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RESEARCH ARTICLE

Ammonotely in a neotropical frugivorous bat as energy intake decreases

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SUMMARY

We tested the role of increased ammonia in urine as an energy- and/or nitrogen (N)-saving mechanism in the great fruit-eating bat *Artibeus lituratus* (Phyllostomidae). We compared N excretion in two groups of bats fed energy-rich (2.75 kJ g^{-1} wet mass) or energy-poor diets (0.7 kJ g^{-1} wet mass). Within each diet, bats were assigned to different N contents. In order to function as an energy-saving mechanism, ammonia production should increase with decreasing energy intake. To function as an N-saving mechanism, ammonia production should increase with decreasing N intake. Because we varied both diet energy density and N content, our study design allowed us to test these two possibilities simultaneously. Bats had higher food intake rate and, consequently, higher N intake rate on the energy-poor diet, but energy intake rate was lower. Most bats on the energy-rich diet were ureotelic whereas on the energy-poor diet bats were ureotelic, ammonotelic or ureo-ammonotelic. Bats fed the energy-poor diet had a higher excretion rate of ammonia and a higher percent of N excreted as ammonia. Percent N ammonia and ammonia excretion rate were inversely related to energy intake, but they were not related to N intake. By favoring ammonia production over urea, bats on the energy-poor diet may save up to 1% of their basal metabolic rate. Consumption of energy-dilute fruits by fruit bats might affect the way in which N wastes are excreted, favoring the excretion of ammonia N when food intake is accompanied by the ingestion of large volumes of water.

Key words: ammonia, *Artibeus lituratus*, bat physiology, nitrogen requirements, nitrogen excretion, urea.

INTRODUCTION

Differences in metabolism of nitrogenous waste among vertebrates have been interpreted as adaptive responses to different life histories (Withers, 1992). The major end products of nitrogen (N) metabolism are ammonia, urea and uric acid. Ammonia is a highly toxic and soluble product of amino acid metabolism and it is the primary form of N excretion found in aquatic animals (Schmidt-Nielsen, 1995; Willmer et al., 2000). Terrestrial animals detoxify ammonia, converting it to urea or uric acid before excretion. Urea predominates in mammals, whereas uric acid is the main form of N excretion in birds and reptiles (Schmidt-Nielsen, 1995; Willmer et al., 2000). Urea and especially uric acid are less toxic than ammonia and require less water for their excretion: 1 g of ammonia requires 400 ml to be diluted below toxic levels whereas urea and uric acid require 10 and 50 times less water, respectively (Wright, 1995). However, urea and uric acid are energetically more expensive to produce than ammonia: 1 mol of urea requires 4 mol of ATP and 1 mol of uric acid requires 9 mol of ATP to be synthesized from ammonia (McNab, 2002).

Several studies have shown that the chemical form in which N is excreted in terrestrial vertebrates is affected by several variables, including water intake, protein intake and ambient temperature. In particular, the presence of ammonotely (>50% of N excreted as ammonia) in birds and mammals has been explored as a physiological strategy to save energy and/or compensate for low-N diets. Preest and Beuchat (Preest and Beuchat, 1997) were the first to report the existence of ammonotely in birds; Anna's hummingbirds (*Calypte anna*) fed an N-free, sugar solution switched to ammonotely when exposed to low ambient temperature (10°C; Preest and Beuchat, 1997). Birds at low temperature increased their food consumption, and five individuals became ammonotelic, four were urico-ammmonotelic and only one was uricotelic. In contrast, all birds at higher temperatures (20 and 40°C) were uricotelic. Ammonotely in *C. anna* was interpreted as an energy-saving mechanism facilitated by increased water turnover rate. A similar interpretation was used to explain increased ammonia excretion when yellow-vented bulbuls (*Pycnonotus xanthopygos*) were exposed to low ambient temperatures (10°C), although no ammonotelic individuals were reported (van Tets et al., 2001). Ammonotely was found in *P. xanthopygos* with low protein intake (Tsahar et al., 2005), and some individuals of Palestine sunbirds (*Nectarinia osea*) had a higher proportion of N excreted as ammonia than as urates (Roxburgh and Pinshow, 2002). In these birds, increased ammonia N was suggested to be due to post-renal urine modification *via* bacterial breakdown of uric acid and/or uric acid reabsorption in the hindgut (Roxburgh and Pinshow, 2002; Tsahar et al., 2005). Apparent ammonotely was considered a common feature of birds with low N requirements as an N salvage mechanism (Tsahar et al., 2005). Alternatively, recycled uric acid might also serve as an antioxidant for these birds (Tsahar et al., 2005).

Among mammals, ammonotely has only been reported in the nectar-feeding Pallas's long-tongued bat (*Glossophaga soricina*) (Herrera M. et al., 2006) and in the Egyptian fruit bat (*Rousettus aegyptiacus*) (Korine et al., 2006). *Glossophaga soricina* showed an increase of ammonia excretion with decreased N intake at moderate ambient temperatures (25–27°C) and two individuals

became ammonotelic (Herrera M. et al., 2006). Ammonia production increased with food intake in *R. aegyptiacus* when the energy content of food was manipulated at low temperatures (12°C), and two individuals became ammonotelic at low N intake. Increased ammonia excretion was interpreted as a potential N-saving mechanism in *G. soricina* (Herrera M. et al., 2006), and it was proposed as a response to increased energy demands in *R. aegyptiacus* (Korine et al., 2006).

We examined the potential role of increased production of ammonia in urine as an energy-saving and/or N-saving mechanism in the great fruit-eating bat *Artibeus lituratus* Olfers 1818 (Phyllostomidae). We compared ammonia excretion in urine in two groups of bats under contrasting energy-density liquid diets with different N content. Because nectarivorous and frugivorous bats increase the intake of liquid diets as energy density decreases but are not able to compensate energy ingestion (Ramírez P. et al., 2005; Ayala-Berdon et al., 2008; Herrera M. and Mancina G., 2008), N intake should increase and energy intake decrease as the energy density of diet decreases. We predicted that N excreted as ammonia would increase as the energy density of the diet decreases because ammonia production would be favored over urea, which is more expensive to synthesize. In contrast, if increased excretion of ammonia N functioned as an N salvage strategy, as suggested in plant-eating birds and bats (Tsahar et al., 2005; Herrera M. et al., 2006), then we expected that ammonia N excretion would increase in the energy-rich diet as N intake decreased.

MATERIALS AND METHODS

Capture of animals and husbandry

Non-reproductive bats (40–60 g) were trapped with mist nets (5×2 m) at the University of Colima central campus in Colima, Mexico (19°14′36″N, 103°43′29″W). Bats were maintained in 80×80×80 cm cages and fed a diet of banana, mango, papaya and guaba for 1 week before the experiment. Water was provided *ad libitum* during this period. Room temperature was maintained at 22°C and illumination was kept at an artificial 12 h:12 h light:dark cycle.

Experimental protocol

The experiments were conducted at the same ambient temperature and light conditions described above. Bats were placed in inverted individual 3 liter plastic containers. Because the smooth plastic sides of the containers provided no perch, the bats could hang only from the plastic mesh placed on the top. Bird feeders were placed on the upper part of the container. We tested the feeders to ensure that food did not drip. We also placed a feeder with the experimental diet outside the cage to control for the volume of food lost by evaporation. Urine and feces drained through the neck of the chamber into a beaker, to which 1 ml of mineral oil was added to prevent evaporation. Bats were assigned to liquid diets with contrasting energy densities: 2.75 kJ g^{-1} wet mass (20 g sucrose 100 ml^{-1} water; 11 females, six males) and 0.7 kJ g^{-1} wet mass (5 g sucrose 100 ml^{-1} water; eight females, six males). We refer to these diets as energy-rich and energy-poor, respectively. N was provided in the form of casein acid hydrolyzate. Bats were randomly assigned to varying amounts of N: 0.1, 0.15 and 0.31 mg ml^{-1} water in both diets, and 0.43 and 0.47 mg ml^{-1} water in the energy-rich and energy-poor diets, respectively. Diets were complemented with fixed amounts of ascorbic acid (0.015 mg ml^{-1}), sodium chloride (0.53 mg ml^{-1}), calcium phosphate (0.53 mg ml^{-1}) and a vitamin complement (0.50 mg ml^{-1}). Bats were offered the experimental diet for 3 days. Bats were weighed (±0.1 g) at 0, 12 and 24 h of the experiment, and they were released at the site of capture at the end of the experiment after 1 day in captivity with the maintenance diet. Only samples from the third day of the experiment were analyzed. Food was offered continuously for 12 h, and the amount consumed during this period was measured to the nearest milliliter and corrected for the volume that evaporated. Urine and feces were collected continuously for 24 h. Urine samples were immediately placed in a freezer at –40°C, and feces samples were kept at –4°C until they were analyzed. Urine was separated from the mineral oil by centrifugation to measure urine volume to the nearest milliliter, and analyzed with clinical diagnostic kits (Diagnostic Chemicals, Charlottetown, PE, Canada) in an automated spectrophotometer (Roche Cobas Mira-S, Basel, Switzerland) to determine urea (catalog 283), ammonia (catalog 233) and uric acid (catalog 237) concentrations. We used concentration values and urine volume to estimate total urea, ammonia and uric acid, and total N excreted in each one of these components. We considered urinary N as the sum of urea, ammonia and uric acid N. Fresh and dry feces were weighed to the nearest milligram and analyzed in an elemental analyzer (PDZ Europa ANCA-GSL, Northwich, UK) to determine the amount of fecal N. Feces samples were dried in a digital oven (Imperial V, Lab-Line Instruments, Melrose Park, IL, USA) at 50°C for 24 h. We determined apparent maintenance N requirements (MNR) using the *x*-intercept of a least squares linear regression of the apparent N balance (N intake minus urinary and fecal N) on N intake (Smith and Green, 1987). We estimated total water gain as the sum of preformed water intake and metabolic water production. Preformed water was estimated by subtracting the mass of sugar from the mass of solution consumed, whereas metabolic water was calculated as 198 g water for every mole (342 g) of sucrose consumed. Evaporative water loss was estimated from the difference between water gain and the sum of fecal water and urine volume. Fecal water was estimated as the difference between the mass of fresh and dry feces.

Statistical analyses

We used *t*-tests to compare body mass, food, energy and N intakes, water gain, urine production, N balance and N excretion between bats on the energy-rich and energy-poor diets. We tested the relationship between N ammonia excretion and water gain, N intake and energy intake using multiple regression analysis followed by estimation of partial correlations (r_p) to assess the effect of each variable on ammonia N concentration, ammonia N excretion rate and percent N ammonia. Values are reported as means ± s.d., and significance was accepted at α=0.05.

RESULTS

Body mass of bats at 0 (t_{29}=0.4, P=0.6), 12 (t_{29}=0.3, P=0.7) and 24 h (t_{29}=0.1, P=0.9) of the experiment did not differ significantly between diets (Table 1). Bats on the energy-poor diet had significantly higher food volumetric intake (t_{29}=2.8, P=0.008), higher N intake (t_{29}=2.5, P=0.01) and lower energy intake (t_{29}=4.1, P=0.0003) than bats on the energy-rich diet (Table 1). Water gain was higher in bats on the energy-poor diet (t_{29}=3.1, P=0.003; Table 1).

Apparent N balance of most bats on the energy-poor diet was negative and significantly lower than that of bats on the energy-rich diet (t_{29}=3.2, P=0.003; Fig. 1). Apparent MNR were 20.9 mg day^{-1} or 194.7 mg $kg^{-0.75}$ day^{-1} for bats on the energy-rich diet (N balance=0.63N ingestion–13.2; r=0.67, $F_{1,15}$=12.3, P=0.003); MNR were not estimated for bats on the energy-poor diet because the regression between apparent N balance and N intake was not significant (N balance=–0.01N ingestion–29.6; r=0.006, $F_{1,12}$=0.0005, P=0.9). The regression between fecal N per unit of

Table 1. Food intake and nitrogen excretion in *Artibeus lituratus* fed energy-rich (20 g sucrose 100 ml^{-1} water; *N*=17) or energy-poor (5 g sucrose 100 ml^{-1} water; *N*=14) diets with varying contents of nitrogen in the form of casein hydrolyzate

	Energy-rich diet	Energy-poor diet	*P*
Initial body mass (g)	50.8±6.1	49.9±5.4	0.6
Body mass at 12 h (g)	52.2±6.9	51.4±6.6	0.7
Body mass at 24 h (g)	49.8±6.8	50.2±6.2	0.9
Food intake (ml day^{-1})	43.8±23.9	69.2±26.5	0.008
Energy intake (kJ day^{-1})*	120.9±63.7	55.3±21.2	0.0003
N intake (mg day^{-1})	11.3±11	23.6±15.9	0.01
Water gain (ml day^{-1})	41.9±21.7	67.8±26	0.003
Urine (ml day^{-1})	19.9±21.4	55.7±24.9	0.001
Feces (mg day^{-1})	72.7±30.1	Trace	–
Water in feces (m day^{-1})	0.3±0.5	Trace	–
N excreted in urine (mg day^{-1})	12.4±7.8	53.4±29.7	<0.0001
N excreted in feces (mg day^{-1})	4.8±1.9	Trace	–
N balance (mg day^{-1})	-6.1±10.3	–29.8±28.4	0.003

Values are means ± s.d.
P-values correspond to *t*-test comparisons between diets.
*Energy intake was estimated assuming 16.5 kJ for 1 g of sucrose and 3.5 kJ for 1 g of casein hydrolyzate in the food.

dry matter intake against the N content of the dry matter in the diet (Bosshardt and Barnes, 1946) was not significant for the bats on the energy-rich diet (r=0.07, $F_{1,15}$=0.08, P=0.8), preventing us from estimating MNR on a truly digestible basis.

A higher volume of urine was produced by bats fed the energy-poor diet (t_{29}=4.2, P=0.0001), and bats on this diet produced almost no feces (Table 1). Virtually all N lost was *via* urine in bats on the energy-poor diet, in contrast to bats on the energy-rich diet (t_{29}=5.1, P<0.0001; Table 1). Most water losses in bats on the energy-poor diet were in urine (79.9±25.5%) whereas evaporative water loss (56.5±17.4%) and urine (42.2±17.3%) accounted for most water losses in bats on the energy-rich diet.

N excretion patterns differed among bats on the two experimental diets. Most bats (94.1%) on the energy-rich diet were ureotelic (% urea N=83.3±9.1), whereas 28.5% of bats on the energy-poor diet were ureotelic (% urea N=76.7±7.2), 28.5% were ammonotelic (% ammonia N=65.4±9.3) and 43% were ureo-ammonotelic (% urea N=50.2±3.9, % ammonia N=49.4±4.1). On average, the percentage of N excreted as ammonia was higher in bats on the energy-poor diet (t_{29}=5.1, P<0.0001), whereas the percentage of N excreted as urea (t_{29}=3.5, P=0.001) and uric acid (t_{29}=3.3, P=0.002) was higher in bats on the energy-rich diet (Table 2). Bats fed the energy-poor diet had a higher concentration of ammonia (t_{29}=3.4, P=0.001) and a lower concentration of uric acid in urine (t_{29}=4.6, P<0.0001) than bats fed the energy-rich diet; urea concentration did not differ between diets (t_{29}=1.1, P=0.2; Table 2). Ammonia N excretion rate was inversely related to energy intake (r_p=–0.71, P<0.0001) and directly related to water gain (r_p=0.77, P<0.0001) but not to N intake (r_p=–0.23, P=0.22; Fig. 2). Ammonia N concentration was related only to energy intake (r_p=–0.53, P=0.002) but not to water gain (r_p=0.23, P=0.22) or N intake (r_p=0.07, P=0.7; Fig. 3). Percent N ammonia was inversely related to energy intake (r_p=–0.64, P=0.0001) and directly related to water gain (r_p=0.53, P=0.002) but not to N intake (r_p=–0.01, P=0.94; Fig. 4).

DISCUSSION

Previous studies have shown a modest increase in ammonia excretion in urine when energy demands of bats were increased by lowering ambient temperature (Korine et al., 2006) or at low N intake (Herrera M. et al., 2006). Our study showed a large increase in ammonia excretion and a switch to ammonotely in some of the bats when energy ingestion was constrained and N intake was high. In the following paragraphs we discuss the implications of our findings for energy and N balance in fruit-eating bats, and for the understanding of plasticity in the excretion of nitrogenous wastes.

Energy intake

Artibeus lituratus increased volumetric food consumption on the energy-poor diet but they were not able to compensate for decreased energy intake, and ingested significantly less energy than bats on the energy-rich diet. Similarly to our frugivore species, the nectarivores *G. soricina* and *Leptonycteris yerbabuenae* and the frugivore *A. jamaicensis* were not able to compensate for decreased food intake when fed low-energy diets (Ramírez P. et al., 2005; Ayala-Berdon et al., 2008; Herrera M. and Mancina G., 2008). Energy intake of *A. lituratus* on the energy-poor diet amounted to ca. 45% of the daily energy ingestion of bats on the energy-rich diet, a lower proportion than the 10 g nectarivore *G. soricina* (ca. 50%; Ramírez P. et al., 2005; Herrera M. and Mancina G., 2008). The inability of *A. lituratus* to compensate for reduced food ingestion is probably due to a combination of morphological constraints on the efficient ingestion of fluid diets, as suggested for *A. jamaicensis* (Ayala-Berdon et al., 2008), and physiological limitations regarding the processing of high water volumes. Previous work with nectarivorous and frugivorous bats (Ramírez P. et al., 2005; Ayala-Berdon et al., 2008; Herrera M. and Mancina G., 2008) suggests that sucrose hydrolysis does not limit the processing of sucrose nectar by *A. lituratus*, but rather the burden of excess water of dilute solutions. Although kidneys of frugivorous bats from the New and Old Worlds, are designed to process large volumes of water (Studier and Wilson, 1983; Studier et al., 1983; Arad and Korine, 1993; Herrera M. et al., 2001; Schondube et al., 2001), there might be a limit to the amount of fluid that they can handle. To compensate for reduced energy ingestion, *A. lituratus* should ingest 178.8 ml of the energy-poor solution, or 3.5 times body mass. In our study, *A. lituratus* increased food consumption from 0.9 times body mass in the energy-rich diet to only 1.4 times body mass in the energy-poor diet. Allometry might also be a limiting factor in *A. lituratus* because *G. soricina*, although unable to compensate for reduced food intake, is able to ingest a volume of 5% sucrose nectar equivalent to four times its body mass (Ramírez P. et al., 2005).

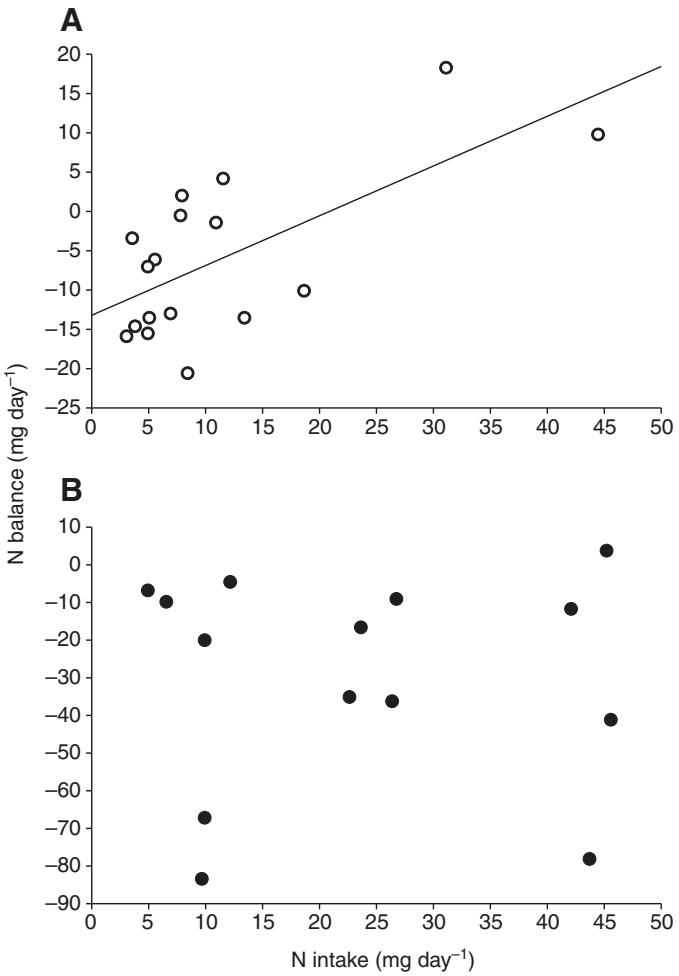


Fig. 1. Relationship between N balance and N intake in *Artibeus lituratus* fed (A) an energy-rich (20 g sucrose 100 ml^{-1} water) or (B) an energy-poor diets (5 g sucrose 100 ml^{-1} water) with varying amounts of N content. N was provided in the form of casein hydrolyzate. The relationship was significant for the bats on the sugar-rich diet (y=0.63x–13.2; r=0.67, $F_{1,15}$=12.3, P=0.003), but not on the sugar-poor diet (y=–0.01x–29.6; r=0.006, $F_{1,12}$=0.0005, P=0.9). We used the x-intercept of the linear regression equation for bats on sugar-rich diets to estimate apparent maintenance N requirements.

N balance

In spite of their higher N intake, bats on the energy-poor diet had a much lower N balance than bats on the energy-rich diet. A similar pattern was found previously in *G. soricina*: bats on energy-poor diets (5 and 10% sucrose) had similar or higher N intakes but lower N balances than bats on energy-rich diets [20 and 30% sucrose (Herrera M. et al., 2006)]. A relationship between energy content of the diet and N balance was previously reported in *A. jamaicensis*: when offered diets with contrasting energy density (2.5–2.9 *vs* 3.1–3.5 kJ g^{-1} wet mass), bats had lower N balance on the diet with less energy density (Delorme and Thomas, 1999). The effect of energy content of diet on N balance in *A. jamaicensis* was far less dramatic than in our study (e.g. most *A. jamaicensis* were in positive balance in both diets) because our energy-rich (2.75 kJ g^{-1} wet mass) and energy-poor diets (0.7 kJ g^{-1} wet mass) had more contrasting energy contents. N balance in *A. lituratus* fed the energy-poor diet did not to follow a linear relationship with N intake and, therefore, apparent MNR were estimated only for bats on the energy-rich diet. Apparent MNR of *A. lituratus* on the energy-rich diet were 194.7 g kg$^{-0.75}$ day^{-1}, a similar value to other nectar and fruit-eating vertebrates fed a casein diet [96–158 g kg$^{-0.75}$ day^{-1} (McWhorter et al., 2003; Tsahar et al., 2005)]. Most bats on the energy-poor diet had a daily N ingestion above MNR, but were in negative balance. N losses in bats on the energy-rich diet were more predominant in urine, and fecal N losses amounted to 28% of total N output. This pattern contrasts with the almost null elimination of N in feces and the large preponderance of urine N in bats on the energy-poor diet. High urinary N output by bats on the energy-poor diet was facilitated by the production of large volumes of urine. The concentration of N in urine of bats fed the energy-poor diet (1.0±0.4 mg N ml^{-1}) was almost identical to the concentration in urine of bats fed the energy-rich diet (0.9±0.8 mg N ml^{-1}). The almost exclusive loss of N *via* urine in these bats, even to levels that double N intake, suggests that virtually all ingested N is digested and that a large portion of N losses must be of endogenous origin.

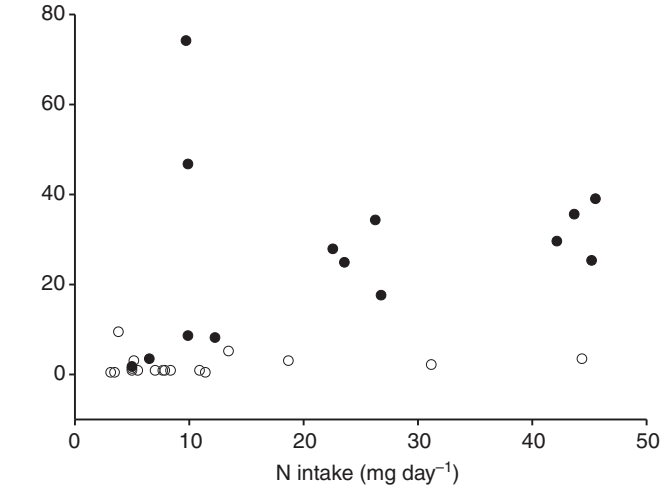
Forms of N excretion

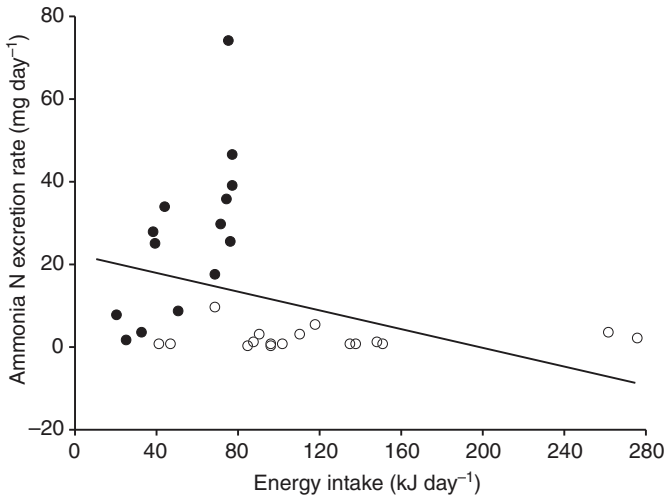
The switch from ureotely to ammonotely in *A. lituratus* fed the energy-poor diet is similar to the changes to ammonotely from ureotely in *C. anna* (Preest and Beuchat, 1997) and *P. xanthopygos* (Tsahar et al., 2005). This shift in *A. lituratus* was accompanied by a threefold increase in ammonia concentration in urine of bats on the energy-poor diet (Table 2). Increased excretion of ammonia N in *A. lituratus* on the energy-poor diet was not related to low N intake, as has been shown in other studies (Roxburgh and Pinshow, 2002; Herrera M. et al., 2006; Tsahar et al., 2005), because bats on this diet had higher N intake rates than those on the energy-rich diet. Ammonotely in our study appears to be related to energy rather than N balance because it occurred when daily energy intake was <40% of the intake when the bats were ureotelic. We estimated the amount of energy saved by bats producing ammonia rather than urea in the energy-poor diet, assuming that it takes 4 mol of ATP to synthesize 1 mol of urea from ammonia (McNab, 2002). The individual that eliminated the highest percentage of N as ammonia (~80%) in the energy-poor diet produced 5.29 mmol day^{-1} of ammonia and 0.68 mmol day^{-1} of urea, and invested 2.72 mmol day^{-1} of ATP or 0.088 kJ day^{-1} during this process. If that individual had eliminated 80% of N as urea, it would have required 0.343 kJ day^{-1}

Table 2. Mean (±s.d.) percentage of the total nitrogen excreted by *Artibeus lituratus* as urea, ammonia and uric acid and the concentration (mg ml^{-1}) of each nitrogenous compound

	% Total N excretion			Urine concentration (mg ml^{-1})			
Diet	Urea N	Ammonia N	Uric acid N	Urea	Ammonia	Uric acid	*N*
Energy-rich	80.3±15.1	16.8±14.7	2.9±2.6	1.6 ±1.8	0.2±0.3	0.04±0.03	17
Energy-poor	53.2±17.8	46.3±18.1	0.4±0.3	1.1±0.5	0.6±0.3	0.01±0.07	14

Two groups of bats were fed either an energy-rich (20 g sucrose 100 ml^{-1} water) or energy-poor (5 g sucrose 100 ml^{-1} water) diet with varying contents of nitrogen in the form of casein hydrolyzate.





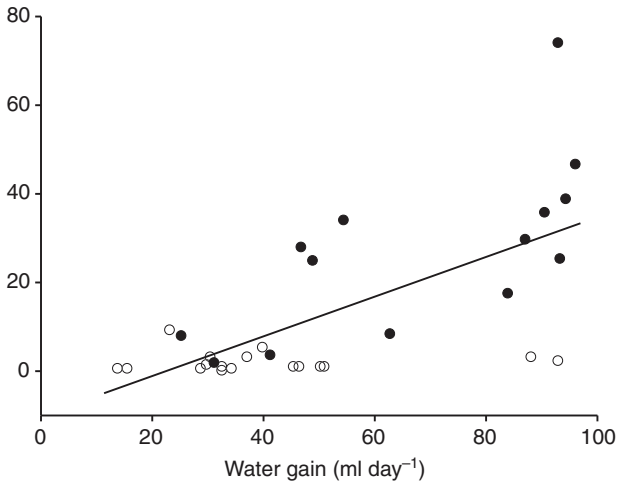
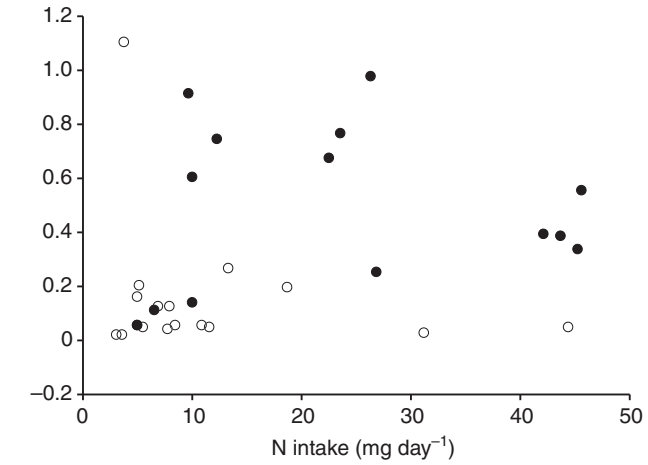
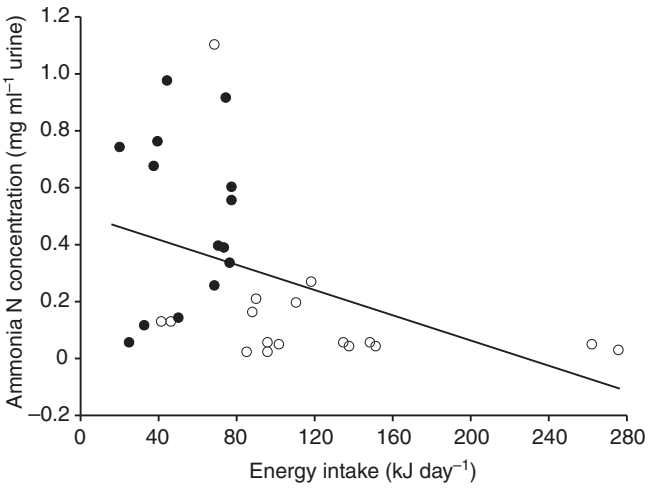


Fig. 2. Excretion rate of ammonia N by *Artibeus lituratus* in relation to N intake (y=4.22+0.53x; r_p=–0.23, P=0.22), energy intake (y=20.89–0.08x; r_p=–0.71, P<0.0001) and water gain (y=0.44x–10.28; r_p=0.77, P<0.0001). Bats were fed energy-rich (20 g sucrose 100 ml^{-1} water; open symbols) or energy-poor diets (5 g sucrose 100 ml^{-1} water; closed symbols). Regression lines are shown only when there were significant correlations after multiple regression analysis followed by partial correlation estimates (r_p).





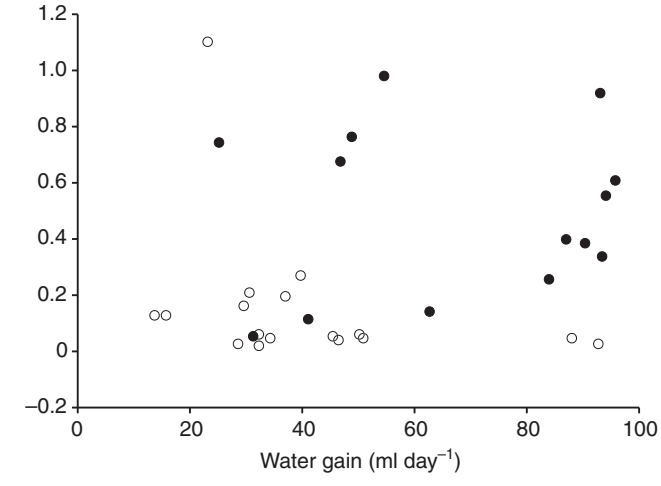


Fig. 3. Concentration of ammonia N by *Artibeus lituratus* in relation to N intake (y=0.23+0.004x; r_p=0.07, P=0.70), energy intake (y=0.52–0.002x; r_p=–0.53, P=0.002) and water gain (y=0.18+0.002x; r_p=0.23, P=0.22). Bats were fed energy-rich (20 g sucrose 100 ml^{-1} water; open symbols) or energy-poor diets (5 g sucrose 100 ml^{-1} water; closed symbols). Regression lines are shown only when there were significant correlations after multiple regression analysis followed by partial correlation estimates (r_p).

to synthesize 2.64 mmol day^{-1} of urea. The amount of energy saved in the ammonotelic individual amounts to 0.255 kJ day^{-1} or ~0.8% of the basal energy requirements for a 51 g *A. lituratus* [32.8 kJ day^{-1} (Cruz-Neto et al., 2001)]. Korine et al. (Korine et al., 2006) assumed that the cost of synthesizing 1 mol of urea was 5 mol of ATP, and estimated that *R. aegyptiacus* saved 2% of its daily

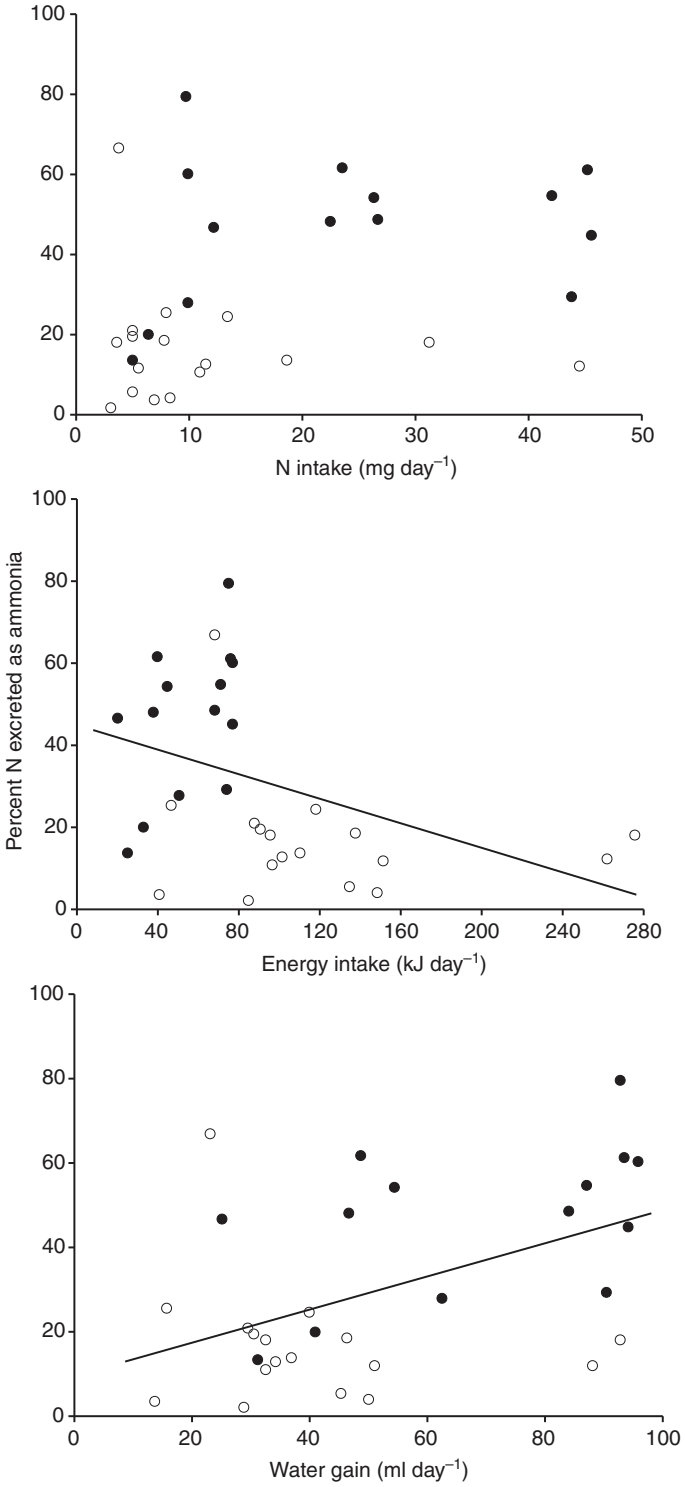


Fig. 4. Percentage of N excreted as ammonia by *Artibeus lituratus* in relation to N intake (y=21.12+0.53x; r_p=–0.01, P=0.94), energy intake (y=43.41–0.14x; r_p=–0.64, P=0.0001) and water gain (y=10.43+0.37x; r_p=0.53, P=0.002). Bats were fed energy-rich (20 g sucrose 100 ml^{-1} water; open symbols) or energy-poor diets (5 g sucrose 100 ml^{-1} water; closed symbols). Regression lines are shown only when there were significant correlations after multiple regression analysis followed by partial correlation estimates (r_p).

metabolic rate by increasing ammonia production when exposed to low ambient temperatures (Korine et al., 2006). When we used the same criteria as Korine et al. (Korine et al., 2006), our estimate increased slightly to 1% of basal energy requirements. Bats on the energy-poor diet not only favored the production of the cheapest N waste, but they also appeared to increase the digestion of dietary N based on the almost null production of feces. Given that bats on this diet had very negative N balances, they probably metabolized dietary and body protein to supplement their energy requirements. Bats on the energy-poor diet ingested 0.6±0.4 kJ day^{-1} of energy derived from casein. Assuming that bats digest most of the casein ingested, the amount of energy provided by casein and the energy saved by favoring ammonia over urea production amounts to 0.85 kJ day^{-1} or ~3% of daily basal energy requirements. The fact that bats on the energy-poor diet had very negative N balance values indicates that body protein was catabolized and used to supplement energy requirements. Furthermore, similar to nectarivorous phyllostomids (Kelm and von Helversen, 2007), bats on the energy-poor diet might have reduced energy expenses entering torpor as a response to limited energy ingestion. The combination of the increased excretion of the cheapest nitrogenous compound, the use of energy from protein catabolism and the potential use of torpor to reduce metabolic rate would compensate for the constraint in energy intake for bats on the energy-poor diet and explain the lack of differences in body mass between bats on the two experimental diets. In addition to the ammonia formed in the liver through amino acid metabolism (Wright, 1995), the kidney produces ammonia to regulate the acid–base balance (Schmidt-Nielsen, 1995). Excretion of renal ammonia increases during acidosis produced by the metabolism of dietary and body protein (Weiner and Verlander, 2011). Because protein metabolism seemed to increase in fruit bats fed the energy-poor diet to compensate for compromised energy balance, increased excretion of urine ammonia in these bats might be a consequence of an increase production of renal ammonia to neutralize metabolic acidosis. Although our study showed that urine ammonia increased at limited energy intake, it is uncertain to what extent renal and liver ammonia contributed to total ammonia excretion.

Concluding remarks

Similarly to birds and bats in which energy expenses were experimentally increased (van Tets et al., 2001; Korine et al., 2006), *A. lituratus* increased the excretion of ammonia when their energy balance was compromised by feeding on energy-dilute diets. Our experiment placed bats in an extreme situation that is probably not confronted very often in the wild. Fruits eaten by *Artibeus* bats are characteristically rich in water with varying amounts of energy. Diet of *Artibeus* bats is largely composed of fruits of several species of figs [*Ficus* spp. (Weldeln et al., 2000)], and their ingestion does not constrain energy balance because they contain energy densities that are similar to those of our energy-rich diet [3.1 kJ g^{-1} wet mass (Weldeln et al., 2000)]. However, low-energy-content fruits are also reported in the diet of *Artibeus* bats. For example, *Carica papaya* contains only 1.7 kJ g^{-1} wet mass (USDA Agricultural Research Service, 2010), an amount that doubles the energy density of our energy-poor diet. A diet based on fruits of *C. papaya* might compromise the energy balance of *Artibeus* bats because energy ingestion is constrained in bats when they feed on nectar with an equivalent amount of energy density [292 mmol l^{-1} (Ayala-Berdon et al., 2008)]. Consumption of energy-dilute fruits in the wild by fruit bats might affect the way in which N wastes are excreted, favoring the production of ammonia when it is accompanied by increased protein catabolism and the ingestion of large volumes of water. Our findings do not necessarily exclude the role of low N intake in ammonotely as suggested in other studies (Roxburgh and

Pinshow, 2002; Herrera M. et al., 2006; Tsahar et al., 2005), but support the view that increased ammonia excretion contributes to the energy budget of the animal, and can also serve to maintain acid–base balance when protein catabolism increases as a result of energy-poor diets.

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