

Changes in the Composition of the Urine of Yellow-Vented Bulbuls (*Pycnonotus xanthopygos*): The Effects of Ambient Temperature, Nitrogen, and Water Intake

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ABSTRACT

Uricotely (uric acid >50% of urinary nitrogen) in birds was once considered ubiquitous. However, Anna's hummingbirds (*Calypte anna*) have been shown to be an exception to this rule; under conditions of low ambient temperature (T_a) and on a nitrogen-free diet, they increased their water intake and often became ammonotelic (ammonia >50% of urinary nitrogen). Our aim was to identify the effects of nitrogen intake, water intake, and T_a on the ammonia excretion of yellow-vented bulbuls (*Pycnonotus xanthopygos*). We chose this predominantly frugivorous species because many of the characteristics of nectarivores that were used to explain increased ammonia excretion by *C. anna* are also characteristics of frugivorous birds. We assayed ureteral urine composition in eight yellow-vented bulbuls (*P. xanthopygos*), each randomly allocated a diet of 20% (0.6 M) sucrose solution supplemented by either 1.03 g/L or 7.23 g/L soy protein and held at a T_a of either 28°C or 10°C. Food, and therefore water, intake rates varied with nitrogen intake but not with T_a . Food intake increased significantly with decreased nitrogen intake, while concentrations of all the excretory compounds in the urine ($P < 0.05$) decreased; yet their proportions in the urine did not change significantly. The lower T_a had no significant effect on food intake or on the concentration of uric acid. However, at 10°C, the ammonia and urea concentrations increased ($P < 0.05$), and this led to a significant

increase in the proportion of ammonia in the urine. Our results demonstrate that, when bulbuls are exposed to low T_a , they are able to save energy by increasing the proportion of the ammonia in their urine.

Introduction

There are three major nitrogenous compounds excreted by vertebrates: ammonia, urea, and uric acid. The form in which most of the nitrogen is excreted varies between animals and is closely related to both phylogenetic origins and environmental factors. Aquatic vertebrates, with the exception of elasmobranchs (Schmidt-Nielsen 1972; Part et al. 1998), tend to be ammonotelic. That is, most of the excreted nitrogen is in the form of ammonia. Their terrestrial counterparts are usually either ureotelic or uricotelic (see reviews by Schmidt-Nielsen and Mackay 1972; Wright 1995).

One environmental link between aquatic and terrestrial environments is often explained as a trade-off between the relative advantages and disadvantages embodied in the use of the different compounds as end products of protein catabolism. Aquatic organisms can excrete ammonia rapidly and efficiently via passive diffusion due to ammonia's high solubility and its high diffusion coefficient. However, large-scale ammonia excretion is usually impractical for terrestrial vertebrates due to the large volume of water needed to dilute ammonia to subtoxic concentrations (Wright 1995).

Urea and uric acid are much less toxic than ammonia and require less water for excretion: 10 times less in the case of urea and 50 times less in the case of uric acid (Wright 1995). However, the synthesis of 1 mol of urea via the uric cycle consumes 2 mol of adenosine triphosphate, and the synthesis of uric acid is even more energetically expensive (Withers 1992).

Birds were once thought to be almost invariably uricotelic. However, it was recently demonstrated that, under conditions of low ambient temperature (T_a) and while eating a nitrogen-free diet, Anna's hummingbirds (*Calypte anna*) increased their water intake and often became ammonotelic (Prest and Beuchat 1997). This phenomenon was attributed to the combination of high water turnover and high energy demands faced by a small hummingbird in a cold environment (Prest and Beuchat 1997). According to Prest and Beuchat (1997), the

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most likely explanation of their observations was that the high water-turnover rate both diluted the soluble, but toxic, ammonia and enabled its rapid and efficient excretion, while the energy demands favored the production of energetically cheaper compounds than uric acid. Unfortunately, their ability to draw firm conclusions was limited by two important factors: first, nitrogen intake, water intake, and T_a were all altered simultaneously in the treatment in which ammonotelism was observed, and because each could potentially have affected the bird's nitrogenous waste excretion, it is impossible to distinguish between their separate effects; and second, the data were based on analysis of excreted cloacal fluid rather than on analysis of ureteral urine.

Avian urine passes from the ureter into the cloaca before excretion. In the cloaca, it is mixed with fecal material and, in some species, is moved by retrograde peristalsis into the lower intestinal tract and ceca where urinary uric acid and urea may then be broken down and converted into ammonia (Mortensen and Tindall 1981; Braun and Campbell 1989; Karasawa 1989; Karasawa and Maeda 1995). Some of this ammonia may be reabsorbed by the bird (Mortensen and Tindall 1981; Karasawa 1984, 1989). However, any that is not absorbed will be excreted with the rest of the cloacal contents. Anna's hummingbirds do not have ceca, but uric acid has been found in their lower intestines (Beuchat et al. 1990), and their intestinal flora include bacteria that are capable of breaking down uric acid, urea, and potassium urate (M. R. Preest, D. G. Folk, and C. A. Beuchat, unpublished data). Therefore, excreted cloacal fluid cannot be used to measure changes accurately in urinary nitrogen composition in birds, and the cloacal fluid of Anna's hummingbirds may contain considerably more ammonia than the ureteral urine.

We address two questions that arise from Preest and Beuchat's (1997) study on hummingbirds. First, does the proportion of ammonia in the urine of birds other than hummingbirds change in response to environmental changes? Second, if so, which of nitrogen intake, water intake, and T_a is responsible for the change in the proportion of ammonia?

We chose a frugivorous passerine, the yellow-vented bulbul *Pycnonotus xanthopygos*, as a model for this study. Like nectarivores, frugivores typically have a diet that is high in water and energy but low in nitrogen (Izhaki 1992; Martínez del Río 1994). Therefore, if increased ammonia excretion is primarily related to environmental factors and is not a phylogenetic trait of hummingbirds alone, then it should occur in these birds.

Material and Methods

The eight bulbuls used in this study were kept in 40 × 60 × 65-cm cages and were fed 90 g of a banana-mash diet per day (Denslow et al. 1987). Water was available ad lib. Ambient temperature in the holding facility was 25°C, and the light cycle was 12L : 12D. Feeding trials were run at two temperatures:

$T_a = 28^\circ\text{C}$ and $T_a = 10^\circ\text{C}$. The birds were weighed, and then they and their cages were transferred to a controlled-environment room set at 28°C, 12L : 12D. Before the start of the trials, the birds were allowed 7 d to acclimate themselves to the experimental conditions. During this period, the birds were weighed every 2 d and were fed a high-nitrogen diet of 20% (0.6 M) sucrose solution supplemented by 7.23 g/L soy protein. Following acclimation, the birds were again weighed, and half of them were randomly allocated to a low-nitrogen diet containing only 1.03 g/L soy protein. The remaining birds continued to be fed the original diet. After a further 4 d, ureteral urine samples were taken from the birds using a handmade plastic catheter following the technique described by Thomas et al. (1984), as modified for small birds by Roxburgh (2000). The diets were subsequently switched so that the birds first fed the higher-protein diet were now on the lower one and vice versa. After a further 4 d, ureteral urine samples were again taken from all the birds, and the birds were weighed and returned to the holding cages at 25°C. The birds were maintained in the normal manner in the holding cages for 2 wk before repeating the whole procedure at 10°C. Ureteral urine collected during the trials was immediately frozen and was spectrophotometrically assayed for ammonia, urea, and uric acid (Sigma kits 685 and 640A) within 6 h of collection.

In light of our small sample sizes, we chose to use a bootstrap randomization method to avoid the limiting assumptions of parametric statistics. Based on resampling programs (Simon 1995) for bootstrap randomization techniques (Manly 1997), we wrote a subroutine analogous to a standard two-factor ANOVA. We used this method for all our comparisons, and it proved more robust than a standard two-factor ANOVA or its nonparametric equivalents. Briefly, data from all four treatments were pooled for a given set of conditions, and then randomly dealt into new "treatments." Equivalents of the F values for the two factors (nitrogen intake and T_a) and for the interaction of the two factors were calculated. The procedure was repeated 10,000 times. Finally, the probability of each of the randomly generated F values being equal to or larger than the measured values was calculated. Where that probability was less than 5%, the relevant factor was considered to have a significant effect.

Results

The mean body mass of the bulbuls did not change significantly during the feeding trials (at the start of the first trial, the mean body mass of the eight bulbuls was 29.2 ± 7.7 g [mean \pm SD], while at the end of the last trial, it was 28.7 ± 7.3 g), and, in general, the results for the bulbuls were similar to those for Anna's hummingbirds (Fig. 1 in Preest and Beuchat 1997), except that their food, and therefore water, intake rates varied with nitrogen intake and not with T_a (Fig. 1A). However, temperature and nitrogen intake had very different effects.

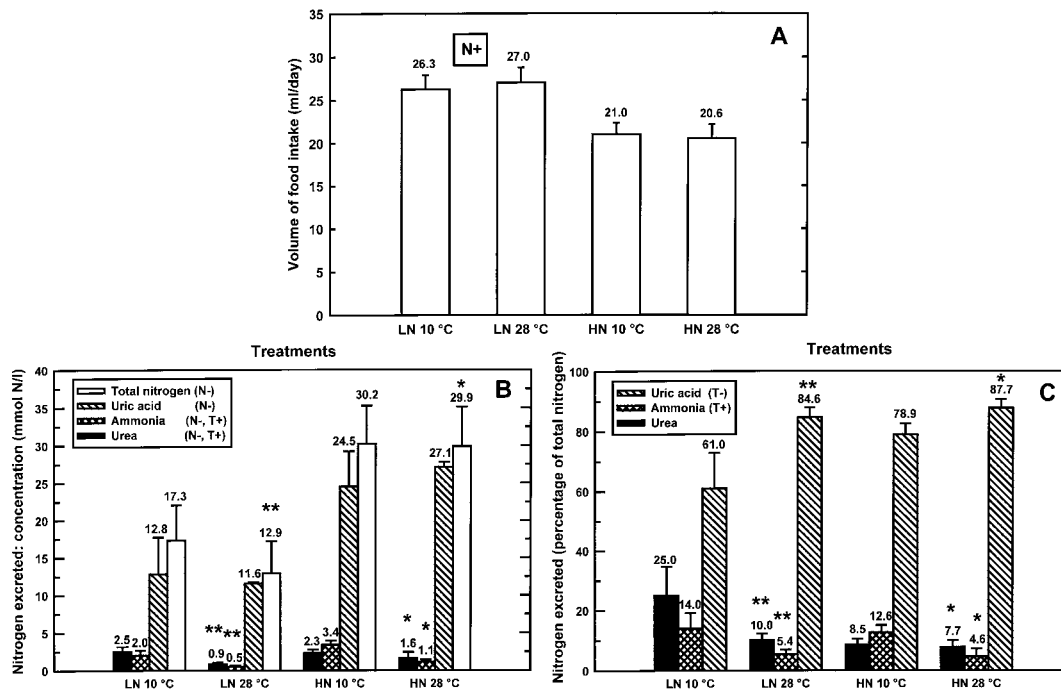


Figure 1. The effect of dietary nitrogen content and ambient temperature (T_a) on (A) food intake; (B) the concentration of uric acid, ammonia, and urea in ureteral urine; and (C) their proportions concentrations in yellow-vented bulbuls. Columns represent means (\pm SE) and have the value of the mean written above them. The sample size is eight unless otherwise indicated above the column (one asterisk indicates $n = 6$; two asterisks indicate $n = 7$). HN = high-nitrogen diet, LN = low-nitrogen diet; 10°C and 28°C are T_a 's. Significant effects ($P < 0.05$) are indicated by the letters N or T. N+ represents a significant increase in response to the low-nitrogen diet; T+ indicates a significant increase in response to the lower T_a . The minus sign is used to indicate significant decreases.

Decreased nitrogen intake was followed by increased food intake ($P < 0.05$; Fig. 1A) and a corresponding decrease in the concentrations of all the excretory compounds in the ureteral urine ($P < 0.05$; Fig. 1B). However, the relative proportions of the three nitrogenous compounds in the urine did not change significantly (Fig. 1C).

Decreasing the T_a had no effect on food intake (Fig. 1A) or on the concentration of uric acid (Fig. 1B). However, it was followed by a significant increase in the concentrations of both ammonia and urea ($P < 0.05$; Fig. 1B). The concentration of ammonia increased from 1.13 mmol N L⁻¹ at $T_a = 28^\circ\text{C}$ to 3.39 at $T_a = 10^\circ\text{C}$ on the high-nitrogen diet and from 0.51 mmol N L⁻¹ at $T_a = 28^\circ\text{C}$ to 2.01 at $T_a = 10^\circ\text{C}$ on the low-nitrogen diet. The concentration of urea increased from 1.62 mmol N L⁻¹ at $T_a = 28^\circ\text{C}$ to 2.28 at $T_a = 10^\circ\text{C}$ on the high-nitrogen diet and from 0.85 mmol N L⁻¹ at $T_a = 28^\circ\text{C}$ to 2.49 at $T_a = 10^\circ\text{C}$ on the low-nitrogen diet ($P < 0.05$; Fig. 1B). These changes led to a significant increase in the proportion of ammonia and a significant decrease in the proportion of uric acid ($P < 0.05$; Fig. 1C). The mean percentage of ammonia in the urinary nitrogen was only 5% for both diets at $T_a = 28^\circ\text{C}$ but increased to 13% and 14% for the high- and low-nitrogen diets,

respectively, at $T_a = 10^\circ\text{C}$, while the mean percentage of uric acid fell from 88% and 85% at $T_a = 28^\circ\text{C}$ to 79% and 61% at $T_a = 10^\circ\text{C}$ (Fig. 1C). Urea comprised a much higher percentage (25%) of the urine on the low-nitrogen-intake, $T_a = 10^\circ\text{C}$ treatment than it did on any of the other three treatments (Fig. 1C). However, the mean percentages of urea in the other three treatments were all very similar (8%–10%; Fig. 1C), and neither nitrogen intake nor T_a had a significant effect on this component of the urine ($P > 0.05$).

Discussion

The observed increase in the proportion of ammonia in the bulbuls' urine was driven primarily by temperature. The diet-linked changes in concentration appear to be the natural consequence of low nitrogen intake and high water intake on total urinary concentration, whereas lowering the T_a led to an increase in ammonia concentration (and therefore proportion) independent of both nitrogen and water intake.

A low nitrogen intake and/or high water intake should lower the concentration of the total nitrogen in the urine, and this was, in fact, observed (Fig. 1B). However, neither was linked

to any significant changes in the proportions of the different urinary compounds (Fig. 1C). Low nitrogen intake and high water intake did not directly cause any increase in the proportion of ammonia in the ureteral urine of the bulbuls.

Nitrogen and water intake may still play an important indirect role for the bulbuls or be important in other bird species. The sucrose/soy protein solution used in our feeding trials had a very high water content, as do nectar and many of the fruits that bulbuls feed on (Izhaki 1992). This high water intake, by mitigating the problems of ammonia storage and facilitating its excretion, may be a necessary prerequisite for any temperature-linked increase in the proportion of ammonia in the bulbuls' urine.

Low nitrogen intake may not have affected the proportions of the different nitrogenous compounds in bulbuls, but it has been shown to be correlated with an increased proportion of ammonia in the excreted fluid (but not ureteral urine) of at least one other bird species: the Palestine sunbird *Nectarinia osea* (Roxburgh 2000). Sunbirds, including this species, have unusually low nitrogen requirements when compared allometrically with other bird species (Roxburgh and Pinshow 2000; van Tets and Nicolson 2000), and the observed effect of low nitrogen intake on the composition of their excrement may reflect one of the ways in which they conserve nitrogen. Hummingbirds also have unusually low nitrogen requirements, comparable to those of sunbirds (Brice and Grau 1991), and it is therefore likely that the nitrogen-free diets fed to Anna's hummingbirds in the earlier research contributed to the high proportion of ammonia observed in their excreted cloacal fluid (Preest and Beuchat 1997).

Unlike nitrogen intake, the lower temperature did have a significant effect on the bulbuls' urine composition, presumably because of a link between urine composition and energy demands. Small birds often have much higher metabolic rates at 10°C than at 28°C. For example, oxygen consumption by Arabian babblers *Turdoides squamiceps* (a 60 g, fruit- and insect-eating passerine species that co-occurs with the bulbuls used in this study) more than doubled when the T_a was reduced from 28°C to 10°C (1.9 mL O₂ g⁻¹ h⁻¹ to 4.6 mL O₂ g⁻¹ h⁻¹; Anava et al. 2000, 2001), while the measured metabolic rates of a number of smaller passerine species (10–25 g) also increased two- to threefold in response to the same change in T_a (Bartholomew et al. 1983; Weathers 1997). Any bird that must increase its metabolic rate to this extent is unlikely to expend energy converting ammonia to uric acid, especially if its water-turnover rate is high enough to alleviate the effects of ammonia accumulation (Preest and Beuchat 1997). Indeed, it is much more likely that such a bird is actually metabolizing uric acid for energetic reasons. Either of these responses to increased energy demands would explain the increase in the proportion of ammonia and the decrease in the proportion of uric acid that we observed in the ureteral urine of bulbuls (Fig. 1C). They would also explain the very similar changes found in the

composition of the cloacal fluid of Anna's hummingbirds when T_a was reduced (Preest and Beuchat 1997). This shift in urinary nitrogen composition from uric acid to ammonia may be further accentuated if the level of amino acid metabolism, and therefore ammonia production, also increases in response to colder temperatures.

In any case, the proportion of ammonia in the urine of bulbuls did increase in response to changes in an environmental variable: T_a . Bulbuls and hummingbirds are by no means the only avian species that have both high metabolic and water-turnover rates. Most nectarivorous and frugivorous species share these characteristics. Our results suggest that, when such birds are exposed to low temperatures, the proportion of ammonia in their urine will increase. High water turnover may be a necessary prerequisite for this to occur, but, in bulbuls at least, the phenomenon is driven by energy demands and not by water or nitrogen intake.

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