrolyte outputs were highest for the most dilute diets, and lowest for concentrated diets without supplementary water, when birds voided minimal volumes of CF. Values are means \pm 1 s.d. ($N=7$). (B) Electrolyte output increased significantly with increasing cloacal fluid volume (note log scale). Values are individual data ($N=7$) for the 14 diet trials with (solid circles) or without (open diamonds) supplementary water; the regression line is fitted to data from all diet trials (electrolyte output = $0.0008[\text{CF volume} + 1] + 0.0047$). The volume of CF excreted per day is given as $\log(\text{ml} + 1)$ since the log of values <1 yields negative values.

nectar concentrations can be lower than the 0.25 mol l^{-1} diet used in this study (Nicolson, 2002).

On dilute diets, our sunbirds voided some of the most dilute CF recorded to date (lowest values averaging 6.2 ± 2.6 mosm kg^{-1} , $N=7$). Tapwater often gives higher osmolality values than this. A minimum field-collected value of 10 mosm kg^{-1} has been recorded for broadtailed hummingbirds *Selasphorus platycercus*, feeding at artificial

Table 3. Values for minimum and maximum urine or voided cloacal fluid osmolality in the three main radiations of nectarivorous birds, compared with some granivorous birds of similar size

| Species | Study site | Diet | Urine/CF | Osmolality (mosm kg ⁻¹) | | | | | | Reference |
|------------------------------------|------------|------|----------|-------------------------------------|-------------------------------------|-----------------------------|-------------------------------------|--------------------------------------|------------------------------------|-----------|
| | | | | Minimum (water-loaded) | | Average (normally hydrated) | | Maximum* (salt-loaded or dehydrated) | | |
| | | | | Values | Treatment/diet | Values | Treatment/diet | Values | Treatment/diet | |
| Granivores | | | | | | | | | | |
| House sparrow (22 g) | L | G | U | | | 325±181 | Seed, W | 825±196 | Seed, 30 h D | 5 |
| Salt-marsh savannah sparrow (19 g) | F | G | U | | | | | 577±164 | Saltmarsh | 7 |
| Whitethroated sparrow (26 g) | L | G | U | | | 476±211 | W | 811±167 | D | 6 |
| House finch (19 g) | F | G | CF | | | | | 183±75 | Desert, ambient temp. 30±4°C | 2 |
| House finch (19 g) | L | G | CF | | | 225 | Seed, W | 850 | Seed, 0.3 mol l ⁻¹ NaCl | 10 |
| Zebra finch (13 g) | L | G | DS | | | 381±25 | Seed, W | 1027±24 | Seed, 0.8 mol l ⁻¹ NaCl | 11 |
| Zebra finch (13 g) | L | G | DS | | | | | 1005±26 | Seed, D | 11 |
| Honeyeaters | | | | | | | | | | |
| Yellow-throated miner (49 g) | F | I | U | | | | | 368±47 | Arid, autumn | 4 |
| New Holland honeyeater (20 g) | F | N,I | U | | | 288±51 | Winter (W) | 187±26 | Mesic, summer (D) | 4 |
| Red wattlebird (97 g) | F | N,I | U | | | 191±21 | Winter (W) | 337±61 | Mesic, summer (D) | 4 |
| Hummingbirds | | | | | | | | | | |
| Broadtailed hummingbird (3 g) | F | N | CF | 10 | Feeders and flowers | 54±43 | Feeders and flowers | 342 | Resource competition stress | 3 |
| Anna's hummingbird (4.5 g) | F | N | CF | 27 | " | 53±18 | " | | | 3 |
| Costa's hummingbird (3 g) | F | N | CF | 34 | " | 57±20 | " | | | 3 |
| Blackchinned hummingbird (3 g) | F | N | CF | 30 | " | 89±52 | " | | | 3 |
| Calliope hummingbird (2.5 g) | F | N | CF | 36 | " | 66±27 | " | | | 3 |
| Rufous hummingbird (4 g) | F | N | CF | 16 | " | 81±77 | " | 383 | Resource competition stress | 3 |
| Rufous hummingbird (4 g) | L | N | CF | | " | 100 | 0.6 mol l ⁻¹ sucrose | 600 | 0.6 mol l ⁻¹ sucrose+ | 8 |
| Rubythroated hummingbird (3 g) | L | N | CF | 10 | 0.2 mol l ⁻¹ sucrose, D | | | | 0.2 mol l ⁻¹ NaCl, D | 1 |
| Sunbirds | | | | | | | | | | |
| Palestine sunbird (6 g) | L | N | U | 15 | 0.15 mol l ⁻¹ sucrose, D | 329 | 1.17 mol l ⁻¹ sucrose, D | | | 9 |
| Palestine sunbird (6 g) | L | N | CF | 12 | 0.15 mol l ⁻¹ sucrose, D | 95 | 1.17 mol l ⁻¹ sucrose, D | | | 9 |
| Whitebellied sunbird (9 g) | L | N | CF | 6±2 | 0.25 mol l ⁻¹ sucrose, W | 56±61 | 1 mol l ⁻¹ sucrose, D | 461±253 | 2.5 mol l ⁻¹ sucrose, D | 12 |

Diets are: G, granivore; I, insectivore; N, nectarivore.

Data are from field (F) and laboratory (L) studies.

U, urine, collected by cannulation; CF, voided cloacal fluid; DS, dropping supernatant.

Trials are: D, without drinking water or dry natural conditions; W, with *ad libitum* drinking water.

Values are means ± 1 s.d., where available.

*Measurements of maximum urine concentrations are limited to diets for which birds maintain measurable levels of urine production, a factor that may particularly affect estimates of the concentrating capacity of granivorous birds. Additionally, spheres of precipitated urates, which do not contribute to measured urinary osmolality, may trap ions (Goldstein and Braun, 1989).

¹Beuchat, 1998; ²Calder, 1981; ³Calder and Hiebert, 1983; Hiebert and Calder, 1986; ⁴Goldstein and Bradshaw, 1998a; ⁵Goldstein and Bradshaw, 1998b; ⁶Goldstein and Braun, 1989; ⁷Goldstein et al., 1990; ⁸Lotz and Martínez del Río, 2003; ⁹T. J. McWhorter, C. Martínez del Río, B. Pinshow and L. Roxburgh, unpublished data; ¹⁰Poulson and Bartholomew, 1962; ¹¹Skadhauge and Bradshaw, 1974; ¹²Present study.

feeders (Calder and Hiebert, 1983), while under laboratory conditions, ruby-throated hummingbirds *Archilochus colubris*, feeding on 0.2 mol l⁻¹ sugar solutions, produced CF with an average osmolality of 10 mosm kg⁻¹ (Beuchat, 1998). Measurement of extremely dilute osmolalities in the present study was made possible by selection of a special thermocouple head for the osmometer and thorough and frequent cleaning. The use of such a thermocouple head was also noted by Beuchat (1998), but other authors have been unable to measure such low osmolalities (Lotz and Martínez del Rio, 2003).

Our minimum values for electrolyte excretion are comparable with the lowest figures recorded for rufous hummingbirds, *S. rufus* (<0.5 mmol l⁻¹ for Na⁺ and K⁺; Lotz and Martínez del Rio, 2003), and lesser doublecollared sunbirds, *N. chalybea* (0.6 mmol l⁻¹ for Na⁺ and 1.5 mmol l⁻¹ for K⁺; Lotz and Nicolson, 1999), fed electrolyte-free diets. These nectarivorous birds have all demonstrated a remarkable ability to produce extremely dilute urine, reabsorbing most electrolytes from excreted fluid. The increase in total Na⁺ and K⁺ excretion with increasing water flux on dilute diets is an interesting result and may pose a problem for birds dealing with dilute nectar diets that are low in electrolytes, particularly Na⁺ (Goldstein and Bradshaw, 1998b). However, means of 3.4 mmol l⁻¹ Na⁺ and 24.7 mmol l⁻¹ K⁺ were measured in nectar of 19 hummingbird-pollinated plant species by Hiebert and Calder (1983) and, in conjunction with arthropod feeding, even these low values may provide adequate electrolyte replacement for birds feeding on natural diets (Lotz and Martínez del Rio, 2003).

Nectarivore kidneys examined to date lack the morphology associated with the concentrating abilities of other birds (Goldstein and Braun, 1989; Johnson and Mugaas, 1970). Kidneys of hummingbirds and honeyeaters contain few mammalian-type concentrating nephrons and a small medullary component (Casotti et al., 1998). They appear to be designed to recover valuable solutes from large quantities of plasma rather than to concentrate urine (Beuchat et al., 1990; Goldstein and Skadhauge, 2000). Sunbird renal morphology is yet to be described. Sunbirds, unlike hummingbirds (McWhorter and Martínez del Rio, 1999), are able to modulate water absorption by the intestine so that excess preformed water is shunted through the gut, and the water load to be processed by the kidneys is correspondingly reduced (McWhorter et al., 2003). A similar modulation of water absorption may exist in honeyeaters (Goldstein and Bradshaw, 1998b). This ability serves to resolve the potential conflict between filtering excess water and retaining solutes. Post-renal modification also plays a significant role in both sunbird and hummingbird osmoregulation (Lotz and Martínez del Rio, 2003; Roxburgh and Pinshow, 2002).

Evaporative water loss

Our data indicate a significant role for evaporative water loss (EWL) in sunbird water balance, and evaporation cannot be discounted as a route for dealing with excess water. While

some of the water we ascribe to EWL could be lost through problems with collection of CF (evaporation from CF droplets prior to sinking under paraffin, or not all the CF droplets reaching the collecting tray), the patterns of EWL show a clear trend that seems unlikely to be produced by methodological errors.

Birds can modulate their EWL in response to heat stress both through panting and control of cutaneous evaporation: the latter is effected by changes in cutaneous vasomotor tone, skin temperature and/or alterations to the disposition of plumage (Hoffman and Walsberg, 1999; Marder and Raber, 1989; Webster and King, 1987; Wolf and Walsberg, 1996). Most research on EWL has been carried out in this thermoregulatory context (reviewed by Dawson, 1982; Dawson and Whittow, 2000; Skadhauge, 1981), while the role of evaporation in water balance has seldom been considered.

Cutaneous EWL is certainly influenced by hydration state. For example, it has been clearly demonstrated that a variety of bird species are capable of reducing EWL through cutaneous or respiratory routes when deprived of drinking water, often at a cost to thermoregulation (e.g. Arad et al., 1987; Maloney and Dawson, 1998). Birds from arid areas also have significantly lower EWL than those from mesic areas (Williams, 1996). However, there are few reports that link water loading (rather than dehydration) with EWL, and the potential interaction between osmoregulation and thermoregulation in this context.

In nectarivorous honeyeaters, EWL is significantly affected by both temperature (Collins et al., 1980) and diet concentration (Collins, 1981). EWL (measured gravimetrically) increased by 21% and 23% for birds fed a dilute (0.4 mol l⁻¹) compared with a more concentrated (1.2 mol l⁻¹) sucrose diet (Collins, 1981). Similarly, Lotz (1999) found that the lesser doublecollared sunbird demonstrated a 115% increase in EWL when switched from a 1.2 to a 0.2 mol l⁻¹ sucrose diet at 20°C (measured with a humidity meter). These changes recorded in flow-through chambers are comparable to the increases in EWL volumes calculated by difference in the present study. In hummingbirds, the third main group of nectarivorous birds, the effect of dilute diet, and therefore waterloading, on EWL has not been examined, and neither have birds been allowed to drink when in respiratory chambers. Published EWL rates are therefore much smaller than the values recorded for feeding sunbirds and honeyeaters. Nevertheless, in response to increasing ambient temperature, various hummingbird species increase EWL (Lasiewski, 1964; Powers, 1992). Furthermore, Lotz and Martínez del Rio (2003) indicated that for rufous hummingbirds fed 0.2–1 mol l⁻¹ sucrose solutions, 50–68% of water intake was lost through EWL.

On the dilute diets, when water-loaded, whitebellied sunbirds were inactive and maintained a posture somewhat similar to that when exposed to low ambient temperatures, feathers being completely piloerect. It is possible that this posture was a response to warming large volumes of cold food to body temperature (Lotz et al., 2003), or else the increased water flux, and potentially greater evaporative losses, increased

heat loss in these birds. Alternatively, their inability to maintain sufficient energy intake (Nicolson and Fleming, 2003) may have affected their thermogenic capacity. Further analysis of the link between evaporative water loss and water loading is required to address these possibilities.

How do sunbirds cope with HIGH concentration nectars?

Sucrose at a concentration of 2.5 mol l⁻¹ is at the uppermost limit of possible nectar concentrations. From allometry, it can be assumed that a 9.3 g bird should consume around 30–35% of body mass (2.8–3.3 ml) of water daily, most of which is lost through respiration (Bartholomew and Cade, 1956). Sunbirds feeding on concentrated sucrose solutions, with water gains of approximately 3.9 and 3.1 ml day⁻¹ (2 and 2.5 mol l⁻¹ sucrose, respectively), therefore may not necessarily be dehydrated; however, their water gain may be almost entirely lost by evaporation alone (Calder, 1979).

Dehydrated birds generally excrete largely solid waste products and switch from production of urine that is iso-osmotic with plasma to a diminished flow of urine with an osmolality 2–3 times that of plasma, avian plasma being 320–370 mmol kg⁻¹ (Goldstein and Braun, 1988, 1989; Goldstein and Skadhauge, 2000; Skadhauge, 1981). Our maximum CF osmolality values, approximately 1.3× estimated plasma osmolality, are comparable to data obtained from normally hydrated granivorous birds on solid diets (Table 3) (Calder, 1981; Goldstein et al., 1990), as well as to maximum values recorded for other nectarivores. For example, field measurements of CF from yellowthroated miners *Manorina flavigula* indicate that these arid-living honeyeaters produce CF with an osmolality of 368±47 mosm kg⁻¹ (mean ± S.E.M.; Goldstein and Bradshaw, 1998a), while Hiebert and Calder (1986) recorded values of 308–426 mosm kg⁻¹ for hummingbirds engaged in intense competition in high-elevation meadows, distant from artificial feeders.

Birds on concentrated diets drank the greatest volumes of supplementary water, when it was available. As a consequence of drinking as little as 5.8±3.6 ml day⁻¹ supplementary water, CF osmolality was reduced dramatically from 461±253 to 80±119 mosm kg⁻¹ on the 2.5 mol l⁻¹ sucrose diets. On concentrated nectars, free water may therefore be an important part of the birds' water balance. Although it is often assumed that nectarivorous birds do not drink free water, field studies in arid Australia have shown that honeyeaters are highly dependent on drinking water (Fisher et al., 1972). Water drinking in the wild may be used to dilute concentrated nectars (Nicolson and Fleming, 2003).

Conclusions

For sunbirds feeding on the most dilute artificial diets, a suite of physiological constraints associated with water loading may limit food intake and thus energy balance. However, for the tenfold range of concentrations from 0.25 to 2.5 mol l⁻¹ sucrose, reflecting natural nectars, sunbirds are eminently capable of maintaining water balance. The birds respond to differences in water availability by altering both CF and

evaporative components of water elimination. Most notably, the scope of CF osmolality in sunbirds is remarkable. Sunbirds are able to produce some of the most dilute CF recorded – their ability to recover electrolytes from CF may be unparalleled by any non-nectarivorous bird, while on concentrated diets with subsequent water shortage, their ability to produce concentrated CF is comparable with that of some granivorous species.

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