

# Physiological and biochemical responses to dietary protein in the omnivore passerine *Zonotrichia capensis* (Emberizidae)

Pablo Sabat<sup>a,b,\*</sup>, Esteban Sepúlveda-Kattan<sup>a</sup>, Karin Maldonado<sup>a</sup>

<sup>a</sup>*Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Casilla 653, Santiago, Chile*

<sup>b</sup>*Center for Advanced Studies in Ecology and Biodiversity, Facultad de Ciencias Biológicas Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile*

---

## Abstract

We studied the physiological, biochemical and morphological responses of the omnivore sparrow *Zonotrichia capensis*, a small opportunistic passerine from Central Chile acclimated to high- and low-protein diets. After 4 weeks of acclimation to 30% (high-protein group) or 7% (low-protein group) dietary casein, we collected urine and plasma for nitrogen waste production and osmometry analysis, and measured gross renal morphology. Plasma osmolality and hematocrit were not significantly affected by dietary treatment, but urine osmolality was higher in the high-protein group than in the low-protein group. Kidney and heart masses were higher in animals acclimated to the high-protein diet. Mean total nitrogen waste was significantly higher in the high-protein group, but the proportions of nitrogen excreted as uric acid, urea and ammonia were unaffected by diet. Our data suggest that the response of *Z. capensis* to an increase in dietary protein content is through greater amounts of total nitrogen excretion and hypertrophy of kidney structures, without any modification of the proportion of excretory compounds.

*Keywords:* Ammonotely; Ammonia; Dietary protein; Osmoregulation; Urea; Uric acid; Urine; Renal function; *Zonotrichia capensis*

---

## 1. Introduction

Many animals change their diet seasonally and during ontogeny, responding to their own ontogenetic program as well as to environmental availability of resources or nutritional requirements (Karasov, 1996). Dietary switches are often coupled with changes in the chemical composition of the resources (Bell, 1990). Phenotypic plasticity, the ability to change and modify the phenotype in response to environmental cues, is crucial for the maintenance of appropriate biological performance

in changing environments (Stearns, 1989; Pigliucci, 2001). Such modifications are expressed at different levels of organization, ranging from systemic to biochemical processes (Karasov, 1996; Sabat et al., 1998; Caviedes-Vidal and Karasov, 1996; Bozinovic et al., 2003). For example it has been documented that birds modify the time of food processing, and levels of digestive enzymes and carriers when they switch from high-protein diets (e.g. animal prey) to carbohydrate-rich diets (fruit and nectar) (Afik et al., 1995; McWilliams and Karasov, 2001). Changes in dietary protein levels not only influence the bird's digestive physiology that allows maximizing energy and nitrogen intake (Karasov 1990; Sabat et al., 1998), but also

---

\*Corresponding author. Tel.: +56-2-6787232; fax: +56-2-2727363.

E-mail address: psabat@uchile.cl (P. Sabat).

exhibit a renal response, as elevated urine flow and medulla hypertrophy are associated with the greater elimination of nitrogen wastes (Goldstein et al., 2001).

The three major end products of protein metabolism are ammonia, urea and uric acid. Because of its toxicity ammonia must be excreted with larger amounts of water than urea and uric acid, but the energy needed to produce urea and uric acid is greater (Withers, 1992). In addition, the amounts of carbon loss per nitrogen associated with uricotelic are increased, as well as a potential increase in protein loss associated with the package of uric acid in urate-containing spheres (Janes and Braun, 1997). As pointed out by Janes and Braun (1997) birds use proteins to facilitate excretion of nitrogen as uric acid, preventing aggregation of uric acid containing spheres in larger masses that would block the renal tubules. Birds are believed to be mainly uricotelic, but studies on nitrogen excretion have often been done in conditions that favored uric acid excretion (e.g. salt loaded and dehydrated birds, see Preest and Beauchat (1997) and references therein). Nevertheless, it has been demonstrated that some birds are able to modify their modality of nitrogen excretion. Indeed, recently van Tets et al. (2001) documented a decrease in the concentration of excretory compounds of the frugivore passerine *Pycnonotus xanthopigos* when protein intake decreased, and a rise in the proportion of ammonia in the urine, associated with high levels of energy demand.

Few studies have explored some aspects of renal function in birds on high- vs. low-protein diets; even fewer have taken an integrative approach (Preest and Beauchat, 1997; Goldstein et al., 2001; van Tets et al., 2001). Consequently, in this study we determined the renal physiological responses of an omnivorous bird fed on high-protein and low-protein diets, and to test if protein intake, modulates the uric acid production. We hypothesized that the omnivore, *Zonotrichia capensis*, fed with high-protein diets might exhibit a higher proportion of nitrogen wastes as uric acid, higher urine concentration and a greater development of kidney tissues.

## 2. Material and methods

We used as a model of study the omnivorous rufous-collared sparrow, *Z. capensis* (Passerine, Emberizidae). *Z. capensis* is a model well suited

for the study of physiological flexibility related to dietary switches, because it has been demonstrated that the species changes its dietary habits seasonally according to the availability of food resources (insects and seeds; Novoa et al., 1996; Sabat et al., 1998). Also, these sparrows can modify their digestive physiology and morphology when their diets are switched in the field and in the laboratory (Sabat et al., 1998). A total of 12 individuals were captured with mist nets in San Carlos de Apoquindo (33°23' S, 70°31' W), a rugged area located in the Andean foothills at an altitude of approximately 1000 m in Central Chile. Animals were transported to the laboratory and maintained at  $22 \pm 2$  °C, L:D 12:12 in separate cages of 50 × 50 × 50 cm with larvae of mealworms (*Tenebrio molitor*), seeds and water ad libitum. After 24 h, animals were weighed with an electronic balance ( $\pm 0.01$  g) and randomly assigned to one of two synthetic diets: a high-protein diet (30% casein, 6% corn oil and 63% starch; 1% salts and vitamins) or a low-protein diet (7% casein, 6% corn oil and 86% starch; 1% salts and vitamins) and water ad libitum for 30 days. Diets were isocaloric; energy content was determined calorimetrically to be near 3850 cal/g using a Parr 1261 calorimetric bomb. The compositions of our artificial diets are in agreement with the protein content expected for an arthropod based-diet and a seed-based diet (Bell, 1990; Karasov, 1990). After the acclimation period, between 08:00 and 10:00 h, a blood sample of 50 to 100  $\mu$ l was collected from the humeral vein into heparinized tubes. Blood samples were centrifuged at  $9000 \times g$  for 5 min, the hematocrit was recorded and the plasma was kept frozen at  $-40$  °C. Ureteral urine was obtained by inserting a small closed-ended cannula into the birds' cloaca. Urine drained into the cannula via a window placed dorsally over the ureteral orifices; the closed end avoids contamination by intestinal fluids (Goldstein and Braun, 1989). A urine subsample of each bird was centrifuged and the supernatant was saved frozen ( $-40$  °C) for later osmometry analysis. The osmolality of the fluids was measured by vapor pressure osmometry (Wescor 5130B). An aliquot of the urine 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 urine was taken for the determination of ammonia and urea using the urease/Berthelot method (Sigma Procedure no. 640). To ensure that urine

Table 1  
Body and organ size of *Z. capensis* acclimated to high-protein and low-protein diets for 30 days

	Low-protein diet	High-protein diet
Body mass (g)	18.57 ± 1.92	19.75 ± 4.05
Liver mass (g)	0.58 ± 0.11	0.66 ± 0.16
Kidney mass (g/pair)	0.21 ± 0.04*	0.30 ± 0.03
Total medullary cones	76.8 ± 15.90	64.45 ± 38.70
Heart mass	0.23 ± 0.03*	0.28 ± 0.06

\* Denotes significant differences between groups (*t*-test) at  $P < 0.05$ .

samples were not alkaline, 100 µl of 10% acetic acid was added to prevent ammonia volatilization. Data of uric acid, urea and ammonia are reported as mg/dl for each treatment, and because uric acid, urea and ammonia have different molecular weights and number of nitrogen atoms, the mean proportion of nitrogen excreted in each form is also reported as mg nitrogen/dl. After trials, animals were weighed, killed by decapitation, dissected and the organs were removed. Kidneys were removed from the synsacrum, weighed ( $\pm 0.001$  g) and preserved in paraformaldehyde–glutaraldehyde. Medullary cones were dissected from both kidneys using a dissecting microscope and the entire collection was counted. We compared physiological and morphological response between dietary treatments using the *t*-test. All statistical comparisons were performed using Statistica for Windows (1997) and data are reported as means  $\pm$  1 S.D.

### 3. Results

Body mass was not significantly affected by diet ( $t_{10} = 0.7$ ,  $P = 0.51$ , Table 1). Kidney and heart masses were higher in animals acclimated to the high-protein diet ( $t_{10} = 4.6$ ,  $P < 0.001$ ;  $t_{10} = 2.3$ ,  $P = 0.04$ , respectively; Table 1). On the contrary, liver mass was not significantly changed by treatments ( $t_{10} = 1.1$ ,  $P = 0.32$ ), nor was the total num-

ber of medullary cones of the kidney ( $t_{10} = 0.65$ ,  $P = 0.53$ ). The number of medullary cones per gram of kidney was higher in the low-protein treatment ( $t_{10} = 2.71$ ,  $P = 0.03$ ). Plasma osmolality and hematocrit were not significantly different between groups ( $t_{10} = 1.3$ ,  $P = 0.22$ ;  $t_{10} = 1.7$ ,  $P = 0.12$ , respectively), but urine osmolality was higher in the high-protein than in the low-protein group. Accordingly, the urine to plasma ratio ( $U/P$ ) was higher in birds from the high-protein group (Table 2).

Nitrogen wastes in *Z. capensis* are excreted mainly in the form of uric acid, but considerable quantities of urea and ammonia were observed in all individuals (Fig. 1). Nitrogen waste excretion varied between groups. The concentration of uric acid and urea in the urine were greater in the high-protein than low-protein group ( $t_{10} = 2.3$ ,  $P = 0.04$ ;  $t_{10} = 3.2$ ,  $P < 0.01$ , respectively), and ammonia excretion was suggestive ( $t_{10} = 1.9$ ,  $P = 0.08$ ; Fig. 1). The mean total nitrogen waste was significantly higher in the high-protein group than in the low-protein group ( $t_{10} = 3.32$ ,  $P < 0.01$ ). However, the proportions of nitrogen excreted in the three compounds were practically identical in the low-protein and high-protein groups; uric acid was 55.7% and 54.5% ( $t_{10} = 0.23$ ,  $P = 0.81$ ) urea was 21.8% and 22.4% ( $t_{10} = 0.12$ ,  $P = 0.9$ ), while ammonia was 22.4% and 23.0% ( $t_{10} = 0.11$ ,  $P = 0.91$ ), respectively, (Fig. 1).

### 4. Discussion

Few studies have examined the effect of dietary protein on renal morphology and physiology of birds; in this study we evaluated the response of an omnivorous passerine to different levels of dietary protein through an experimental approach. The observed increase of kidney mass on high-protein diets agrees with previous reports in sparrows. Recently, Goldstein et al. (2001) documented changes in renal structure associated

Table 2  
Urine and blood composition of *Z. capensis* acclimated to high-protein and low-protein diets for 30 days

	Low-protein diet	High-protein diet
Urine osmolality (mOsm/kg)	480 ± 184*	786 ± 48
Plasma osmolality (mOsm/kg)	375 ± 10	382 ± 8
$U/P$ ratio	1.28 ± 0.32*	2.06 ± 0.14
Hematocrit (% red cell)	47.2 ± 3.70	53.17 ± 5.90

\* Denotes significant differences between groups (*t*-test) at  $P < 0.05$ .

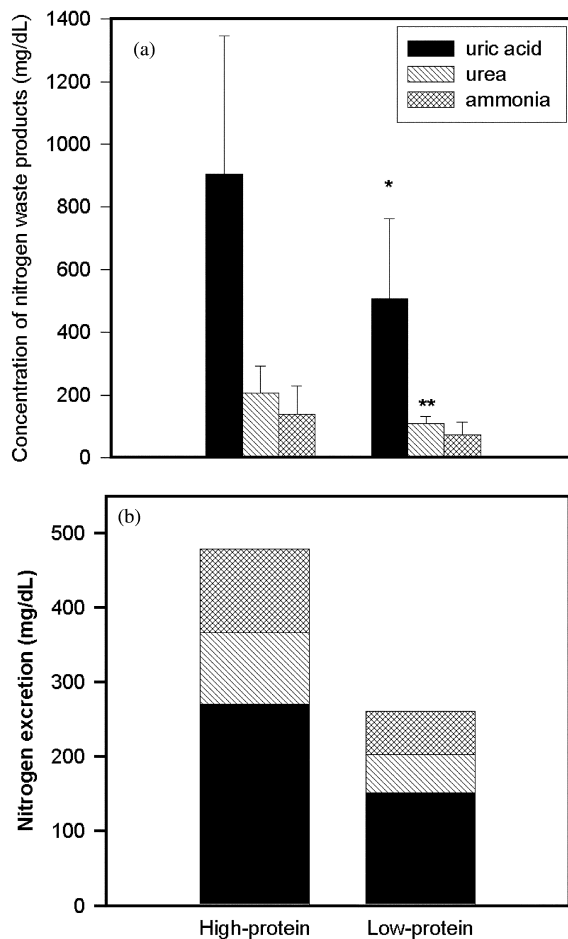


Fig. 1. Changes in concentration of urinary compounds of *Z. capensis* acclimated to high- and low-protein diets for 30 days. (a) Both uric acid and urea concentrations are higher in birds acclimated to high-protein diet than in birds acclimated to the low-protein diet. Ammonia concentration was only marginally significant between groups. (b) Concentration of nitrogen excreted as ammonia, uric acid or urea in the urine remains unchanged among groups.

with experimental changes in dietary protein. These authors found that the house sparrow (*Passer domesticus*) fed with high protein diet for 30 days increased the medullary tissue and exhibited larger medullary cones, associated with changes of urinary flux and the elimination of nitrogen wastes. It seems that the need for the elimination and concentration of large amounts of nitrogen wastes elicits this response in kidney morphology, producing a hypertrophy of medullary tissue. Our data differ from those of Goldstein et al. (2001) in that house sparrows do not change kidney size significantly, whereas *Z. capensis* did. Although we did

not quantify the amount of medullary tissue, the total number of medullary cones was unaffected by diet; and, although the medullary cones per gram of kidney increased with the high-protein diet, this was probably an effect of the observed differences in kidney size. We cannot determine whether the response of kidney to high-protein diet involves an increase of medullary or cortical tissue or both, but the hypertrophy of the whole organ is probably coupled with the high urine flow (see McNabb et al., 1972), and the need to eliminate greater amounts of nitrogen wastes.

Plasma osmolality can change due to diet, water availability and by interspecific differences in how animals respond to these factors (Goldstein and Zahedi, 1990; Goldstein et al., 1990). *Z. capensis* seems to be able to regulate plasma osmolality closely. Also, the hematocrit remained similar in both diets. Coupled with this, urine osmolality increased under the high-protein diet, and accordingly, so did the *U/P* ratio. Our results are consistent with those reported by Goldstein et al. (2001).

Nitrogen waste concentration in the urine was strongly affected by dietary protein. Total excreted nitrogen was higher in birds consuming the high protein diets, as the concentration of the combined nitrogenous waste products was almost double that of low-protein diet birds. Goldstein et al. (2001) found similar results in uric acid concentration in the urine of house sparrows, although these authors did not report results for other nitrogen wastes. Changes in total nitrogen excretion as a function of dietary protein were previously demonstrated in several birds (McNabb et al., 1972; Ward et al., 1975a,b; Singer, 2003 and references therein) and it seems reasonable that a decrease in nitrogen (protein) intake is associated with a decrease in total uric acid, urea and ammonia production.

Due to the potential cost of high protein loss through the urine associated with uricotelic, we expected a decrease in the proportion of nitrogen excreted as uric acid in birds consuming low-protein diets. Our data do not support the hypothesis that *Z. capensis* modulate the proportions of nitrogen wastes as a function of moderate changes in protein intake. We found that all birds were predominantly uricotelic in spite of the dietary acclimation. Indeed, the proportion of nitrogen excreted as uric acid, urea and ammonia did not change as a function of diet (Fig. 1). In a previous report on pigeons, McNabb et al. (1972) suggested that changes in dietary protein content do not alter

the proportion of the total excretory nitrogen that appears as ammonia. Also, McNabb and McNabb (1975) reported the same trend in *Gallus gallus* fed on high (33%) and low-protein (11%) diets. Unfortunately, in the former study the authors only determined the ammonia content in the urine, which precludes a proper evaluation of this result. Also, both studies were done apparently on non-acclimated birds, i.e. animals were fed with different diets and immediately tested for nitrogen excretion, which makes it difficult to compare with our results. In addition, our experimental diets were both probably above the minimal requirements of nitrogen for *Z. capensis* (estimated from Robins, 1981). Hence, it is possible that a more drastic decrease in protein intake would lead to the changes in the modality of nitrogen excretion observed in other species (e.g. McNabb et al., 1980).

To our knowledge, most of the recent studies on nitrogen excretion were conducted on frugivorous and nectarivorous birds using liquids diets as experimental treatments (Prest and Beauchat, 1997; van Tets et al., 2001; Roxburgh and Pinshow, 2002). Data for omnivorous and granivorous birds are scarce. Also, since our experimental approach does not assess the possible effect of water availability and reduced temperature on nitrogen excretion, further studies in granivorous and omnivorous birds are needed to determine how energetic demands and/or water availability may lead to modification in the pattern of nitrogen excretion. Our data suggest that moderate differences in protein intake do not lead to changes in the proportion of nitrogen wastes excreted in rufous-collared sparrows. Indeed, the response of *Z. capensis* to an increase in dietary protein content is through greater amounts of total nitrogen excretion and hypertrophy of kidney structures (probably medulla), without any modification of the modality of nitrogen excretion.

### Acknowledgments

We thank F. Bozinovic, L. Eaton and two anonymous referees for useful comments on an earlier version of the manuscript. This research was funded by FONDECYT grant No. 1010647 to PS.

### References

Afik, D., Caviedes-vidal, E., Martínez del río, C., Karasov, W., 1995. Dietary modulation of intestinal hydrolytic

- enzymes in Yellow-rumped warblers. *Am. J. Physiol.* 269, R413–R420.
- Bell, G.P., 1990. Birds and mammals on an insect diet: a primer on composition analysis in relation to ecological energetics. In: Morrison, M.L., Ralph, C.J., Verner, J., Jehl, J.R. (Eds.), *Avian Foraging: Theory, Methodology and Applications*. Studies in Avian Biology, Vol. 13. Cooper Ornithological Society and Allen Press, Lawrence, KS, USA, pp. 416–422.
- Bozinovic, F., Gallardo, P.A., Visser, G.H., Cortés, A., 2003. Seasonal acclimatization in water flux rate, urine osmolality and kidney water channels in free-living degus: molecular mechanisms, physiological processes and ecological implications. *J. Exp. Biol.* 206, 2959–2966.
- Caviedes-Vidal, E., Karasov, W.H., 1996. Glucose and amino acid absorption in house sparrow intestine and its dietary modulation. *Am. J. Physiol.* 271, R561–R568.
- Goldstein, D.L., Braun, E.J., 1989. Structure and concentrating ability in the avian kidney. *Am. J. Physiol.* 256, R501–R509.
- Goldstein, D.J., Zahedi, A., 1990. Variation in osmoregulatory parameters of captive and wild house sparrows, *Passer domesticus*. *Auk* 107, 533–538.
- Goldstein, D.L., Williams, J.B., Braun, E.J., 1990. Osmoregulation in the field by salt-marsh savannah sparrows *Passerculus sandwichensis beldingi*. *Physiol. Zool.* 63, 669–682.
- Goldstein, D.L., Guntle, L., Flaugh, C., 2001. Renal response to dietary protein in the house sparrow *Passer domesticus*. *Physiol. Biochem. Zool.* 74, 461–467.
- Janes, D.N., Braun, E.J., 1997. Urinary protein excretion in red jungle fowl (*Gallus gallus*). *Comp. Biochem. Physiol.* 118A, 1273–1275.
- Karasov, W.H., 1990. Digestion in birds: chemical and physiological determinants and ecological implications. In: Morrison, M.L., Ralph, C.J., Verner, J., Jehl, J.R. (Eds.), *Avian Foraging: Theory, Methodology and Applications*. Studies in Avian Biology, Vol. 13. Cooper Ornithological Society and Allen Press, Lawrence, KS, USA, pp. 391–415.
- Karasov, W.H., 1996b. Digestive plasticity in avian energetics and feeding ecology. In: Carey, C. (Ed.), *Avian Energetics and Nutritional Ecology*. Chapman and Hall, New York, pp. 61–84.
- McNabb, F.M.A., McNabb, R.A., 1975. Proportion of ammonia, urea, urate and total nitrogen in avian urine and quantitative methods for their analysis on a single urine sample. *Poult. Sci.* 54, 1498–1505.
- McNabb, F.M.A., McNabb, R.A., Ward Jr, J.M., 1972. The effects of dietary protein content on water requirements and ammonia excretion in pigeons, *Columba livia*. *Comp. Biochem. Physiol. A* 43, 181–185.
- McNabb, F.M.A., McNabb, R.A., Prather, I.D., Conner, R.N., Adkinson, C.S., 1980. Nitrogen excretion by turkey vultures. *Condor* 82, 219–223.
- McWilliams, S.R., Karasov, W.H., 2001. Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. *Comp. Biochem. Physiol. A* 128, 579–593.
- Novoa, F., Veloso, C., López-Calleja, M.V., Bozinovic, F., 1996. Seasonal changes in diet, digestive, morphology and digestive efficiency in the Rufous-collared Sparrow (*Zonotrichia capensis*) in Central Chile. *Condor* 98, 873–876.

- Pigliucci, M., 2001. Phenotypic Plasticity. Beyond Nature and Nurture. Johns Hopkins University Press, Baltimore, Maryland.
- Preest, M., Beauchat, C.A., 1997. Ammonia excretion by humming birds. *Nature* 386, 561–562.
- Robins, C., 1981. Estimation of the relative protein cost of reproduction in birds. *Condor* 83, 177–179.
- Roxburgh, L., Pinshow, B., 2002. Ammonotelicity in a passerine nectarivore: the influence of renal and post-renal modification on nitrogenous waste product excretion. *J. Exp. Biol.* 205, 1735–1745.
- Sabat, P., Novoa, F.F., Bozinovic, F., Martínez del Rio, C., 1998. Dietary flexibility and intestinal plasticity in birds: a field and laboratory study. *Physiol. Zool.* 71, 226–236.
- Singer, M.A., 2003. Do mammals, birds, reptiles and fish have similar nitrogen conserving systems? *Comp. Biochem. Physiol. B* 134, 543–558.
- Statistica, 1997. STATISTICA (Quick Reference) for the Windows 95 operating system. StatSoft, Inc., Tulsa, Oklahoma.
- Stearns, S.C., 1989. The evolutionary significance of phenotypic plasticity. *Bioscience* 39, 436–445.
- van Tets, I.G., Korine, C., Roxburgh, L., Pinshow, B., 2001. Changes in the composition of the urine of yellow-vented bulbuls (*Pycnonotus xanthopygos*): the effects of ambient temperature, nitrogen and water intake. *Physiol. Biochem. Zool.* 74, 853–857.
- Ward Jr, J.M., McNabb, R.A., McNabb, F.M.A., 1975a. The effects of changes in dietary protein and water availability on urinary nitrogen compounds in the rooster, *Gallus domesticus*-I. Urine flow and the excretion of uric acid and ammonia. *Comp. Biochem. Physiol. A* 51, 165–169.
- Ward Jr, J.M., McNabb, R.A., McNabb, F.M.A., 1975b. The effects of changes in dietary protein and water availability on urinary nitrogen compounds in the rooster, *Gallus domesticus*-II. Diurnal patterns in urine flow rates, and urinary uric acid and ammonia concentrations. *Comp. Biochem. Physiol. A* 51, 171–174.
- Withers, P.C., 1992. Comparative Animal Physiology. Saunders College Publishing, Orlando, Florida.