



Revista Chilena de Historia Natural

ISSN: 0716-078X

editorial@revchilhistnat.com

Sociedad de Biología de Chile

Chile

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Revista Chilena de Historia Natural, vol. 80, núm. 4, 2007, pp. 447-454

Sociedad de Biología de Chile

Santiago, Chile

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Osmoregulatory responses to dietary protein and water intake in the granivorous *Zonotrichia capensis* (Passerine, Emberizidae)

Respuestas osmorregulatorias a la proteína dietaria y al consumo de agua en el granívoro *Zonotrichia capensis* (Paseriformes, Emberizidae)

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ABSTRACT

We studied the osmoregulatory responses of the granivore *Zonotrichia capensis*, acclimated to high- and low-protein diets as well as two levels of water intake. We tested whether *Z. capensis* has the ability to modify the proportion of nitrogenous waste in the excreta when protein intake and water intake varies. After 21 days of acclimation, plasma osmolality was not significantly affected by dietary treatment; however, excreta osmolality was higher in the high-protein group compared to the low-protein group. Nitrogenous wastes in *Z. capensis* are excreted mainly in the form of uric acid. The proportion of kidney devoted to medullary tissue was 40 % higher in dehydrated birds than in hydrated birds. Excreta osmolality was higher in dehydrated birds, and in all cases higher than plasma concentration by more than 300 mOsm kg⁻¹. Our data do not support the hypothesis that *Z. capensis* can switch nitrogen excretion pathways. We hypothesize that the low water content of the seed-base diet, the comparatively low water intake, and the large difference between urine and plasma concentrations may minimize the retrograde flux of urine to the lower intestinal tract, thereby reducing the potential for post-renal urine modification.

Key words: granivory, kidney, nitrogenous waste, osmoregulation, passerines.

RESUMEN

Estudiamos la respuesta osmorregulatoria del granívoro *Zonotrichia capensis*, aclimatado a dietas con alta y baja proteína como también a dos niveles de ingestión de agua. Evaluamos si *Z. capensis* tiene la capacidad de modificar la proporción de los desechos nitrogenados en la excreta cuando la ingestión de proteína y agua varía. Después de 21 días de aclimatación, la osmolalidad del plasma no fue afectada significativamente por el tratamiento dietario. Sin embargo, la osmolalidad de la excreta fue mayor en el grupo alta-proteína comparado con el grupo baja-proteína. Los desechos nitrogenados en *Z. capensis* son excretados mayoritariamente en forma de ácido úrico. La proporción del riñón ocupado por tejido medular fue un 40% mayor en las aves deshidratadas que en las aves hidratadas. La osmolalidad de la excreta fue mayor en las aves hidratadas y en todos los casos excedió la concentración del plasma por más de 300 mOsm kg⁻¹. Nuestros datos no apoyan la hipótesis que *Z. capensis* puede modificar las vías de excreción de nitrógeno. Hipotetizamos que el bajo contenido de agua de la dieta basada en semillas, la ingestión de agua comparativamente baja y la gran diferencia entre las concentraciones de orina y el plasma podría minimizar el flujo retrógrado de la orina hacia la porción posterior del intestino, reduciendo el potencial de modificación postrenal de la orina.

Palabras clave: desechos nitrogenados, granivoría, osmorregulación, passeriformes, riñón.

INTRODUCTION

Many birds switch seasonally as well as during ontogeny, responding to the environmental availability of resources or nutritional requirements (Karasov 1996). Dietary switches

are often coupled with changes in the protein content of the resources (Bell 1990). Goldstein et al. (2001) and Sabat et al. (2004) documented changes in renal structure associated with experimental changes in dietary protein. It seems that the consumption by

sparrows of high protein diets increased the medullary tissue and exhibited larger medullary cones, associated with changes of urinary flux and the elimination of nitrogen wastes. Changes in dietary protein levels not only influence the kidney morphology and physiology, but also the metabolic pathways that are needed to cope with the greater elimination of nitrogen wastes (Goldstein et al. 2001).

The primary form in which most birds excrete nitrogen is uric acid (Singer 2003). Because uric acid has a low water solubility, uricotelic promotes water conservation in birds (Braun 1981). However, the carbon lost per nitrogen excreted is relatively high with uricotelic, and uricotelic has the potential to increase protein loss from the packaging of urate-containing spheres (Janes & Braun 1997). Some bird species have been shown to switch from uricotelic to ammonotelic depending on water availability, ambient temperature and diet (Preest & Beauchat 1997, Roxburgh & Pinshow 2002). For example, Van Tets et al. (2001) documented that the frugivorous passerine *Pycnonotus xanthopygos* exposed to low environmental temperature is able to save energy increasing the proportion of nitrogenous waste excreted as ammonia. To maintain higher levels of energy intake when exposed to low environmental temperature (10 °C), *P. xanthopygos* must increase the consumption of food. Because birds fed on energetically-dilute solutions (sucrose more soy protein), the observed increase in the proportion of nitrogenous waste excreted as ammonia occurred when food (and thus water) intake was high (Van Tets et al. 2001). Although the patterns of nitrogen excretion in fruit- and nectar-feeding passerines on high- versus low-protein diets have been studied, relatively little is known about how nitrogen excretion in granivorous birds is affected by rates of protein and water intake. Besides, most studies have been performed in birds offered with experimental diluted diets. We addressed the question if the proportion of ammonia in the urine of birds other than frugivorous and necativorous birds may switch in response to environmental changes. In this sense, the study of how nitrogen waste is affected by diet and water in bird species that fed on solid diets may illustrate about the mechanism involved in the

ability to modify the proportions of urinary wastes. In this study, we fed the granivorous Rufous-collared Sparrow, *Zonotrichia capensis* (Passerine, Emberizidae), both high- and low-protein diets with different levels of water availability to test if rates of protein and water intake affect both uric acid production and renal morphology.

Previously, it was shown that in *Z. capensis* the proportions of nitrogenous compounds (uric acid, urea, and ammonia) excreted in ureteral urine were not affected by diet (Sabat et al. 2004). However, in that study, the low-protein, artificial diet was probably not limiting in nitrogen (Bell 1990, Karasov 1990). In this study, we included an artificial diet composed of 4 % protein. In addition, we manipulated the water offered to birds in order to assess the possible effect of an interaction between water availability and protein intake on both nitrogen excretion and renal morphology. Because we were unable to increase the drinking rate without increasing the environmental ambient temperature (i.e., increasing the total evaporative water loss rate), and hence manipulating the energy demands for thermoregulation, we decided to manipulate the drinking regime. Therefore, rather than increasing water availability to the high water intake groups, we decreased the availability of water to our low water intake groups. In order to gauge how water availability and dietary protein affects patterns of nitrogen excretion, we measured nitrogenous compounds in voided urine (i.e., excreta). We predicted that birds fed high-protein diets with low water intake would excrete a higher proportion of nitrogenous waste as uric acid; whereas birds fed low-protein diets with ad libitum water would excrete nitrogen mainly as ammonia and urea.

MATERIAL AND METHODS

A total of 24 individuals were captured with mist nets in Quebrada de la Plata (33°31' S, 70°50' W), a rugged area located at an altitude of about 800 m in central Chile. Animals were transported to the laboratory and maintained at 22 ± 2 °C, L:D 12:12 in separate cages of 50 x 50 x 50 cm with larvae of mealworms (*Tenebrio molitor*), seeds and water ad libitum. After 24 h, animals were weighed with an

electronic balance (± 0.01 g) and randomly assigned to one of two synthetic diets: a high-protein diet (HP diet; 30 % casein, 1.8 % corn oil, 44 % starch, 24 % fructose and 0.2 % salts and vitamins) or a low-protein diet (LP diet; 4 % casein, 1.8 % corn oil, 72 % starch, 22 % fructose and 0.2 % salts and vitamins). Diets were isocaloric; energy content was determined to be roughly $4,200 \text{ cal g}^{-1}$ using a Parr 1261 calorimetric bomb. Additionally, the LP and HP groups were separated into groups with either ad libitum water (hydrated birds, HYD) or restricted water (dehydrated birds, DEH). DEH birds were offered approximately one half of the water consumed by HYD birds as determined by measuring the consumption of 12 individuals using graduated tubes containing tap water for 24 h in a separate experiment. Consumed volumes ($11 \pm 1.8 \text{ mL}$ each day) were determined and corrected for evaporation using control tubes located outside the experimental cage. Hence, we supplied 6 mL day^{-1} of water to the DEH groups.

After the 21 day acclimation period, a blood sample (ca. $50 \mu\text{L}$) from the humeral vein was collected with heparinized tubes. Blood samples were centrifuged at $9,000 \text{ rpm}$ for 7 min. We recorded hematocrit and froze the plasma at -40°C for subsequent analyses. Birds were placed in individual $25 \times 25 \times 30 \text{ cm}$ plastic cages and were continuously observed (12 h beginning at 07:00) and excreta samples were taken each time the birds voided using eppendorf micropipettes. All samples were pooled within individual birds before aliquots were taken for analysis of different compounds. An excreta sub-sample of each bird was centrifuged and the supernatant was frozen (-40°C) for osmometry analysis. Osmolality was measured by vapor pressure osmometry (Wescor 5130B). An aliquot of the excreta was diluted with LiOH and analyzed for the concentration of uric acid using the uricase/peroxidase method (Sigma Procedure no. 684). A second aliquot of excreta was taken for the determination of ammonia and urea using the urease/Berthelot method (Sigma Procedure no. 640). To ensure that urine samples were not alkaline, $100 \mu\text{L}$ of 10 % acetic acid was added to prevent ammonia volatilization. Uric acid, urea and ammonia data are reported in mg dL^{-1} . After our experimental trials, we weighed the birds,

killed them by decapitation, and then performed dissections. Kidneys were removed from the synsacrum, weighed ($\pm 0.001 \text{ g}$) and preserved in paraformaldehyde-glutaraldehyde. Medullary cones were dissected from the kidneys using a dissecting microscope and the entire collection was weighed ($\pm 0.001 \text{ g}$) and counted. We compared the physiological responses to dietary treatments using ANOVA, excepting for kidney mass and medullary cones, which were analyzed with an ANCOVA with body mass as a covariate. Because the percentage of medulla was dependent on kidney mass, we performed an ANCOVA with kidney mass as a covariate. To determine the effect of experimental conditions on body mass, we compared pre-trial body mass to that at the end of the trial. All statistical analyses were performed using Statistica for Windows (1997) and data are reported as means \pm SD.

RESULTS

The ratio between the final body mass and the initial body mass ($\text{mb}_{\text{final}}/\text{mb}_{\text{initial}}$) was significantly affected by dietary protein ($F_{1,20} = 29.109$, $P < 0.001$). There was, however, no effect of water intake ($F_{1,20} = 0.7$, $P = 0.70$) and no interaction between protein and water intake ($F_{1,20} = 1.55$, $P = 0.23$) ($F_{1,20} = 29.109$, $P < 0.001$). The $\text{mb}_{\text{final}}/\text{mb}_{\text{initial}}$ ratio in HP groups was near unity, whereas this ratio in the LP groups was 0.64 and 0.74 for LP/DEH and LP/HYD groups, respectively. Hence, compared with the initial value, body mass at the end of the acclimation period was lower in the LP groups (Table 1).

Nitrogenous waste in *Z. capensis* was primarily excreted as uric acid; however, considerable quantities of urea and ammonia were observed in all individuals. The mean concentration of nitrogen excreted as uric acid was roughly 60 % higher in DEH birds (HP/DEH = $658 \pm 343 \text{ mg dL}^{-1}$ and LP/DEH = $492 \pm 268 \text{ mg dL}^{-1}$) compared to hydrated birds (HP/HYD = $387 \pm 283 \text{ mg dL}^{-1}$ and LP/HYD = $289 \pm 65 \text{ mg dL}^{-1}$, $F_{1,20} = 4.84$, $P = 0.04$). However, there was no effect of dietary protein ($F_{1,20} = 0.10$, $P = 0.76$) and no interaction between factors ($F_{1,20} = 1.53$, $P = 0.23$). Accordingly, the concentration of ammonia nitrogen in the urine of *Z. capensis* was 67 %

higher in DEH birds (HP/DEH = 401 ± 155 mg dL⁻¹ and LP/DEH = 343 ± 153 mg dL⁻¹) compared with HYD birds (HP/HYD = 296 ± 172 mg dL⁻¹ and LP/HYD = 147 ± 79 mg dL⁻¹, $F_{1,20} = 6.58$, $P = 0.018$). There was no effect of dietary protein, and no interaction between diet and water intake ($F_{1,20} = 0.62$, $P = 0.44$ and $F_{1,20} = 2.97$, $P = 0.10$, respectively). The concentration of nitrogen excreted as urea was not affected by experimental treatment ($F_{\text{protein } 1,20} = 1.75$, $P = 0.20$, $F_{\text{water } 1,20} = 0.01$, $P = 0.92$ and $F_{\text{interaction } 1,20} = 2.25$, $P = 0.15$; 73.87 ± 52.88 mg dL⁻¹). However, the proportions of nitrogen excreted in the three forms were practically identical across all experimental groups. The main nitrogen waste excreted by *Z. capensis* was uric acid, which represented over 50 % of all components (ANOVA on arcsin square root transformed data, $F_{\text{protein } 1,20} = 2.78$, $P = 0.11$, $F_{\text{water } 1,20} = 0.003$, $P = 0.96$, $F_{\text{interaction } 1,20} = 0.93$, $P = 0.35$) followed by ammonia with 36% ($F_{\text{protein } 1,20} = 1.11$, $P = 0.30$, $F_{\text{water } 1,20} = 0.54$, $P = 0.47$, $F_{\text{interaction } 1,20} = 0.23$, $P = 0.64$) and urea with approximately 10 % ($F_{\text{protein } 1,20} = 1.47$, $P = 0.24$, $F_{\text{water } 1,20} = 1.15$, $P = 0.30$, $F_{\text{interaction } 1,20} = 0.82$, $P = 0.38$, Fig. 1). Urine osmolality was higher in DEH birds ($F_{1,20} = 1.20$, $P = 0.02$), but was not affected by protein ($F_{1,20} = 1.47$, $P = 0.24$) or by the interaction

($F_{1,20} = 1.82$, $P = 0.18$, Table 1). No treatment effect was observed on plasma osmolality ($F_{\text{protein } 1,20} = 1.29$, $P = 0.27$, $F_{\text{water } 1,20} = 0.45$, $P = 0.50$, $F_{\text{interaction } 1,20} = 2.41$, $P = 0.13$, Table 1). The hematocrit was higher in the HP than in LP acclimated groups ($F_{1,20} = 9.35$, $P = 0.006$), but was not affected by water availability ($F_{1,20} = 0.09$, $P = 0.75$) or by the interaction ($F_{1,20} = 2.56$, $P = 0.12$, Table 1).

Kidney mass was affected by the interaction of water intake and protein level ($F_{1,19} = 7.09$, $P = 0.015$), but not by each factor in isolation ($F_{\text{protein } 1,19} = 1.62$, $P = 0.22$, $F_{\text{water } 1,19} = 0.47$, $P = 0.83$). In the DEH groups, kidney mass was roughly 30% higher in HP groups compared to the LP groups after correcting for body mass. There were, however, no differences between HYD groups (Table 1). The proportion of kidney devoted to medullary tissue was ca. 40 % higher in DEH than in HYD birds ($F_{1,19} = 4.44$, $P = 0.048$), but this effect is mainly due to the significant difference in the LP group (Table 1). Medullary tissue was not affected by protein ($F_{1,19} = 0.07$, $P = 0.79$) or by the interaction between factors ($F_{1,19} = 0.32$, $P = 0.57$). The number of medullary cones remained constant among groups ($F_{\text{water } 1,20} = 3.06$, $P = 0.96$, $F_{\text{protein } 1,19} = 1.25$, $P = 0.278$, $F_{\text{interaction } 1,19} = 0.10$, $P = 0.30$).

TABLE 1

Body mass and osmoregulatory features of *Z. capensis* acclimated for 21 days to four experimental treatments (two experimental diets and two levels of water availability). Similar letters indicate no difference between groups based on analyses using Tukey tests

Masa corporal y características osmorregulatorias de *Z. capensis* aclimatados por 21 días a cuatro tratamientos experimentales (dos dietas experimentales y dos niveles de disponibilidad hídrica). Letras similares indican ausencia de diferencias significativas entre grupos basados en el análisis utilizando una prueba de Tukey

	Experimental groups			
	LP/ HYD	LP/ DEH	HP/ HYD	HP/ DEH
Body mass (g)	14.50 \pm 1.23 ^a	15.23 \pm 1.98 ^a	19.31 \pm 0.81 ^b	20.16 \pm 1.26 ^b
Kidney mass (g)	0.13 \pm 0.03 ^{a,c}	0.10 \pm 0.02 ^a	0.15 \pm 0.02 ^{b,c}	0.17 \pm 0.04 ^b
Number of medullary cones	43.0 \pm 5.3 ^a	49.6 \pm 5.1 ^a	48.2 \pm 6.0 ^a	49.8 \pm 6.7 ^a
Proportion of medulla	3.8 \pm 1.33 ^a	5.8 \pm 2.87 ^b	3.3 \pm 1.6 ^a	4.4 \pm 1.4 ^{a,b}
E _{osm} (mOsm kg ⁻¹)	771.5 \pm 241.6 ^{a,b}	875.5 \pm 454.8 ^a	662.0 \pm 176.1 ^b	995.16 \pm 256.1 ^a
P _{osm} (mOsm kg ⁻¹)	334.2 \pm 89.2 ^a	308.2 \pm 79.9 ^a	321.8 \pm 63.2 ^a	388.0 \pm 52.9 ^a
Hematocrit (%)	38.22 \pm 3.78 ^a	40.71 \pm 5.47 ^{a,c}	47.26 \pm 3.72 ^b	43.54 \pm 5.68 ^{b,c}

LP: low-protein diet; HP: high-protein diet; HYD: water ad libitum; DEH: 50 % of ad libitum water consumption. E_{osm}: excreta osmolality; P_{osm}: plasma osmolality

LP: dieta baja en proteínas; HP: dieta alta en proteínas; HYD: agua ad libitum; DEH: 50 % del consumo ad libitum; E_{osm}: osmolalidad de la excreta; P_{osm}: osmolalidad del plasma

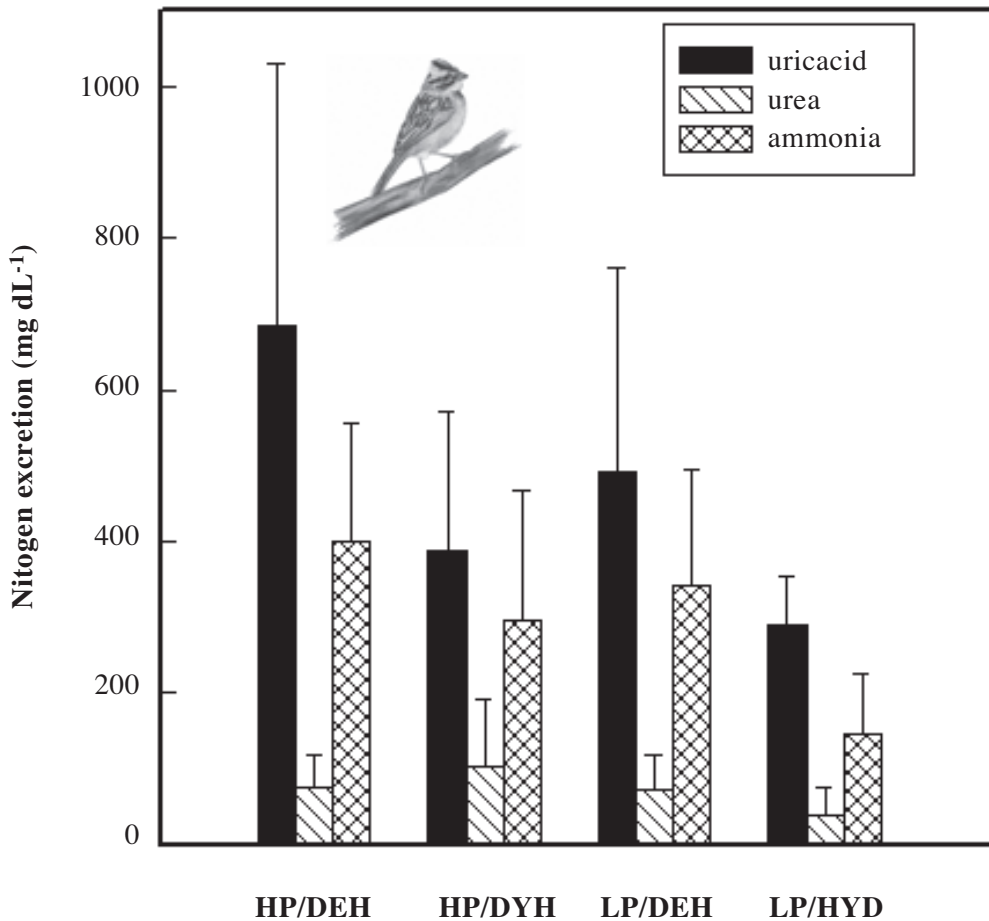


Fig 1: Changes in the concentration of nitrogenous waste forms in *Zonotrichia capensis* acclimated to high-protein (HP) and low-protein (LP) diets as well as two levels of water intake (hydrated, HYD; offered 50 % of the normal consumption, DEH) for three weeks. Both uric acid and urea concentrations are higher in DEH birds than in HYD birds. Ammonia concentration was only marginally significant between groups. Proportions of nitrogen excreted as ammonia, uric acid and urea in the urine remains unchanged among groups.

Cambios en la concentración de desechos nitrogenados en *Zonotrichia capensis* aclimatados a dietas ricas en proteína (HP) y pobres en proteína (LP) y a dos niveles de consumo de agua (hidratadas, HYD; y restringidas al 50 % del consumo normal, DEH) por tres semanas. Las concentraciones de ácido úrico y urea fueron mayores en el grupo DEH que en el grupo HYD. La concentración de amonio fue solo marginalmente significativa entre los grupos. Las proporciones de nitrógeno excretado como amonio, ácido úrico y urea se mantuvieron constante entre los grupos.

DISCUSSION

In this paper, we explored osmoregulatory responses to dietary protein and water intake in a granivorous passerine. We tested whether *Z. capensis* has the ability to switch nitrogen excretion pathways when protein intake decreases and water intake varies. However, we did not find support for this hypothesis: independent of experimental treatment, all birds were uricotelic (i.e., uric acid account for

more than the 50 % of the nitrogen excreted and the proportions of each compound remained constant among experimental treatments, Fig. 1).

Recently, some bird species have been shown to modify the proportions of nitrogenous wastes in the excreta. For example, as a response to protein and water intakes and to different levels of energy expenditure (Preest & Beuchat 1997, Van Tets et al. 2001, Roxburg & Pinshow 2002, Zanutto & Bicudo 2005, Tsahar

et al. 2005b). However, this ability is far from universal in birds (McWhorter et al. 2003, Tsahar et al. 2005a). Roxburg & Pinshow (2002) have suggested that birds can only augment the ammonia in the urine when there are drastic increases in water intake. They observed that Palestine sunbirds (*Nectarinia osea*) fed low-protein diets modified urine, which decreased the proportions of uric acid and increased the proportions of ammonia in excreta. In this way, Tsahar et al. (2005a) documented that the frugivorous, *Pycnonotus xanthopygos*, exhibits "apparent ammonotelic" when protein intake is low and water intake is high as a result of post-renal urine modification. Tsahar et al. (2005a) postulated that apparent ammonotelic probably requires the ability to modify urine in the lower digestive tract. The mechanism suggested for this modification includes bacterial breakdown of uric acid and/or direct absorption of uric acid (Karasawa et al. 1988, Tsahar et al. 2005a). It is therefore possible that the absence of nitrogen excretion pathway switching in *Z. capensis* is because the water consumed by granivorous birds does not exceed a minimum threshold that permits significant post-renal urine modification. This hypothesis is supported by our finding that water consumption by HYD birds was 20-30 % of that reported for passerines showing apparent ammonotelic (Tsahar et al. 2005a). Because we did not measure simultaneously the nitrogen compounds in the ureteral and voided urine for each experimental treatment, our experimental design precludes the evaluation of the post-renal modification of the urine.

Even though *Z. capensis* did not change the proportion of metabolites in the urine, total concentration was affected by the availability of water. DEH birds had higher uric acid and ammonia concentration than HYD birds (Fig. 1). Accordingly, both the urine osmolality and the percentage of the kidney devoted to medullary tissue were higher in DEH birds compared to HYD birds. Our results agree with those found in other studies (Johnson & Mugaas 1970, McNabb et al. 1972, Van Tets et al. 2001, Singer 2003, Tsahar et al. 2005a) and confirm the integrated response of kidney form and function to water deprivation. The increase in the proportion of medullary tissue at an intraspecific level in *Z. capensis* corroborates

the pattern documented for birds at an interspecific level (Travis 1994, Sabat & Martínez del Río 2002, Tsahar et al. 2005a).

Zonotrichia capensis responded to low-protein diets decreasing body mass, kidney mass and hematocrit (Table 1). These results suggest that a 4 % protein diet is probably nitrogen limiting in *Z. capensis*, and that the decreases in muscle and tissues masses are likely related to an increase in endogenous protein catabolism (Bosque & Pacheco 2000). Our results also indicate that *Z. capensis* acclimated to a high-and-low protein diets can maintain an adequate osmotic state even though the access to water is limited. Although plasma osmolality can be influenced by diet and water availability, and by interspecific differences in how animals respond to these factors (Goldstein & Zahedi 1990, Goldstein et al. 1990), *Z. capensis* maintained a constant plasma concentration between treatments at levels typical of other passerines (Braun 1999, Casotti & Braun 2000, Casotti 2001, Fleming & Nicolson 2003).

Our data suggest that protein intake and water intake does not lead to any modification of nitrogenous waste proportions in the excreta of Rufous-collared Sparrows. Instead, *Z. capensis* responds to water restriction by increasing both the concentration of nitrogenous waste in excreta and the quantity of renal medullary tissue without modifying the pattern of nitrogen excretion. Finally, it has been suggested that nitrogen excretion pathways can be influenced by the thermal environment due to energetic demands (Prest & Beuchat 1997, Van Test et al. 2001). Although our experimental approach did not allow us to evaluate this possibility, we suspect that water intake, water flux and urine concentration explains why facultative ammonotelic is not widespread among birds. Although a correlation between high water intake and high levels of ammonia excretion is expected because ammonia requires large amounts of water for its excretion, apparent ammonotelic in birds is usually coupled with a decrease of uric acid concentration in the excreta but without a significant increase of ammonia concentration (Tsahar et al. 2005a, Roxburg & Pinshow 2002). The mechanism underlying the positive correlation between water intake and ammonotelic remains obscure. However, we

hypothesize that the ability to modify urine in the lower intestinal tract depends on the magnitude of retrograde urine flow. As pointed out by Braun (1999), if the osmotic potential of the urine exceeds the plasma osmolality by more than 100-200 mOsm kg⁻¹, refluxing of urine into the colon is reduced (Goldstein & Braun 1988, Braun 2003). This phenomenon is probably regulated by the hydration state of birds, which controls retrograde colonic motility (Brummermann & Braun 1995). Hence, urine may undergo significant post-renal modification in birds when water flux is high and the urine osmolality is low; however, not in birds that must conserve water.

Although our experimental design precludes the evaluation of the post-renal modification of the urine in the lower intestine, we hypothesize that the low water content of granivorous diets (compared with the high content of water in fruits and nectar), the comparatively low water intake, and the large difference between urine and plasma osmolalities (in *Z. capensis* is more than 300 mOsm kg⁻¹, Table 1) prevents the retrograde flux of urine to the lower intestinal tract, which, in turn, reduces any potential for post-renal urine modification.

ACKNOWLEDGEMENTS

We thank Carlos Martínez del Río and Bradley Bakken for useful comments on an earlier version of this manuscript. We thank Annie Hartman Bakken for the bird illustration. This research was founded by FONDECYT grant 1050196 to PS and PG-21/2004 to PA.

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Associate Editor: Francisco Bozinovic

Received April 4, 2007; accepted May 16, 2007