EFFECTS OF NUTRITIONAL AND ANTI-NUTRITIONAL PROPERTIES OF SEEDS ON THE FEEDING ECOLOGY OF SEED-EATING BIRDS OF THE MONTE DESERT, ARGENTINA

JUAN MANUEL RÍOS[1, 2, 5], ANTONIO MANGIONE[1, 2], AND LUIS MARONE[2, 3, 4]

[1]*Laboratory of Nutritional Ecology, Multidisciplinary Institute of Biological Research (IMIBIO), CCT–CONICET San Luis, Ejército de los Andes 950, San Luis, Argentina*
[2]*Desert Community Ecology Research Team (Ecodes), Argentine Institute for Arid Zones Research (IADIZA), CCT–CONICET Mendoza, Casilla de Correo 507, 5500 Mendoza, Argentina*
[3]*Instituto de Ciencias Básicas, Universidad Nacional de Cuyo, Mendoza, Argentina*
[4]*Center for Advanced Studies in Ecology and Biodiversity (CASEB), Pontificia Universidad Católica de Chile, Santiago, Chile*

Abstract. Food quality and physiological capacities and limitations in recognizing and processing food are among the factors influencing the choices of desert birds in feeding. Seed-eating birds of the central Monte desert generally select grass seeds rather than forb seeds. We studied some of the mechanisms underlying seed-selection patterns in seed-eating birds of the Monte desert, analyzing nutrients and secondary compounds of the 15 most abundant seeds in the soil and their relationship with the diet of six species of seed-eating birds. Grass seeds contained more starch and less total phenols than did forb seeds and were free from alkaloids. The diet of the most graminivorous birds was correlated with seeds' starch concentration, while generalists' diet was correlated with seed abundance. To assess the plausible mechanisms underlying birds' selection of seed, we experimented with three species differing in the breadth of their diet: a generalist, the Rufous-collared Sparrow (*Zonotrichia capensis*), and two graminivores, the Many-colored Chaco-Finch (*Saltatricula multicolor*) and Common Diuca-Finch (*Diuca diuca*). We postulated that the level of starch and the presence of phenolic compounds and alkaloids influence food preference. Results suggest that most graminivorous birds prefer high-starch diets and avoid diets with phenols and alkaloids. In contrast, the generalist foraged regardless of starch content, and its food intake was reduced only by some of the phenols and alkaloids tested. Seed chemistry may explain some important features of seed selection by birds in the Monte desert.

Key words: alkaloids, avian food choice, diet composition, granivory, phenols, seeds, starch.

Efectos de las Propiedades Nutricionales y Antinutricionales de las Semillas en la Ecología Alimentaria de Aves Granívoras del Desierto de Monte, Argentina

Resumen. La calidad del alimento como así también las capacidades y limitaciones fisiológicas de los animales para reconocer y procesar el alimento forman parte de los factores que influencian las decisiones alimentarias de las aves del desierto. Las aves granívoras del desierto del Monte central seleccionan—en conjunto—semillas de gramíneas sobre semillas de dicotiledóneas herbáceas. El objetivo de este trabajo fue estudiar algunos de los mecanismos que subyacen ese patrón de selección de semillas. Analizamos nutrientes y compuestos secundarios de las 15 especies de semillas más abundantes en el banco de semillas del suelo y su relación con la dieta a campo de seis especies de aves granívoras. Los resultados indicaron que las semillas de gramíneas contienen más almidón y menos fenoles totales que las semillas de dicotiledóneas herbáceas, y están libres de alcaloides. La dieta de campo se correlacionó con la concentración de almidón en las aves con dietas más granívoras, mientras que la dieta de la especie generalista se correlacionó con la abundancia de las semillas. Con el fin de evaluar plausibles mecanismos que subyacen a la selección de semillas, realizamos pruebas de cafetería con tres especies de aves que difieren en su amplitud de dieta: una generalista (*Zonotrichia capensis*) y dos granívoras (*Diuca diuca* y *Saltatricula multicolor*). Postulamos que (1) el nivel de almidón y (2) la presencia de compuestos fenólicos y alcaloides influencian la preferencia por el alimento. Los resultados sugieren que las aves más granívoras prefirieron dietas ricas en almidón y evitaron dietas con fenoles y alcaloides. En cambio, el generalista no se alimentó según el contenido de almidón, y redujo la ingesta de alimento sólo frente a algunos de los fenoles y alcaloides ensayados. La química de semillas podría explicar algunas características importantes de la selección de semillas por las aves del desierto del Monte.

INTRODUCTION

Animals that feed on seeds consume components of plants that are nutritionally complete because they contain proteins, lipids, carbohydrates, minerals (Díaz 1996, Banko et al. 2002), and water (Carrillo et al. 2007). Seeds also contain relatively high concentrations of secondary compounds, some of which have anti-nutritional properties and may be toxic to certain consumers (Janzen 1971, Banko et al. 2002, Karasov and Martínez del Río 2007). Therefore, it is often assumed that animals consuming substantial quantities of seeds have developed, throughout their evolutionary histories, strategies to profit from the benefits of their nutrients while avoiding the negative effect of their secondary compounds (Janzen 1971, Díaz 1996, Jaksic and Marone 2007).

Numerous factors are involved in seed selection by birds. Some studies report positive associations between the proportion of seeds eaten by birds and the seeds' content of energy (Glück 1985, Carrillo et al. 2007), lipids (Greig-Smith and Wilson 1985), protein (Valera et al. 2005), carbohydrates (Kelrick et al. 1986), and water (Carrillo et al. 2007). These correlations suggest causal relationships linked to physiological mechanisms, which have been independently corroborated by numerous studies. For example, some bird species need to store dietary lipids during periods of high energy demand such as winter and preparation for migration (Bairlein 2002), while other species, such as the White–crowned Sparrow (*Zonotrichia leucophrys*) or the Serin (*Serinus serinus*), incorporate protein to properly maintain their nutritional balance (Murphy 1993, Valera et al. 2005). Soluble sugars and starch are efficient sources of digestible energy for birds (Klasing 1998, Brzęk et al. 2010, Kohl et al. 2011). Nectar- and fruit-eating birds get energy from foods that contain primarily monosaccharides and disaccharides (Martínez del Río 1990, Schaefer et al. 2003a), but many seed-eating birds obtain immediate digestible energy from complex polysaccharides like starch (Karasov 1990). Starch is a key nutrient for the ontogenetic development and survival of the Zebra Finch (*Taeniopygia guttata*), a specialist granivore (Brzęk et al. 2010).

The effect of anti-nutritional secondary compounds in the diet can also be an important issue that influences seed selection by birds (Díaz 1996). Some such compounds diminish energy acquisition, reduce food digestibility, or can even be lethal (Karasov and Martínez del Río 2007). Optimal defense theory (Rhoades 1979) assumes that diet composition is negatively associated with secondary compounds' concentration and that the reproductive organs of plants (e.g., seeds) are highly defended (Janzen 1971, Banko et al. 2002). Phenol concentration, for instance, could affect some animals' diet selection (Guglielmo et al. 1996, Schaefer et al. 2003b, Ríos et al. 2008). Simple phenols and tannins frequently found in seeds may deter some granivorous birds (Avery and Decker 1992, Matson et al. 2004), mainly because these secondary compounds negatively affect both intake rate (Greig-Smith and Wilson 1985) and energy balance (Koenig 1991). Alkaloids, in turn, could be the most restrictive secondary compounds for some animals because they are toxic even at low concentrations (Levey and Cipollini 1998, Matson et al. 2004). The alkaloids from *Heliotropium dolosum* and *Datura ferox* seeds are toxic to quails (Eröksüz et al. 2002) and chickens (Kovatsis et al. 1993). Finally, saponins can act as anti-nutrients because of their bitter taste and may have detrimental effects on the performance of poultry (Dei et al. 2007).

Several bird families have diets based partially or entirely on seeds (Wilson et al. 1999), diets in which grass seeds usually prevail over forb seeds (Marone et al. 1998, 2008, Desmond et al. 2008). The six most abundant species of Emberizidae at the Biosphere Reserve of Ñacuñán, central Monte, Argentina, feed on a diet composed of 55% to 99% grass seeds, although only 30% of the seeds available in the soil seed bank are from grasses (Marone et al. 1998, 2004, 2008). Birds' preference for grass seeds was experimentally confirmed by Cueto et al. (2006), who, following Pulliam (1980) and Díaz (1996), suggested that grass seeds' having a lower content of secondary compounds than do forb seeds could be the cause of birds' preferences.

It is quite accepted that the production and distribution of seeds are among the most important factors influencing the diet composition and winter abundance of granivorous birds in arid environments (Pulliam and Dunning 1987, Schluter and Repasky 1991, Desmond et al. 2008). In this study, we propose that seed chemistry is also an important factor influencing seed consumption and selection by wintering birds. Our first objective was to assess whether the content of nutrients and secondary compounds in seeds can explain the patterns of seed selection reported previously for the Monte's birds. For this purpose, to identify the chemical compounds that may be affecting food selection, we analyzed the chemical properties of the 15 most abundant seeds in the soil seed bank and correlated these results with the proportion of those seeds birds' diets (Marone et al. 2008). We also used data on the abundance of various species in the soil seed bank to explore its association with the patterns of birds' seed consumption. Our second objective was to test the hypothesis that certain specific nutrients and secondary compounds, identified in the exploratory analyses, encourage or deter intake by seed-eating birds. We tested this objective through trials in which we manipulated various diets, including specific nutrients and secondary compounds.

METHODS

We studied the chemical properties of seeds of the grasses *Sporobolus cryptandrus*, *Trichloris crinita*, *Eragrostis pilosa*, *Stipa ichu*, *Setaria leucopila*, *Digitaria californica*, *Aristida mendocina*, and *Pappophorum* spp. Two species of the

genus *Pappophorum* (*P. caespitosum* and *P. philippianum*) are found at Ñacuñán. Birds probably consume more seeds of *P. caespitosum*, the more abundant species (Roig 1981), so we collected and processed a mix of *Pappophorum* seeds in which *P. caespitosum* prevailed. We also studied the chemical properties of seeds of the forbs *Chenopodium papulosum* (Chenopodiaceae), *Lappula redowskii* (Boraginaceae), *Parthenium hysterophorus* (Asteraceae), *Glandularia mendocina* (Verbenaceae), *Sphaeralcea miniata* (Malvaceae), *Phacelia artemisioides* (Hydrophyllaceae), and *Plantago patagonica* (Plantaginaceae) to determine whether some of the seeds' properties may be guiding the feeding behavior of the following seed-eating birds (Emberizidae): Ringed Warbling-Finch (*Poospiza torquata*, body weight 10.5 g)*,* Cinnamon Warbling-Finch (*P. ornata*, 12.7 g), Carbonated Sierra-Finch (*Phrygilus carbonarius*, 16.1 g), Rufous-collared Sparrow (*Zonothrichia capensis*, 19.0 g), Many-colored Chaco-Finch (*Saltatricula multicolor*, 22.4 g), and Common Diuca-Finch (*Diuca diuca*, 25.0 g).

STUDY AREA, SEED COLLECTION, AND
SEED-SAMPLE PREPARATION

During 2007 and 2008, we collected seeds by hand from various patches of vegetation located randomly within the Biosphere Reserve of Ñacuñán (34° 02′ S 67° 58′ W). The reserve has a tree stratum composed of scattered *Prosopis flexuosa* and *Geoffroea decorticans*, within a dense matrix of tall shrubs of *Larrea divaricata*, *Capparis atamisquea*, *Condalia microphylla*, and *Atriplex lampa* and low shrubs of *Lycium* spp., *Verbena aspera*, and *Acantholippia seriphiodes*. Grass cover is 25–50%. The most common grasses are C$_4$ perennials: *Pappophorum* spp., *Trichloris crinita*, *Setaria leucopila*, *Digitaria californica*, *Sporobolus cryptandrus*, *Aristida mendocina*, *Diplachne dubia*, and *Neobouteloua lophostachya*. Most forbs are annual or biennial and include *Chenopodium papulosum*, *Phacelia artemisioides*, *Descurainia* sp.*, Glandularia mendocina*, *Sphaeralcea miniata*, *Conyza* spp., *Parthenium hysterophorus*, *Lappula redowskii*, *Heliotropium mendocinum*, and *Plantago patagonica*. Mean January (summer) and July (winter) temperatures at Ñacuñan are 30 °C and 4 °C, respectively. Mean annual precipitation is 342 mm, of which ~75% falls in the warm months from October through March (21.2 ± 1.4 °C), coinciding with the growing season. Average rainfall during this season is 266 mm (*n* = 32 years). Most grass and forb seeds are dispersed and enter the soil in late summer and early autumn (Marone et al. 2008).

In the laboratory, we pooled seeds of the same species to obtain a mass sufficient for all chemical analyses. We prepared the propagules (i.e., the seeds and accompanying structures dispersing) to render them to a form similar as to when they are consumed by birds. This meant, in many cases, removing all accompanying structures from the seeds. In some species, because these structures are tightly attached to the seed,

granivores usually swallow at least part of them along with the seed. To condition the seeds, we used a hand-operated threshing machine, tweezers, and a binocular magnifying glass. We then oven-dried the seeds at 40 ºC until they reached a constant mass, then determined their water content. Once dried, seeds were ground with an electric mill into a homogeneous powder (particle size ≤ 0.5 mm, DeLonghi mill model 30, Italy) and stored in airtight bags with silica gel, which were placed in caramel-colored chambers until chemical analyses. The subsamples we used to measure total phenols and condensed tannins were not oven dried to prevent the oxidation of these chemicals (Waterman and Mole 1994).

We analyzed caryopses, i.e., seeds with no accompanying structures, of *Sporobolus cryptandrus*, *Pappophorum* spp., *Trichloris crinita*, *Eragrostis pilosa*, *Stipa ichu*, *Chenopodium papulosum*, *Lappula redowskii*, *Parthenium hysterophorus*, *Glandularia mendocina*, *Sphaeralcea miniata*, *Phacelia artemisioides*, *Plantago patagonica*), caryopses wrapped in proximal or inner glumes of *Setaria leucopila*, and caryopses with remains of inner glumes of *Digitaria californica* and *Aristida mendocina*.

In order to explore the importance of seed abundance in birds' pattern of food selection, we used data from Ñacuñán Reserve on seeds' relative abundance (mean percentage of seeds in the soil seed bank in year-round samples from 1993 to 1998) and the composition of birds' diet (mean percentage of seeds in the digestive tract in year-round samples from 1993 to 2000) (Marone et al. 2008). Briefly, seed relative abundance was analyzed by Marone et al. (2008) as follows: we took 73 replicate seed samples over three or four 2-ha plots within the reserve's open woodland. Soil cores, 3.2 cm diameter and 2 cm deep, were extracted with a cylindrical sampler, air-dried in the laboratory, and sifted through a sieve of 0.27 mm mesh. We recorded and identified the seeds under a stereoscopic microscope. We obtained seeds from birds' digestive tracts by flushing, which allows the effective collection of different food items (Lopez de Casenave 2001, Marone et al. 2008).

CHEMICAL ANALYSES OF SEEDS

For all chemical analyses we used at least 1 g of prepared sample mass for each seed species, with the exception of total phenolic compounds and condensed tannins, which we determined by using only 100 mg of sample mass. We estimated crude protein by determining nitrogen with the Kjeldahl method and multiplying the result by 6.25 (Valera et. al. 2005). We determined total lipid content by the Soxhlet procedure, using a Soxhlet Fat Extractor with ethyl ether as the solvent (Earle and Jones 1962). We analyzed soluble sugars such as monosaccharides, disaccharides, and oligosaccharides with the phenol–sulphuric acid method (Dubois et al. 1956) and starch content with the hot iodine–NaOH procedure (Mohammadkhani et al. 1999). Total phenols and condensed tannin contents were analyzed in 85% and 50% methanol:water

extracts, respectively. Total phenols were assessed with the Folin–Ciocalteu method (Waterman and Mole 1994), and condensed tannins with the proanthocyanidin method (Watterson and Butler 1983). Duplicate analyses were done for water, protein, and lipid contents, and triplicate analyses were performed for the photometric analyses of soluble sugars, starch, total phenols, and condensed tannins. We estimated the gross energy content of seeds from their nutrient content by using the following values: protein (23.4 kJ g^{-1}), lipid (39.3 kJ g^{-1}) and total carbohydrates (soluble sugars plus starch = 17.1 kJ g^{-1}) (Suttie 1977, Klasing 1998). Saponins were determined by foam index (WHO/PHARM/92559, 1992). This procedure estimates the saponin content in a sample by taking into account the height of the foam formed after an assay tube containing a decoction of seed is shaken. To detect alkaloid presence in the seeds we used a conventional qualitative method (Harborne 1998) by thin-layer chromatography and Dragendorff spray reagent. When a seed sample was positive for alkaloids, an intense orange spot (Vitale et al. 1995) was formed over the chromatography plate (Merck, silica gel 60 F$_{254}$ UV indicator HX887500, Germany) after being sprayed with Dragendorff reagent (Merck, lot 2934, OC548958, Germany).

BIRD CAPTURE AND MAINTENANCE IN THE LABORATORY

In the autumn of 2009, we captured adult Rufous-collared Sparrows ($n = 7$), Common Diuca-Finches ($n = 7$), and Many-colored Chaco-Finches ($n = 7$) with mist nets in an open woodland of *Prosopis flexuosa* in Ñacuñán Reserve. Birds were transported to the laboratory and housed in single cages ($40 \times 40 \times 50$ cm) under a constant temperature of 20 °C and a light regime of 12 hr light, 12 hr dark. During the period of acclimation to laboratory conditions (one month), the birds were fed on commercial fox-tail millet seeds (*Setaria italica*) and given water ad libitum supplemented with vitamins and minerals once a week (Vigorex labyes #80313). After the experiments, we released all birds in the same area where we had caught them.

FEEDING TRIALS IN THE LABORATORY

Experiment 1 assessed the role of nutrients in the food preference of the Rufous-collared Sparrow, Common Diuca-Finch, and Many-colored Chaco-Finch; experiment 2 assessed these species' feeding response to secondary compounds. In both experiments, we used two-choice trials in which birds could choose between a control diet and a treatment diet. Both diets were provided ad libitum. Feeders were located randomly in the cages to prevent association of feeder site with type of treatment. The measured response variable was the amount of food consumed from both diets per individual. In both experiments, the control diet consisted of dry fox-tail millet seeds ground into powder. We analyzed the nutritional and secondary-compound content of fox-tail millet by the procedures described above.

Experiment 1: *Starch trials.* We used powdered fox-tail millet seeds (46% of starch, Table 1) as a control or high-starch diet. The treatment or low-starch diet was also based on powdered fox-tail millet seeds but with starch diluted to half of its original concentration (23%) by addition of a known amount of the diluent mixture (non-nutritive bulk + soluble sugars + protein + lipids, each in appropriate proportions; see Table 1) to the powdered dry fox-tail millet seeds, so that the low-starch diet contained the same amount of all nutrients except starch (reduced to one half) as the high-starch diet. Even though these diets were not isocaloric, their formulation was the only one—among other synthetic diets previously tested—that allowed all birds of all species to feed. All other synthetic formulations offered to the birds were rejected, especially by the Many-colored Chaco-Finch. After a 3-hr fast, birds were exposed to the experimental arena, choosing between a control diet and a treatment diet during a 2-hr trial.

Experiment 2: *Response to secondary compounds.* To test the responses of feeding birds to secondary compounds we used treatment diets with two kinds of alkaloids and two kinds of phenolic compounds. To prepare each treatment diet we weighed known amounts of the secondary compounds and diluted them in absolute ethanol (EtOH) to facilitate the

TABLE 1.   Procedure to dilute a diet high in starch (powdered fox-tail millet seeds) to obtain a low-starch diet with equal amounts of other nutrients.

	Lipids (g)	Protein (g)	Soluble sugars (g)	Starch (g)	Non-nutritive bulk (g)	Total (g)
High-starch diet						
Powdered fox-tail millet seeds (100 g)	3.16	10.50	13.93	46.53	25.88	100.00
Diluent mix (100g)	3.16[a]	10.50[b]	13.93[c]	0.00	72.41[d]	100.00
High-starch diet (100 g) + diluent mix (100 g)	6.32	21.00	27.86	46.53	98.29	200.00
Low-starch diet (100 g)	3.16	10.50	13.93	23.27[e]	49.15	100.00

[a] Corn oil.
[b] Casein (Sigma Chemical Co., #037K0202, New Zealand).
[c] Glucose (Cicarelli, #37684, Argentina).
[d] Commercial ash (Calcimer, #3769, Argentina).
[e] Starch dilution 1:2.

proper mixture with the dry powdered fox-tail millet seeds used as the base food of both the control and treatment diets. The EtOH was allowed to evaporate under a bell jar for 36 hr at room temperature with no exposure to light. In this experiment, the control diet was likewise treated with EtOH but without the addition of a secondary compound. The control diet and each treatment diet were stored in airtight bags and kept at –25 °C until used. We assessed the feeding responses of the same species used in experiment 1 by allowing them to choose between a control diet and a treatment diet. We tested two phenolic compounds, the hydrolyzable tannin tannic acid (Sigma Chemical Co., St. Louis, MO), and the condensed tannin quebracho (Tannin Corporation, Peabody, MA), as well as two alkaloids, caffeine (Parafarma #9934700268/008, China) and scopolamine (Sigma Chemical Co., #97H0384, USA). We chose these compounds because (1) tannic acid, condensed tannin, and caffeine were previously used in bitterness-detection tests of granivorous birds and other animals and showed deterrent effects (Nelson and Sanregret 1997, Matson et al. 2004, Avery et al. 2005), (2) caffeine and scopolamine are commonly found in wild seeds (Vitale et al. 1995, Silvarolla et al. 2000), and (3) all of them are commercially available.

In order to use ecologically realistic concentrations of secondary compounds in feeding trials, we assessed the two phenolic compounds at 1% (% mass) and the two alkaloids at 0.05% (% mass). We chose a tannic acid concentration of 1% because this is the total phenol concentration in *Trichloris crinita* seeds, the lowest concentration among all seeds tested in the central Monte (see Results, Table 2). We assessed condensed tannin at the same concentration for purposes of comparison. In the case of alkaloids, we chose 0.05% of scopolamine, which is the lowest concentration measured in seeds (Vitale et al. 1995). We assessed caffeine at the same concentration for comparison.

In the experiments, after the birds had fasted 3 hr, we simultaneously supplied them with a control diet and a treatment diet (1% tannic acid) for 6 hr. After a nonexperimental period of 3 days, during which birds remained under usual maintenance conditions, we repeated the procedure with diet containing 1% condensed tannin. After another nonexperimental period of 3 days and fast of 3 hr, birds were offered a choice between a control diet and a diet with 0.05% caffeine for 2 hr. Finally, we repeated the same procedure to assess the birds' response to a diet containing 0.05% scopolamine. In agreement with the protocols in the research permits, we

TABLE 2. Chemistry of 15 species of seeds from the Monte desert and of commercial fox-tail millet seed used in experiments.

Seeds	Water (%)	Protein (%)	Lipids (%)	Soluble sugars (%)	Starch (%)	Energy[a] (kJ g^{-1})	Total phenols (%)	Condensed tannins (%)	Saponins[b] (FI)	Alkaloids (+/−)	Availability[c] (%)
Grasses											
Sporobolus cryptandrus	5.55	19.75	0.81	11.17	46.83	14.90	1.64	0	NF	−	15.1
Pappophorum spp.	7.67	26.70	4.98	43.71	48.70	24.07	2.90	0	<100	−	6.0
Setaria leucopila	7.43	12.70	2.13	12.23	8.70	7.41	2.78	0	<100	−	1.4
Trichloris crinita	11.01	24.71	3.43	17.50	51.33	18.95	1.02	0.69	NF	−	1.6
Digitaria californica	6.24	19.76	6.22	31.89	33.40	18.28	2.15	0	<100	−	3.1
Aristida mendocina	6.87	10.05	2.04	20.36	43.65	14.14	2.38	0	<100	−	0.2
Eragrostis pilosa	10	16.83	0.35	21.79	49.86	16.37	2.15	0	NF	−	0.0
Stipa ichu	9.16	22.51	2.85	10.52	48.50	16.52	1.84	0.08	<100	−	0.0
Fox-tail millet[d]	7.07	10.5	3.16	13.93	46.53	14.02	1.57	0	<100	−	
Forbs											
Chenopodium papulosum	6.94	14.16	4.71	11.50	30.26	12.34	3.26	0	<100	+	58.7
Glandularia mendocina	14.24	16.25	10.35	43.73	0.86	15.53	3.54	0.07	NF	−	1.9
Lappula redowskii	6.71	15.40	19.59	12.65	0.36	13.49	4.98	0.06	<100	−	0.3
Parthenium hysterophorus	4.93	23.85	28.92	35.40	1.06	23.22	2.91	0	<100	+	0.3
Sphaeralcea miniata	4.16	23.07	13.69	45.59	0.93	18.77	1.26	0.50	<100	+	1.5
Phacelia artemisioides	6.34	15.93	2.53	9.86	1.76	6.72	2.90	0.99	NF	−	3.8
Plantago patagonica	6.38	18.12	5.11	46.53	2.90	14.74	2.36	0.03	<100	−	0.2

[a]Energy content derived as 23.44 kJ g^{-1} protein, 39.34 kJ g^{-1} lipids, and 17.16 kJ g^{-1} total carbohydrates (i.e., soluble sugars plus starch) (Suttie 1977).
[b]FI (foam index) 1000/a, a = mL of filtrate in the tube that reached 1 cm of foam. Foam index <100 is when no tube exhibited 1 cm of foam. NF when no tube exhibited foam.
[c]Data on availability of seeds in the Monte desert from Marone et al. (2008) and expressed as percentage of relative abundance in soil seed bank.
[d]Commercial seed of *Setaria italica*. Values in the table not always add up or exceed 100%, in part because the techniques involved in determinations of nutrients and anti-nutrients give slight differences and because of averaging of results of triplicates or duplicates.

limited the test with alkaloids to 2 hr because alkaloids can be highly toxic to animals even at low concentrations (Matson et al. 2004), and the seed alkaloid scopolamine is highly toxic or lethal to other granivorous birds (Kovatsis et al. 1993, and references therein).

STATISTICAL ANALYSES

To emphasize differences and similarities among seed species and to detect the main variables involved, we carried out a principal components analysis of the seeds' chemical characteristics (water, protein, lipids, soluble sugars, starch, gross energy, total phenols, and condensed tannins; Schaefer et al. 2003b). The values reported for seed chemistry are an average of triplicates for the photometric analyses of phenols, condensed tannins, soluble sugars, and starch, and of duplicates for analyses of water, protein, and lipids. We used Pearson's correlation to explore relationships between seed chemistry, seed abundance in soil, and percentages of seed in a bird's diet. Each correlation was adjusted by Bonferroni correction. Variables were arcsin transformed (Zar 1996) because raw data did not fit a normal distribution (Shapiro–Wilks W test, $P < 0.05$). Normality was assessed after transformation. Transformed results of feeding trials (experiments 1 and 2) did not fit normality, so we used Wilcoxon´s matched-pairs test to compare the birds' intake of control and treatment food. The values reported in feeding trials represent averages ± SE. We used Statistica version 6.0 (StatSoft 2001) for all analyses. We considered a value of $P < 0.05$ significant, except for Pearson's correlations, which were adjusted by Bonferroni and alpha error was divided by the number of comparisons, in our case, six species (i.e., 0.05/6). So, we considered all correlations significant at $P < 0.0083$.

RESULTS

CHEMICAL CHARACTERISTICS AND ABUNDANCE OF SEEDS: THEIR RELATIONSHIP WITH PROPORTION OF SEEDS IN THE DIET

Data on seeds' chemical characteristics are shown in Table 2. The principal components analysis identified three components that accounted for 76% of the total variance (Table 3). The first component accounted for 32% of the variation and was positively associated with protein, lipids, soluble sugars, and energy (Table 3, Fig. 1). The second component accounted for 27% of the variance and showed a high positive loading for starch content and a high negative loading for content of total phenols (Table 3, Fig. 1). Seeds with the greatest amount of condensed tannins scored high on the third component, which represented 18% of total variance. We found three contrasting chemical characteristics between grass and forb seeds: grass seeds contained more starch (Mann–Whitney U-test: $U = 1.00$, $P = 0.001$) and less total phenols than forb seeds (Mann–Whitney U-test: $U = 10.50$, $P = 0.04$), and all

TABLE 3. Principal components analysis based on chemical characteristics of 15 seed species from central Monte desert, Argentina. Loadings for the most heavily weighted variables are shown with an asterisk. Loading factors >0.45 provide information regarding important chemical variables of the seeds in this study and do not overestimates weak patterns (Hair et al. 2010).

Seed chemistry	PC 1	PC 2	PC 3
Water	0.17	0.17	−0.11
Protein	*0.46	0.31	0.19
Lipids	*0.46	−0.40	−0.01
Soluble sugars	*0.49	0.01	−0.24
Starch	−0.24	*0.58	−0.21
Energy	*0.48	0.32	−0.29
Total phenols	−0.10	−0.53	−0.40
Condensed tannins	0.08	−0.05	*0.78
Eigenvalues	2.53	2.13	1.44
Variance (%)	32	27	18
Cumulative variance	32	58	76

grass seeds were free from alkaloids whereas three of the forb seeds studied had alkaloids (Table 2).

Starch content was correlated significantly with the diet of the Ringed Warbling-Finch, Carbonated Sierra-Finch, and Many-colored Chaco-Finch but showed no relation with the diet of the Cinnamon Warbling-Finch, Common Diuca-Finch, or Rufous-collared Sparrow (Fig. 2). The contents of lipids, protein, water, energy, soluble sugars, total phenols, condensed tannins, or saponins were not correlated with the proportion of seeds in the diet of any species studied. The proportion of seeds in the diet of the Rufous-collared Sparrow was correlated with seed abundance in the soil seed bank
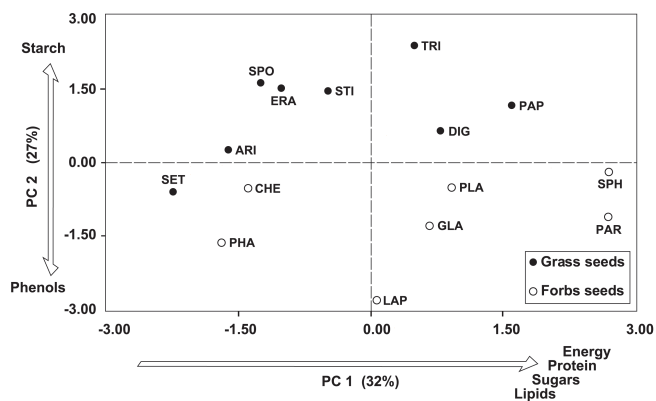

FIGURE 1. Position of seed species from central Monte desert in the plane defined by the first two axes obtained from a principal components analysis carried out with seed chemistry. Abbreviations: SPO (*Sporobolus cryptandrus*), PAP (*Pappophorum spp.*), SET (*Setaria leucopila*), TRI (*Trichloris crinita*), DIG (*Digitaria californica*), ARI (*Aristida mendocina*), ERA (*Eragrostis pilosa*), STI (*Stipa ichu*), CHE (*Chenopodium papulosum*), GLA (*Glandularia mendocina*), LAP (*Lappula redowskii*), PAR (*Parthenium hysterophorus*), SPH (*Sphaeralcea miniata*), PHA (*Phacelia artemisioides*), PLA (*Plantago patagonica*).

($r = 0.69$, $P = 0.006$), while the diet of the other five species showed no relation with seed abundance.

FEEDING TRIALS

Experiment 1: starch. Rufous-collared Sparrows consumed the high-starch and low-starch diets indiscriminately ($z = 0.62$, $P = 0.52$), whereas Common Diuca-Finches and Many-colored Chaco-Finches consumed more of the high-starch than of the low-strach diet ($z = 2.20$, $P = 0.027$ and $z = 2.02$, $P = 0.04$, respectively) (Fig. 3).

Experiment 2: secondary compounds. Rufous-collared Sparrows, Common Diuca-Finches, and Many-colored Chaco-Finches consumed more of the control diet than of the diet with 1% tannic acid ($z = 2.19$, $P = 0.027$, $z = 2.36$, $P = 0.017$ and $z = 2.36$, $P = 0.017$, respectively) (Fig. 4a). When the birds selected between the control and 1% condensed-tannin diets, the Common Diuca-Finch and Many-colored Chaco-Finch consumed more control diet ($z = 2.19$, $P = 0.027$ and $z = 2.36$, $P = 0.017$, respectively), but the Rufous-collared Sparrow consumed both similarly ($z = 1.52$, $P = 0.12$) (Fig. 4b). In alkaloid
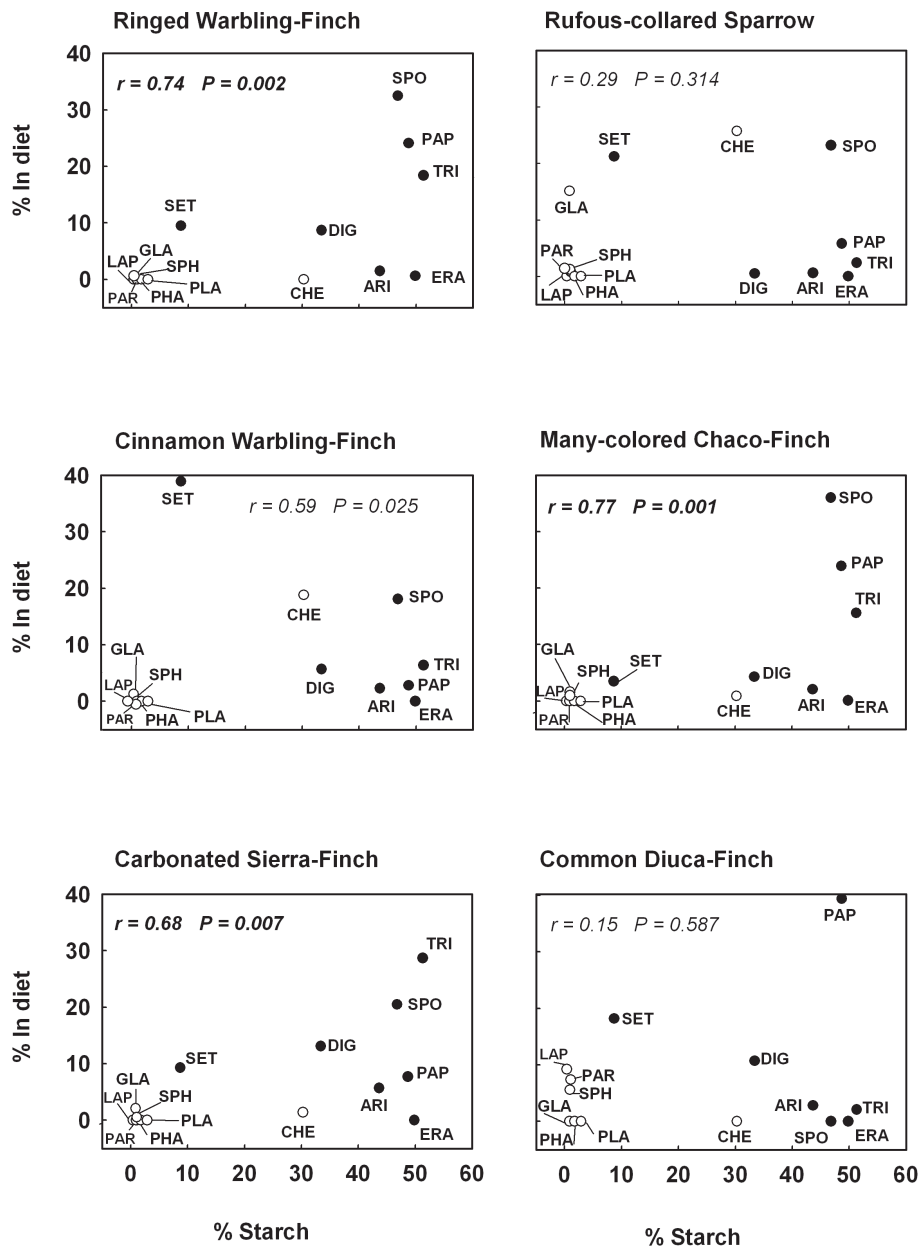
FIGURE 2.   Relationships between mean percentage of seeds in the diet of the Ringed Warbling-Finch, Cinnamon Warbling-Finch, Carbonated Sierra-Finch, Rufous-collared Sparrow, Many-colored Chaco-Finch, and Common Diuca-Finch and percent starch concentration in these seeds. Black circles correspond to grass species, white circles to forb species. See Fig. 1 for abbreviations. Data on percentage of seeds found in the diet of birds were taken from Marone et al. (2008). Values of r in bold differ significantly (Bonferroni correction $P < 0.0083$).

trials, Rufous-collared Sparrows and Many-colored Chaco-Finches consumed more control diet than diet with 0.05% caffeine ($z = 2.20$, $P = 0.027$ and $z = 2.36$, $P = 0.017$, respectively), and Common Diuca-Finches also tended to consume more control diet ($z = 1.78$, $P = 0.07$) (Fig. 4c). Finally, Common Diuca-Finches and Many-colored Chaco-Finches consumed more control diet than diet with 0.05% scopolamine ($z = 2.19$, $P = 0.027$ and $z = 2.36$, $P = 0.017$, respectively), but Rufous-collared Sparrows consumed both indiscriminately ($z = 0.16$, $P = 0.86$) (Fig. 4d).

DISCUSSION

Seeds with relatively high starch content predominated in the diet of three of the six species we studied, the most solid and consistent pattern we observed. Starch is a complex polysaccharide that is common and abundant in many wild seeds (Karasov and Martínez del Río 2007). In North American
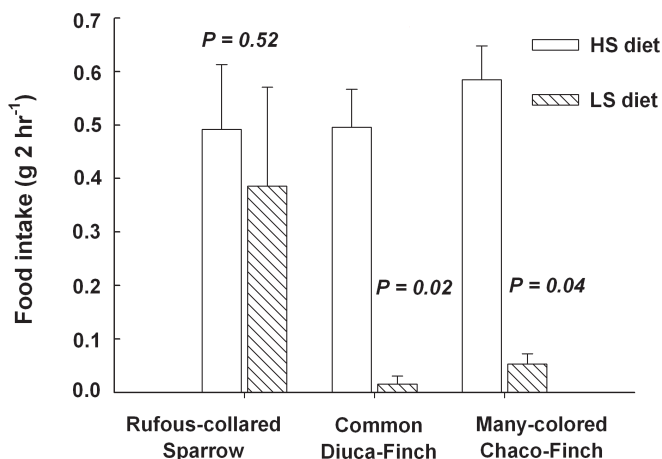
FIGURE 3. Food intake by the Rufous-collared Sparrow, Common Diuca-Finch, and Many-colored Chaco-Finch feeding for 2 hr on a high-starch diet (HS) vs. a low-starch diet (LS).
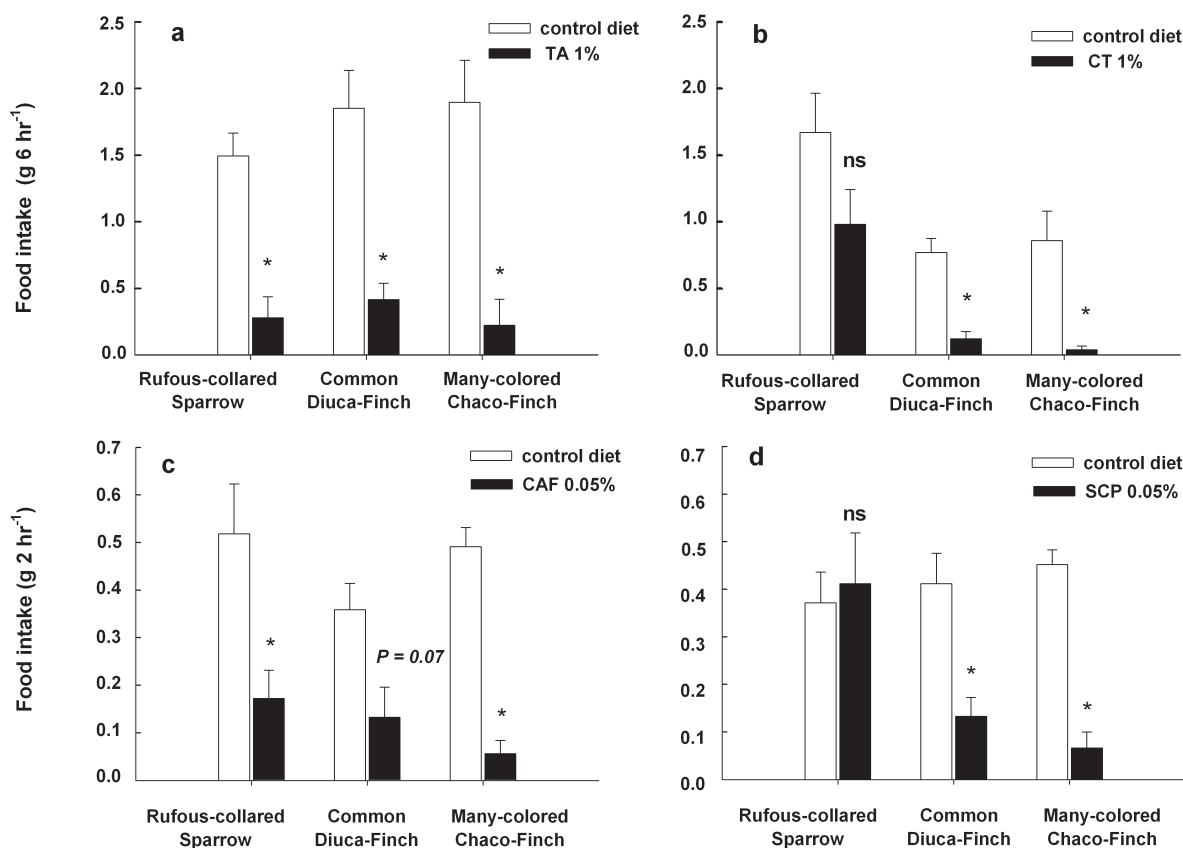

FIGURE 4.   Food intake by the Rufous-collared Sparrow, Common Diuca-Finch, and Many-colored Chaco-Finch feeding for 6 hr on a control diet vs. a treatment diet with 1% tannic acid (a) or 1% condensed tannin (b) or feeding for 2 hr on a control diet vs. a treatment diet with 0.05% caffeine (c) or 0.05% scopolamine (d). Asterisks indicate significant differences between intake of the control diet vs. intake of the treatment (*$P < 0.05$; ns, not significant).

shrub-steppe, Kelrick et al. (1986) also found strong positive correlations between the rate at which birds removed various seed types and the seeds' total carbohydrate content. They concluded that nutritional quality has a notable effect on birds' selection of seeds and that the percentage of carbohydrates is a good indicator of easily digestible energy in food. Recently, Brzęk et al. (2010) proposed that the level of intake of starch is a key factor for the development, maintenance and survival of the Zebra Finch, which feeds only on grass seeds in its natural Australian desert environment. Given that survival of Zebra Finches fed a diet poor in or free of starch dropped dramatically, Brzęk et al. (2010) concluded that the amount of starch in the diet may be a key factor in the feeding ecology of birds specialized for eating seeds.

In our experiments we tested a strictly graminivorous bird (Many-colored Chaco-Finch; 99% of its diet is grass seeds), a generalist (Rufous-collared Sparrow, which consumes similar proportions of grass and forb seeds) and a species of intermediate diet breadth (Common Diuca-Finch, which consumes 78% grasses and 22% forbs) (Marone et al. 2008). The seeds most consumed by the Many-colored Chaco-Finch in nature, *Sporobolus cryptandrus*, *Pappophorum* spp., and *Trichloris crinita*, have high starch content, and the chaco-finch also preferred a high-starch diet in the experiments. By contrast, the Rufous-collared Sparrow neither consumed the seeds according to starch content nor preferred the starch-rich diet at the laboratory. In turn, the Common Diuca-Finch preferred the starch-rich diet, although this behavior is not reflected in the natural diet. Results of the exploratory analysis and experiments became then solidly integrated in this study, corroborating that high trophic specialization of seed-eating birds can be a consequence, at least in part, of a digestive adaptation to processing a starch-rich diet (Karasov 1990, Brzęk et al. 2010). In addition, Kohl et al. (2011) have reported that pancreatic and intestinal carbohydrases are matched to dietary starch level in some wild passerines and that those birds that specialize on seeds have a higher carbohydrase activity than do birds with broader diets.

Lipids are the other important source of energy. Some species of birds feed heavily on lipid-rich food owing to its great energy density per unit of mass, which is needed for fattening previous to migration or to increase the chances of birds' overnight survival in winter (Greig-Smith and Wilson 1985, Bairlein 2002). In the Monte, however, the composition of birds' diet was not correlated with the lipid content or with gross energy content of seeds. This last pattern probably arose because lipids—not correlated with diet composition—yield more gross energy per dry matter unit than does starch (39.3 kJ g^{-1} and 17.1 kJ g^{-1}, respectively; Suttie 1977). The stronger association of diet composition with starch than with lipid content may be due, in turn, to the different pathways by which the two compounds are digested. Lipids require a process involving several biochemical steps that tends to increase the retention time in the digestive tract (Afik and

Karasov 1995): emulsification, hydrolysis, and absorption (Griminger 1986). Starch digestion is simpler: starch is directly hydrolyzed by pancreatic and intestinal enzymes and mostly absorbed in a passive manner with no energy expense (Caviedes-Vidal and Karasov 1996). In addition, the body-heat increment through feeding is lower for dietary lipids than for starch (Klasing 1998): around 20% of the metabolizable energy for starch and 10% for lipids (Scott et al. 1982, Sedinger et al. 1992). Thus birds' choice of food with high starch content entails two advantages: obtaining easily digestible energy with low cost of absorption and a relative gain in terms of thermogenesis during the cold season. Still, given that the birds' nutritional requirements can change seasonally, the time of the year in which food preferences are studied is a critical point to be considered before more general conclusions are drawn (Karasov and Martínez del Río 2007).

In our study birds' diet did not correlate with the concentration of soluble sugars in the seeds. It seems surprising that granivores from the Monte desert did not select sugar-rich seeds because emberizids can metabolize monosaccharides as well as disaccharides (Martínez del Río et al. 1990, Sabat et al. 1998) and the three species studied did select high-starch seeds. The lack of pattern, notwithstanding, seems to indicate that the seed-eating birds we studied might not use sweetness as an indicator of seed quality and that several of them could select only starch or total carbohydrate content for the purpose of gaining assimilable energy quickly (Kelrick et al. 1986, Karasov and Martínez del Río 2007).

The birds' feeding behavior was not associated with the seeds' protein content. This might be so because seeds in the Monte (10–26% of protein, Table 2) usually surpass passerines' minimum requirement threshold for protein. Díaz (1996) showed that the moderate protein levels in seeds (10–17%) are enough for these birds to satisfy their protein requirements and nitrogen balance. For example, the protein requirements of *Spizella arborea* and *Zonotrichia leucophrys*, birds of the family Emberizidae similar in body size to the species we studied, are, respectively, 8% and 7.3% (Martin 1968, Murphy 1993). Under these assumptions and also taking into account that birds can also include in their diets some insects (Blendinger 2001, Lopez de Casenave et al. 2008) with high protein levels, it is not surprising that we did not find any correlation between seed protein level and proportion of seeds in the diet.

The concentration of secondary compounds in leaves, stems, buds, and seeds is usually higher in dicots than in monocots (Earle and Jones 1962, Bernays 1990, Dearing et al. 2005, Iason and Villalba 2006), and the pattern in seeds of the Monte desert seems similar (e.g., phenols). We found no evidence that the proportion of seeds in the birds' diet in the field was negatively correlated with the concentration of the several secondary compounds assessed (i.e., total phenols, condensed tannins, saponins). Such lack of association could obscure, however, the effect of some specific compounds

because our chemical analyses focused on a gross phenolic chemical composition whose individual constituents (specific phenols) may have contrasting effects on birds' food selection (e.g., some phenols like tannins may deter feeding but others like antioxidant flavonoids may not since they have beneficial humoral immune responses in fruit-eating birds; Catoni et al. 2008). On the basis of previous knowledge of the deterrent effect on birds of some specific secondary compounds (Avery and Decker 1992, Levey and Cipollini 1998, Eröksüz et al. 2002, Matson et al. 2004), which may be present in several seeds of the Monte desert (Greig-Smith and Wilson 1985, Díaz 1996, Banko et al. 2002), we tested the responses of birds to tannic acid and condensed tannin (phenols), and caffeine and scopolamine (alkaloids), in order to assess whether such specific compounds could deter birds' feeding and affect—together with nutrients like starch—birds' feeding decisions. The two types of phenols we tested were clearly deterrents for the graminivorous Many-colored Chaco-Finch and also for the Common Diuca-Finch but not for the generalist Rufous-collared Sparrow, which was affected by tannic acid only. In turn, all alkaloids tested deterred the most graminivorous Many-colored Chaco-Finch from feeding, but their effect on the feeding behavior of the Rufous-collared Sparrow and Common Diuca-Finch was less clear. We conclude that the highly specialized feeding behavior of Many-colored Chaco-Finch could be a result of both the selection of seeds with high starch content and the low tolerance of seeds with some specific secondary compounds, probably linked to a low capacity for detoxification such compounds. The mechanistic foundation of this hypothesis deserves, however, further analyses.

The understanding of patterns of seed selection by birds in their natural habitats is a challenging task. We integrated different approaches—chemical, behavioral and ecological—which proved useful in unveiling the underlying mechanisms involved in seed selection. Exploratory and correlational analyses showed that starch predominates in grass seeds and that it is selected by the most graminivorous birds. Phenolic compounds (which are present in higher concentration in forb seeds than in grass seeds) and alkaloids (present only in some forb seeds) were both avoided by the most graminivorous species. These results, added to the fact that grass seeds are only one third as abundant as forb seeds in the soil seed bank in Ñacuñán (Marone et al. 1998, 2008), suggest that birds' selection of grass seeds can be heavily conditioned by the seeds' chemical composition combined with the animal's physiological capacities to process nutrients and toxins. Information regarding the chemical identity of all secondary compounds in seeds, the interaction among them, and other physical traits of seeds (i.e., seed hardness and color; Díaz 1990, 1996) should be obtained before broader generalizations on birds' selection of seeds is attempted. Notwithstanding, the combined knowledge of both the nutritional and anti-nutritional composition of seeds, coupled with information on the birds' feeding ecology, provided elements key to understanding the possible multiple—but not inscrutable—causes of diet selection in desert birds.

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