The Influence of Ambient Temperature and the Energy and Protein Content of Food on Nitrogenous Excretion in the Egyptian Fruit Bat (*Rousettus aegyptiacus*)

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ABSTRACT

The diets of frugivorous and nectarivorous vertebrates contain much water and generally have high energy but low protein contents. Therefore, we tested the prediction that to save energy under conditions of high energy demands and high water intake, frugivorous Egyptian fruit bats (*Rousettus aegyptiacus*) will increase both the absolute quantity and the proportion of ammonia in their urine. We also examined whether such changes occur when protein intake is low and water intake is high. We did three feeding trials. In trials 1 and 2, bats were fed one of four liquid diets containing constant soy protein concentrations but varying in sucrose concentration and were kept at ambient temperatures (T_a) of 30°C and 12°C, respectively. In trial 3, bats were kept at $T_a = 12$°C and fed one of four liquid diets with equal sucrose concentrations but varying protein concentrations. In trial 1, food intake at a sucrose concentration of 256 mmol/kg H_2O was initially high but decreased to a constant rate with further increases in sucrose concentration, while in trial 2, food intake decreased exponentially with increasing sucrose concentration. As predicted, at 12°C with varying sucrose concentration, both the absolute quantity and the fraction of ammonia in the bats' urine increased significantly with food intake ($P < 0.02$), while the absolute quantity of urea and the fraction of urea nitrogen excreted decreased significantly with food intake ($P < 0.03$). Varying sucrose concentration had no significant effect on nitrogen excretion at $T_a = 30$°C. Varying protein concentration had no significant effect on nitrogen excretion at $T_a = 12$°C. We suggest that Egyptian fruit bats can increase ammonia excretion in response to increased energetic demands, and we calculate that they can save energy equal to ~2% of their daily metabolic rate by doing so.

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Introduction

Patterns of nitrogen excretion in animals are strongly correlated with phylogeny and linked to the environment in which the animals live. Gill-breathing aquatic animals tend to be ammonotelic (i.e., most excreted nitrogen is in the form of ammonia), while terrestrial animals are usually ureotelic or uricotelic (i.e., most waste nitrogen is excreted as urea or uric acid; Campbell 1991). Mammals are largely ureotelic, although some desert-dwelling rodents excrete allantoin as well (Buffenstein et al. 1985; Downs and Perrin 1991), and birds are generally uricotelic. There are energy and water cost trade-offs for the excretion of each of the three most common compounds. In terms of energy, ammonia is the cheapest to produce, but it requires the highest amount of water for its excretion. Uric acid is energetically the most expensive to synthesize (Voet et al. 1999); however, as urates have low solubility, they require the least amount of water to excrete. Urea is intermediate in both energy and water costs (Withers 1992).

The possibility that nectarivorous and frugivorous birds become ammonotelic in response to changes in water intake, nitrogen intake, and/or changes in their energy requirements has been explored by several investigators. Preest and Beuchat (1997) reported that at low ambient temperature (T_a), when water and energy intake rates were increased, the fraction of ammonia in the excreted fluid of Anna's hummingbirds (*Calypte anna*) increased beyond 50%, making them, by their definition, ammonotelic rather than uricotelic. These authors postulated that high energy demands at cold T_as might have triggered an energy conservation mechanism that enabled the hummingbirds to switch their nitrogen excretion from the energetically expensive uric acid to cheaper ammonia. This switch would be made possible by high water turnover that would dilute the toxic ammonia and enable its rapid excretion (Preest and Beuchat 1997).

However, an alternative explanation was proposed by van Tets et al. (2001), who found that in yellow-vented bulbuls (*Pycnonotus xanthopygos*), the proportion of ammonia in ureteral urine increased at relatively low T_a, but water turnover rate did not alter this proportion. They suggested that whenever frugivorous birds that have high metabolic and water turnover rates are exposed to low T_a, an increase in ammonia excretion will occur. Roxburgh and Pinshow (2002) found that the proportion of ammonia in ureteral urine of Palestine sunbirds (*Nectarinia osea*) was not correlated with T_a, water, or protein intake. They did, however, observe an increase in the proportion of ammonia in excreted fluid when dietary protein levels were decreased, leading to what they termed apparent ammonotely.

McWhorter et al. (2003*b*) examined three species of hummingbirds with a range of body mass (m_b) from 2.7 to 7.9 g. None of these birds became ammonotelic, and there was no effect of water turnover rate on the proportion of ammonia in their excreta. The smallest bird studied, *Archilocus alexandri*, had a significantly higher level of ammonia as a fraction of total nitrogen excreted than the other two, *Eugenes fulgens* and *Lampornis clemenciae* (25.7% vs. 4.5% and 3.1%, respectively). McWhorter et al. (2003*b*) concluded that their results did not support McNab's (2002) facultative ammonotely hypothesis, which states that although animals excrete primarily urates, they excrete ammonia under certain conditions, such as increased water intake in birds.

Sabat et al. (2004) also did not find a change in the fractions of excretory nitrogen compounds in the rufous-collared sparrow (*Zonotrichia capensis*) after 4 wk of exposure to high- and low-protein diets. And recently, Tsahar et al. (2005*b*), in their study of the effect of protein and water intake on the nitrogenous waste composition in yellow-vented bulbuls and Tristram's grackles (*Onychognathus tristrami*), found that grackles were uricotelic and that the chemical composition of their nitrogenous waste products was relatively independent of trial conditions; the bulbuls were apparently ammonotelic. This apparent ammonotely was related to low protein intake and high water flux and was the result of postrenal urine modification (Tsahar et al. 2005*b*).

As in birds, frugivorous bats have diets that are high energy, high water, and low protein in content (Mattson 1980) and often have low nitrogen requirements (birds: Brice and Grau 1991; Witmer 1998; Roxburgh and Pinshow 2000; van Tets and Nicolson 2000; Pryor et al. 2001; Lopez-Calleja et al. 2003; McWhorter et al. 2003*b*; Tsahar et al. 2005*a*; bats: Law 1992; Delorme and Thomas 1996, 1999; Korine et al. 1996; Herrera et al. 2002). Due to the high content of water in their diet, frugivorous and nectarivorous species of both taxa also have high water turnover rates (birds: Rooke et al. 1983; Beuchat et al. 1990; McWhorter et al. 2003*a*; Nicolson and Fleming 2003; bats: Helversen and Reyer 1984; Geiser and Coburn 1999; Korine et al. 2004). Any changes in ammonia excretion observed in bats may be attributed to changes in their urine composition, as the urine excreted by bats, unlike that excreted by birds, does not undergo cloacal mixing and thus cannot be modified by hind-gut bacteria.

Mammals regulate nitrogenous excretion by adjusting urea excretion in response to dietary composition and energy requirements (Klahr 1989; Delgiudice et al. 1995; Hammond and Janes 1998; Singer 2003). Further, ammonia excretion in mammals increases with both acute and chronic metabolic acidosis (Hamm and Simon 1987); this results from increased renal production of ammonia in the proximal tubular cells (Remer 2000). It is not known whether increased ammonia excretion in mammals is related to regulation of energy balance.

In light of the above, we explored patterns of nitrogen excretion in the Egyptian fruit bat (*Rousettus aegyptiacus*; Megachiroptera, Pteropodidae). We tested the prediction that under conditions of high energy demands and high water intake, Egyptian fruit bats increase the proportion and amount of ammonia in their urine to save energy. We also examined the effect of protein intake on the proportion and amount of ammonia excreted in the urine of the Egyptian fruit bats.

Material and Methods

Animals and Housing Condition

Adult Egyptian fruit bats were captured with mist nets in an underground parking garage in Beer Sheva, Israel, in March and April 2000 and were housed in an outdoor flight cage (7 m × 3 m × 2.5 m) on the Sede Boqer Campus of Ben-Gurion University of the Negev (30°51′N, 34°47′E; 475 m above sea level), where they were acclimated for a month before trials began. During this period, they were fed a mixed fruit diet, and trials began when the bats maintained constant body mass (m_b). Individuals were marked with aluminum ear tags. Four nonreproductive female and four male bats ranging in m_b from 130 to 160 g were used in the three trials.

Trial Protocol

In the first trial, bats were exposed to a T_a of 30°C. Four pairs of bats were randomly assigned to each of four diets having the same protein concentration, 7.23 g soy protein/kg H_2O, enough to fulfill the animals' protein requirements, but varying in sugar concentration (256, 513, 769, 1,026 mmol sucrose/kg H_2O). The protein-to-sucrose ratio ranged from 0.02 to 0.09. The second trial differed from the first in T_a alone; bats were exposed to 12°C and offered the same diets as in trial 1. In the third trial, run at $T_a = 12$°C, four pairs of bats were randomly assigned to each of four diets with the same sugar concentration, 769 mmol sucrose/kg H_2O, sufficient sucrose to fulfill energy requirements, but varying in protein concentration (2.58, 4.13, 5.68, 7.23 g soy protein/kg H_2O). Here, the protein-to-sucrose ratio ranged from 0.01 to 0.04. In all of the trials,

all of the diets contained the same 840 mg/L KCl, 659 mg/L NaCl, and approximately 50 μL/kg H_2O of vitamin supplement (A-D-VIT3000, Koffolk) and sugar and protein concentrations within the range found in fruits eaten by Egyptian fruit bats in the wild (Korine et al. 1998). In all three trials, pairs of bats were randomly assigned to a single diet each. This design allowed us to use the same eight bats simultaneously in each of the three trials without having to repeat each diet treatment for all four pairs (McWhorter et al. 2003*b*; Tsahar et al. 2005*b*).

Each of the three trials was preceded by 5 d of habituation to the trial conditions, and each trial lasted 5 d. Between trials, we returned the bats to their flight cage for 1 mo of recovery. Bats were acclimated to either a T_a of 30°C, which is the lower critical temperature (T_{lc}) for this species, or a T_a of 12°C, well below the T_{lc} (Noll 1979; Korine and Arad 1993). At T_a = 30°C, therefore, bats experienced conditions of low thermoregulatory energy demands and were not heat stressed, while at T_a = 12°C, they experienced high thermoregulatory energy demands. At the start of habituation, we transferred the bats to individual cages that were darkened with black cloth hoods in a controlled-environment room with a 12L : 12D cycle. During the first 3 d of habituation, we fed the bats the same mixed fruit diet they received in the outdoor flight cage. During the following 2 d, the bats were fed the same diet as in the 5-d trial, ad lib. From the second day of the trial, food intake was measured each morning.

At 1800 hours on each night of a trial, weighed amounts ($\pm$0.01 g) of food were placed in two plastic feeders inside each cage and removed at 0600 hours the following morning, when they were reweighed. The difference between the masses was assumed to be the quantity of food eaten, after correction for evaporation. To control for evaporation, we placed three feeders with the same amount of food in cages without bats in the controlled-environment room and weighed them along with the bat feeders. At 0400 hours on the fifth day, we placed plastic-lined paper on the bottom of the cages. Every 15 min for 4 h we collected freshly voided urine by aspiration into a glass pipette. The urine samples were placed in Eppendorf tubes containing 0.1 mL of 10% (v/v) glacial acetic acid to acidify samples and prevent volatilization of ammonia. The Eppendorf tubes were immediately cooled in an ice bath and then refrigerated until analysis later that day. We did not collect urine that was contaminated with feces or that had partially evaporated.

Urine Analysis

Urine was assayed for ammonia, urea, and uric acid concentrations (Sigma kits 685 and 640A) within 4 h of collection. We did not measure urine volume in this study because we could not separate urine from spilled food. Therefore, we estimated urine volume from reported relationships between water intake and urine volume, which are independent of diet composition in Egyptian fruit bats (Arad and Korine 1993; Korine 1996). From these relationships, we calculated the urine volume and the total amount of nitrogen excreted as uric acid, ammonia, and urea per day.

Statistical Analysis

We compared food intake among the bats in the three trials, and the relationship between food intake and sucrose concentration was best described by a power function (Martínez del Rio et al. 2001). We examined the relationships between the quantities and fractions of nitrogen excreted as urea, uric acid, and nitrogen and food or nitrogen intake by regression analysis. All fractions were arcsine transformed before analysis. We chose $P < 0.05$ as the minimum acceptable level of significance.

Results

Relationships between Food Intake and Sucrose or Protein Concentration

In trial 1, at T_a = 30°C, there was a steep decrease in food intake between 256 and 513 mmol sucrose/kg H_2O. At higher sucrose concentrations, food intake was constant (Fig. 1). In trial 2, at T_a = 12°C, food intake (y, g/d) decreased exponentially with sucrose concentration (x, mmol/kg H_2O; $y = 22{,}347x^{-0.82}$, $r^2 = 0.93$, $F_{1,7} = 40.9$, $P < 0.0007$; Fig. 1). In contrast, in trial 3, food intake was not affected by protein concentration (T_a = 12°C, $P > 0.69$).

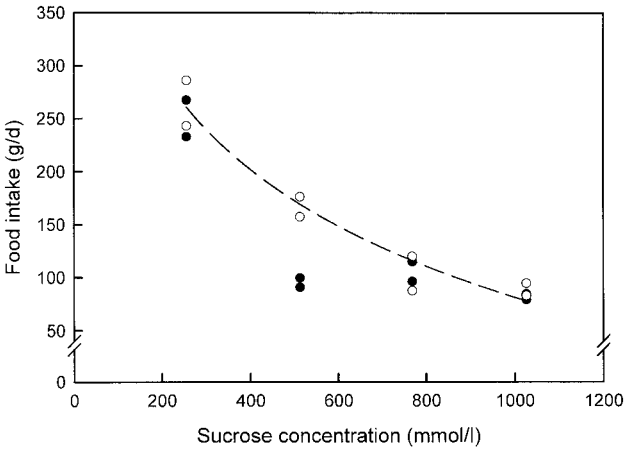

Figure 1. Food intake as a function of food sucrose concentration in Egyptian fruit bats acclimated to two ambient temperatures (T_a). A filled circle represents measures for bats exposed to a T_a of 30°C (trial 1); an unfilled circle and the dashed line represent measures of bats at a T_a of 12°C (trial 2). In both trials, bats were offered diets containing soy protein at a constant concentration but varying in sucrose concentration. See text for statistics.

Food Intake and the Composition of Excreta Nitrogen

In trial 1, at $T_a = 30°C$, with varied sucrose and constant protein, the fractions of nitrogen excreted as uric acid, ammonia, or urea were independent of food intake ($P > 0.51$, $P > 0.46$, $P > 0.58$, respectively). In trial 2, at $T_a = 12°C$, with varied sucrose and constant protein, the fraction of nitrogen excreted as uric acid was also independent of food intake ($P > 0.96$). In contrast, the fraction of nitrogen excreted as ammonia (y) increased with food intake (x, g/d; $y = 0.0005x - 0.01$, $r^2 = 0.61$, $F_{1,7} = 8.88$, $P < 0.02$; Fig. 2*A*), and the fraction of nitrogen excreted as urea (y) decreased with food intake ($y = 0.0005x + 1.00$, $r^2 = 0.62$, $F_{1,7} = 9.22$, $P < 0.03$; Fig. 2*B*). In trial 2, the fraction of nitrogen excreted as ammonia increased with food intake, but none of the bats became ammonotelic (Fig. 3). In trial 3, at $T_a = 12°C$, and where protein concentration varied, the fractions of nitrogen excreted as uric acid, ammonia, and urea were independent of food intake because food intake did not change ($P > 0.66$, $P > 0.67$, $P > 0.66$, respectively).

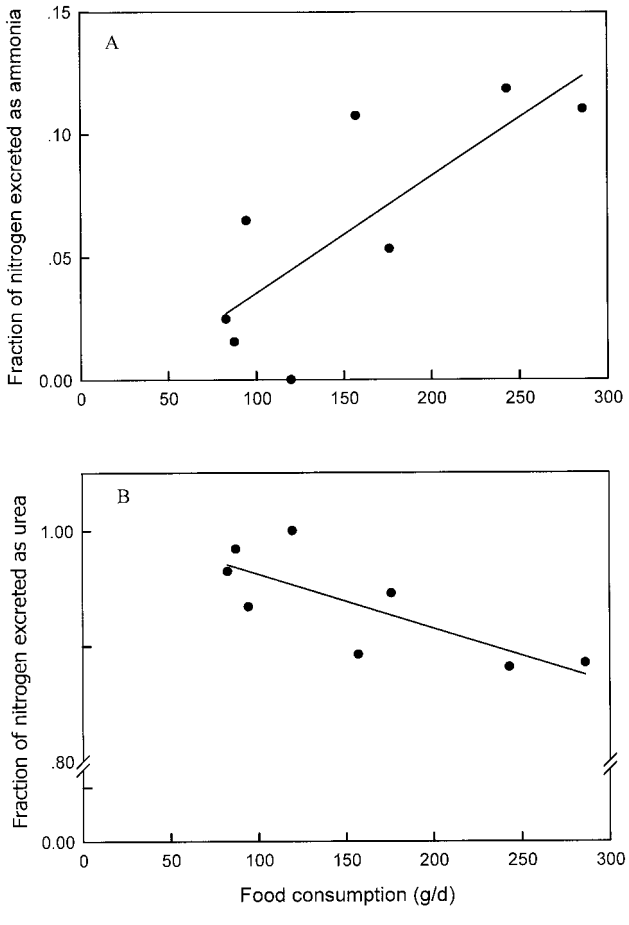

Figure 2. Fraction of nitrogen excreted as ammonia (*A*) and fraction of nitrogen excreted as urea (*B*) as a function of food intake in the urine of Egyptian fruit bats. See text for statistics.

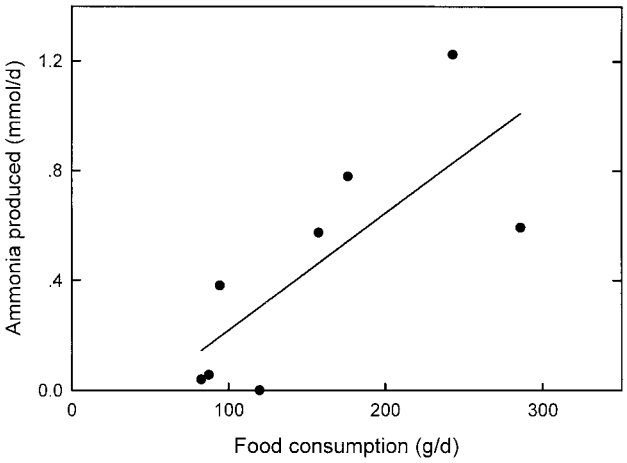

Figure 3. Daily amount of ammonia excreted in urine as a function of food intake in Egyptian fruit bats acclimated to an ambient temperature of 12°C and offered diets with constant soy protein concentration but varying in sucrose concentration. See text for statistics.

Sucrose or Protein Intake and the Composition of Excreta Nitrogen

In trials 1 and 2, the fraction of nitrogen excreted as uric acid, ammonia, and urea was independent of sucrose intake (all $P > 0.20$). In trial 3, at $T_a = 12°C$, with constant sucrose and varied protein concentration, the fraction of nitrogen excreted as uric acid was independent of protein intake ($P = 0.1$). However, protein intake (x, g/d) was positively correlated with the fraction of nitrogen excreted as urea (y; $y = 1.25x - 0.023$, $r^2 = 0.88$, $F_{1,7} = 21.5$, $P < 0.004$) and negatively correlated with fraction of nitrogen excreted as ammonia ($y = -1.09x + 0.88$, $r^2 = 0.78$, $F_{1,7} = 9.3$, $P < 0.02$). In addition, in trial 3, the two bats that had the lowest protein intake became ammonotelic. In these two individuals, the fraction of nitrogen excreted as ammonia was 0.78 and 0.61, respectively.

Food Intake and the Quantity of Nitrogen Excreted

Food intake in trial 1 was not correlated with the quantity of uric acid or ammonia excreted per day ($F_{1,7} = 1.36$, $P > 0.28$; $F_{1,7} = 1.66$, $P > 0.22$, respectively). Urine urea excreted by the six bats that did not change the amount of food they ate (Fig. 1) remained constant but increased in the two bats that increased their food consumption when feeding on the lower sucrose concentration (Fig. 1). Food intake in trial 2 was not correlated with the quantity of uric acid or urea excreted daily ($F_{1,7} = 1.05$, $P > 0.34$; $F_{1,7} = 1.27$, $P > 0.30$, respectively) but was positively correlated with the quantity of ammonia ($r^2 = 0.58$, $F_{1,7} = 7.78$, $P < 0.03$, $y = 0.004x - 0.21$; Fig. 3). In trial 3, food intake was not correlated with the quantities of uric acid, ammonia, or urea excreted daily ($F_{1,7} = 0.15$, $P > 0.71$; $F_{1,7} = 0.04$, $P > 0.86$; $F_{1,7} = 0.01$, $P > 0.91$, respectively).

Table 1: Comparison of quantities of ammonia, urea, and uric acid excretion in the Egyptian fruit bat and in other mammals

Animal	Body Mass (kg)	Ammonia (mmol/kg/d)	Urea (mmol/kg/d)	Uric Acid (mmol/kg/d)
Egyptian fruit bat:				
Trial 1	.149	0–5.44	1.4–27.1	.007–.2
Trial 2	.147	0–8.05	4.9–46.7	0–.1
Trial 3	.151	0–22.4	.5–59.5	.001–.1
Cat	2.5	3.53	12.9–64.5	.001–.09
Cattle	500	.1–8.33	.8–1	.007–.03
Dog	12	.9–3.54	4.8–8.1	0–.03
Goat	50	.1–.35	2.3–7.5	.003–.05
Monkey	12	.1–.59	3.2–11.3	.007–.01
Rabbit	2	.2–.3	19.3–24.2	.003–.04
Rat	.33	.6–1.76	16.1–25.8	.06–.08
Sheep	60	0–8	1.7–2.7	.001–.03

Note. Data for other mammals are from Altman and Dittmer (1974). Values are presented as minimum and maximum concentrations and reflect a range of conditions.

Discussion

Since the artificial food used in the present study was liquid, food intake and water intake by the bats were highly and positively correlated in the first two trials (both $r^2 > 0.99$), enabling us to test the prediction that Egyptian fruit bats, under conditions of high energy demands and water intake, increase the proportion of ammonia in their urine to save energy. This prediction was in accord with the view that it is energetically cheaper to produce ammonia than urea or uric acid (Voet et al. 1999) and that animals capable of making the switch will do so (Preest and Beuchat 1997; van Tets et al. 2001). The results of the present study support this prediction.

Fruit bats that were exposed to 12°C in trial 2 (constant protein, varied sucrose) responded in two ways. First, food intake, and thereby energy and water intake, increased, and second, the fractions of nitrogenous waste compounds excreted in urine changed. However, although the fraction of ammonia in urine increased, none of the bats became ammonotelic (Fig. 2*A*).

In contrast to other mammals that increase the amount of urea produced when eating high-protein diets or that adjust urea excretion in response to dietary composition and energy requirements (Klahr 1989; Delgiudice et al. 1995; Hammond and Janes 1998; Singer 2003), Egyptian fruit bats increased the proportion and the amount of ammonia excreted in their urine (Figs. 2, 3). To substantiate this finding, we plotted the fraction of ammonia excreted at 12°C against the fraction of ammonia excreted at 30°C and did the same for urea. Seven of eight points for ammonia were above the line of equality, while seven out of eight points for urea were below the line of equality, indicating that the proportion of ammonia was higher and that of urea lower at 12°C compared with 30°C. This response may be unique to fruit bats because of the combination of their high dietary water intake and high energy demands for flight.

One possible explanation for the two types of response observed in trial 2 is that the bats with increased energy demands increased ammonia production to save energy. It is likely that bats exposed to $T_a = 12$°C excreted more urea in their urine because their protein intake was high. However, compared with bats exposed to $T_a = 30$°C, they excreted proportionately less urea (0.88 urea at 12°C vs. 0.96 urea at 30°C). We therefore estimated how much extra energy a bat would expend if it responded by increasing only urinary urea, as has been found in other mammals, and not ammonia concentration (Singer 2003).

In Egyptian fruit bats, the maximum measured urea production at $T_a = 12$°C was 14 mmol urea/d. The synthesis of 1 mol of urea requires between 3 (Voet et al. 1999) and 8 mol of ATP (Pattabiraman 1995). Our calculation is based on 5 mol of ATP per mole urea produced (Hochachka and Somero 2002). Thus, at 12°C, the extra energy required to synthesize 14 mmol of urea from ammonia and CO_2 would be (14 mmol urea × 5 mmol ATP/mmol urea × 32.5 J/mmol ATP) × 0.96/0.88, namely, 2.5 kJ/d. This is approximately 2% of an Egyptian fruit bat's daily metabolic rate at 12°C (Noll 1979).

Egyptian fruit bats are active all year (Galil et al. 1976), and they do not enter torpor, even under laboratory conditions (Noll 1979). In Israel, Egyptian fruit bats experience a winter that lasts 3–4 mo when temperatures can drop below 12°C and food supplies are limited and of low quality (Korine et al. 1999). These conditions result in a general decrease in body condition in the population (Makin 1990) and may indicate that the bats are in poor body condition. In addition, Egyptian fruit bats begin their reproductive period during winter (Makin 1990).

Winter is energetically challenging to fruit bats, and any mechanism that results in energy savings would be advantageous.

We compared the amount of ammonia excreted daily by the fruit bats with that of other mammals (Table 1). Fruit bats with high energy requirements at $T_a = 12°C$ excreted more ammonia than at 30°C (their T_{lc}), at which they excreted ammonia in quantities similar to those excreted by other mammals. The large quantity of ammonia excreted at 12°C is likely to reflect the high water intake of these bats at that T_a (Table 1).

In contrast to studies on birds that reported apparent ammonotely (Roxburgh 2000; van Tets et al. 2001; Roxburgh and Pinshow 2002; McWhorter et al. 2003*b*; Tsahar et al. 2005*b*), our results and calculations show that Egyptian fruit bats not only increased the proportion of ammonia in their urine but also increased the total amount of excreted ammonia per day.

Mammals may increase urinary ammonia excretion as a result of either high or low protein intake. Humans increased urinary ammonia excretion in response to high dietary protein to balance acid-base status through glutamine catabolism (Vander 1995; Remer 2000). In sunbirds, hummingbirds, and bulbuls (Roxburgh and Pinshow 2002; McWhorter et al. 2003*b*; Tsahar et al. 2005*b*), low dietary nitrogen leads to apparent ammonotely. Egyptian fruit bats may experience large variations in dietary protein content (Korine et al. 1998), but in the present study, they did not increase excreted ammonia in urine in response at any of the measured protein intake levels. We found that Egyptian fruit bats fed a low-protein diet increased the proportion of ammonia in their urine. Indeed, the two bats that had the lowest protein intake also became ammonotelic. However, when we calculated the total amount of ammonia excreted per day from the concentrations that we measured and the urine volumes that we estimated, we did not find any relationship between total ammonia excreted in the urine and protein intake. We found that even at high levels of water intake, protein intake did not affect total ammonia excreted by the fruit bats. Thus, ammonia excretion in Egyptian fruit bats was not related to protein intake. Ammonotely is usually defined in terms of the proportion of ammonia N to total N in urine. However, we suggest that it is not enough to ascribe ammonotely based on proportions alone but that the total amount of N excreted as urea, urates, and ammonia should all be accounted for in order to determine whether an animal is truly or only apparently ammonotelic.

The results of the present study are also in accord with reports that fruit- and pollen-eating bats from both the Old and New Worlds regulate food intake based on its energy rather than its protein content (Thomas 1984; Law 1992; Delorme and Thomas 1996, 1999; Korine et al. 1996). Namely, in trials 1 and 2, where food protein concentration was constant but sucrose concentration varied, food intake was negatively correlated with sucrose concentration, as observed in the nectarivorous microchiropteran Pallas's long-tongued bat (*Glossophaga soricina*; Ramírez P. et al. 2005). In contrast, similar relationships were not found in trial 3, where protein concentration was varied.

In summary, our results generally support our predictions that the proportion of ammonia in the urine of Egyptian fruit bats increased in response to low T_a (high energy demand and consequently high water intake) to save energy, but the bats did not change the proportion of ammonia in their urine in response to changes in dietary protein. Therefore, these results indicate that the switch in nitrogenous compounds excreted is not driven by a low protein intake but rather is an energy-saving mechanism that may be unique to fruit bats.

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