



Discrimination abilities for nutrients: which difference matters for choosy birds and why?

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Fine-tuned discrimination abilities are a prerequisite for optimal diet theory (ODT) and the concept of nutrient regulation as alternative models of food choice. These abilities have rarely been determined, and neither has been applied to frugivorous consumers. We investigated nutrient choices of four tanagers (*Tachyphonus cristatus*, *Dacnis cayana*, *Chlorophanes spiza* and *Cyanerpes nitidus*) for different lipid or carbohydrate concentrations, and determined discrimination abilities by reducing in steps the difference between two foods ranging from 2.5 to 12%. Three species detected differences in sugar concentrations of only 1% and differences in lipid content of 2%. Hence, frugivorous tanagers were able to select the more rewarding food based on fine-scale differences in nutritional content. Tanagers also consumed more carbohydrates than proteins or lipids in isocaloric trials, but were indifferent to equicaloric solutions of 20% of either glucose or sucrose. In contrast to foods with low sugar contents, intake of foods high in lipids or carbohydrates was limited to a protein intake of 0.75 g/7 h. Lipid and carbohydrates were treated as though they were interchangeable nutrients for birds at low, but not at high, sugar concentrations. Furthermore, the tanagers discriminated foods containing the same amount of protein but differing in the type of protein. Preference for boviserine serum albumin (BSA) over casein was not related to moult. We supplemented the food consisting of casein with the essential amino acid cysteine to match cysteine concentrations of BSA. The birds then consumed more of the casein food than of the cysteine-supplemented casein food. Overall, the birds' consistent choices in the experiments with foods of different energy density are interpretable by both ODT and the nutrient regulation theory. However, only the latter accounts for the birds' choices of isocaloric foods differing in macro- or micronutritional content. Models of food selection should therefore focus on nutrient geometry and consumer-specific requirements.

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The influence of pulp chemistry on fruit choice in birds is controversial, with some studies reporting a positive correlation between lipid or carbohydrate pulp contents and diets of free-ranging birds (Fuentes 1994; Herrera 1998), and feeding trials yielding inconsistent results with species and even individuals preferring different fruit species (Johnson et al. 1985; Borowicz 1988; Jung 1992; Whelan & Willson 1994; Lepzyk et al. 2000). The inconsistencies between studies have led to the opinion that contextual circumstances rather than pulp chemistry and profitability determine fruit choice or that birds consume fruits at random (Poston & Middendorf 1988).

Consequently, optimal diet theory (ODT), which assumes the maximization of net energy intake, has had a comparatively minor influence on studies on fruit choice,

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with only one study listed in a recent review of ODT (Sih & Christensen 2001). A common critique of ODT is that it neglects the complexities of digestion and nutritional requirements, focusing on energy as sole currency (Bozinovic & Martinez del Rio 1996; Levey & Martinez del Rio 2001). An alternative model of food choice is the geometric framework of nutrient regulation which differs from ODT in addressing multiple nutritional needs and the current state of the forager (Simpson & Raubenheimer 1999, 2001). In this model, nutrients are not treated a priori as interchangeable, and resource quality is thus measured multidimensionally. Because food items differ in nutrient ratio and concentration, nutrient regulation is operating when consumers choose those food items that meet their current multiple nutritional requirements (Raubenheimer & Simpson 1993; Behmer et al. 2001). This model has been useful in explaining food choice in insects and some

vertebrates (Raubenheimer & Simpson 1997; Simpson & Raubenheimer 1997) but it has not yet been applied in detail to birds and frugivores.

A prerequisite for both models of food choice is the ability to distinguish between different nutritional concentrations in foods. Poston & Middendorf (1988) argued that birds do not possess such sensory capabilities. So far, discrimination abilities of consumers have not been determined for the different macronutrients but only for the main nutrient source, for example protein in granivores and carbohydrates in nectarivores (Murphy & King 1987, 1989; Lotz & Nicholson 1996; Blem et al. 2000). Based on the results of studies on nectarivores, it has been hypothesized that passerines in general prefer hexose over sucrose because of its faster assimilation (Martinez del Rio & Karasov 1990). Because nutrient ratios of food items vary, however, it is essential to determine the discrimination abilities for different nutrients to understand the mechanisms underlying food choices. For instance, some fruits are high in carbohydrates (mostly hexoses) or lipids (Herrera 1987; Baker et al. 1998), but most fruits have low protein content or low proportions of digestible protein (Foster 1978; Izhaki & Safrieli 1989). Relative to the amount of essential amino acids required for maintenance in passerines, fruits are most deficient in the sulphur-containing amino acids methionine and cysteine (Izhaki 1998). Because of the low protein content of fruits, frugivorous birds use a foraging strategy of protein complementation (Witmer 2001).

To establish the basis for an evaluation of the alternative models on food choice, we investigated the discrimination abilities of four frugivorous tanagers (Emberizidae, Thraupini) for the main nutrients. By studying whether birds discriminated between semisynthetic foods differing only in lipid, protein, or carbohydrate content, we tested the hypothesis that frugivorous birds are unable to choose precisely between foods differing in nutritional content. We designed experiments to examine (1) which concentrations of lipids and carbohydrates were preferred within a range of 4–12% and 2.5–12%, respectively. We (2) then reduced, in steps, the differences in lipid or sugar concentrations to assess birds' detection abilities. By offering foods with different nutrient ratios, we (3) investigated the importance of nutrient regulation for food choice in these trials. We (4) also tested the hypothesis of sucrose avoidance in passerines, by comparing intake rates of sucrose and glucose. To assess protein discrimination abilities, we (5) analysed whether birds were able to discriminate between different types of proteins (casein versus albumin). We (6) then determined whether they discriminated between different concentrations of the essential amino acid cysteine.

METHODS

We used four species of tanagers: flame-crested tanager, *Tachyphonus cristatus* (20 g, range 17–23 g), blue dacnis, *Dacnis cayana* (12 g, range 10–14 g), green honeycreeper, *Chlorophanes spiza* (17 g, range 15–18 g) and short-billed honeycreeper, *Cyanerpes nitidus* (8 g, range 7–9 g). The latter three species belong to the subfamily Dacninae.

Birds were kept individually in cages (62 × 40 cm and 39 cm high) under constant conditions (12:12 h light:dark, 25 ± 1°C, ca. 70% relative humidity). The experiments were done between February 1999 and February 2001. Although birds might shift their diets seasonally, we assumed that the ability to discriminate between nutrient concentrations does not. Birds were kept on a standardized food of dried insects (20%), vegetable oil, protein, carbohydrates (glucose and sucrose), cellulose (14%), minerals and vitamins (2.5%), and water (50%) (Bairlein 1986; Table 1). Water was available ad libitum. For the three nectar-consuming species of Dacninae, the maintenance food was supplemented with sugar water (20% sugar concentration) or artificial nectar designed for small nectarivores (Nectar-Plus, Enderle Production, Pforzheim, Germany). With this diet the birds remained in good health. We tested 29 individuals altogether. Because birds were also tested in fruit colour experiments (V. Schmidt, unpublished data), we used a different number of birds for different trials, which we report below for each trial. All individuals were bought as captive bred in trade, most of them as juveniles, and were held under licence of the Weser-Ems District Government, Germany. During the experimental period, birds maintained or increased their body mass. When not involved in trials, they were kept in an aviary (6 × 4 m and 3 m high). After the experiments in our laboratory, the birds will be moved to the neotropical aviary in the Zoological Garden in Vienna, Austria.

We repeated three trials (lipid: 4 versus 6%; carbohydrate: 8 versus 12% and 5 versus 6%) with seven wild-caught birds (three blue dacnis and four short-billed honeycreepers) at the Surumoni Station, Venezuela (3°10'N, 65°40'W) in November 1999 at the beginning of the dry season. Birds were observed 1 month before capture in a mixed-species flock and were mist netted after the breeding season, to ensure that they were in nonbreeding condition. Permits for trapping and housing birds were given by the SADA-Amazonas Department, Pto Ayacucho, Venezuela. These birds were acclimated for 2 weeks to the standard laboratory diet before we did the tests, and they were released within 4 weeks of capture. We observed these colour-ringed birds continually until the end of our stay, about 4 months after release into the same mixed-species flock in which we caught them. We therefore assume that they were unharmed and did not alter their behaviour because of the experiment. At the field station in Venezuela, we used the same cage dimensions, 12:12 h L:D, ca. 25°C, ca. 90% relative humidity (see Anhuf & Winkler 1999 for a general description).

Experimental Design

All experiments were designed as standard pairwise trials (Van der Meer 1992) with birds offered a choice between two foods. The advantage of this design is that both discrimination abilities and nutrient regulation can be studied simultaneously, avoiding the complexities of varying nutritional compositions of multiple foods. We ran each feeding experiment on 4 successive days, for 7 h each day. Between trials, the birds were kept on the

Table 1. Composition of the maintenance and isocaloric foods used to test nutrient choice of tanager species

Food type	Ingredients (%)						kJ/10 g
	Lipid	Protein	Sugar	Fibre	Water	Mineral mix	
Maintenance	10	14.1	5.0	18.4	50	2.5	71.8
First isocaloric trial							
Lipid	8	14.1	2.0	23.4	50	2.5	58.8
Sugar	2.6	14.1	14.2	16.6	50	2.5	58.8
Second isocaloric trial							
Protein	10	14.1	2.1	21.3	50	2.5	66.8
Sugar	10	2.1	14.1	21.3	50	2.5	66.8

Energy density expressed per g wet mass.

maintenance food. During feeding trials they were simultaneously offered two identical cups containing alternative foods. We switched locations of the two foods daily. To control for weight loss from evaporation during the trials, we used a control cup containing the same amount of food. We corrected for spills of food and calculated food intake by subtracting the mass of uneaten food from the mass of food offered to the bird. For each food type, the daily food intake was calculated and used separately for statistical analyses.

Food Composition

Sugar preference

We used 20% solutions of glucose or sucrose, a level that matches sugar concentrations in nectar, to test preferences for glucose versus sucrose. For all other experiments we used the maintenance food and changed nutrient concentrations within a narrow range commonly found in fruits.

Nutrient choices in isocaloric food

In isocaloric foods, we determined the birds' selection for sugar over protein and lipid. We calculated the energetic value of each food type by using the average gross energy equivalents of protein (17.2 kJ/g), fat (38.9 kJ/g) and carbohydrates (17.2 kJ/g; [Karlson 1972](#)). We modified the proportions of nutrients and cellulose so that alternative foods had the same energy density per wet mass ([Table 1](#)).

Carbohydrate and lipid choice trials

We tested food choices for different lipid and carbohydrate concentrations in a range from 4 to 12% and 2.5 to 12%, respectively. In these experimental foods, we altered the nutritional concentrations of just one primary nutrient (i.e. lipid or sugar) and changed the amount of cellulose accordingly. For instance, when comparing foods containing 4 and 12% lipids, the 4% food contained 8% more cellulose than the 12% food. To assess discrimination abilities for lipids and carbohydrates, we reduced, in steps, the differences in concentration levels of the two alternative foods. Protein content of all foods was held constant at 14.1%; in lipid trials carbohydrate

content was 5% and lipid content of all carbohydrate foods was 10%.

Protein choice trials

To test whether tanagers were able to discriminate type of protein, we fed two foods containing the same amount of protein (14.1%), but consisting of different proteins. One food contained 98% pure casein and the other 98% pure boviserine serum albumin (BSA). BSA and casein differ mainly in the amount of cysteine: BSA contains 5.8% cysteine ([Friedli 1996](#)) and casein 1.9% ([Murphy 1993](#)). [Murphy & King \(1989\)](#) found no effect of the form of amino acids (crystalline or proteinbound) on the pattern of food selection. We hypothesized that BSA would be preferred because of casein's relative cysteine deficiency. We tested this conjecture by adding 99% crystalline L-Cysteine (Sigma, Deisenhofen, Germany) to casein, to match its composition to that of BSA. Because birds have specific amino acid requirements during moult we tested whether the birds' preference was related to moult, by repeating the experiment after 6 months when the birds were at different moult stages.

Statistical Analysis

The differences in the preferences for foods were tested in permutation tests (see [Good 1994](#)). Because the design was hierarchical (treatments, species, individuals) and repeated over individuals, permutations were carried out on values centred at zero within individuals ([Mundry 1999](#)). The statistic evaluated in 10 000 permutations was between-treatments variance versus within-treatments variance. Permutation tests were performed with software from the Konrad Lorenz-Institute for Comparative Ethology (H. Winkler, unpublished). Other tests were performed with SPSS software.

RESULTS

Nutrient Choice

With isocaloric foods, we tested the choices of blue dacnis (BD), short-billed honeycreeper (SH) and green honeycreeper (GH) for carbohydrates versus lipids or

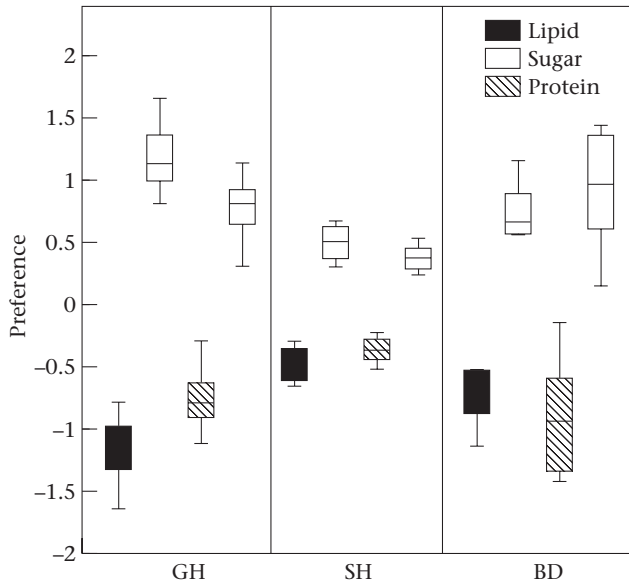


Figure 1. Nutrient selection of three tanager species on isocaloric foods for one of the main nutrients: carbohydrate (sugar) versus lipid in trial 1 and carbohydrate versus protein in trial 2. Preference values are centred at zero. Indicated are median, 2nd and 3rd interquartiles and 5th and 95th percentiles. GH: Green honeycreeper; SH: short-billed honeycreeper; BD: blue dacnis.

proteins. In trial 1, we tested the birds' choices for a food rich in carbohydrates or lipids (Table 1); all species consumed more of the sugar-rich food (sugar: $\bar{X} \pm SE = 0.84 \pm 0.08$; lipid: -0.84 ± 0.05). To test choices for one of the foods we used permutation tests for all of the following comparisons unless otherwise stated (GH: $N=4$, $P < 0.0001$; SH: $N=3$, $P < 0.001$; BD: $N=5$, $P < 0.0001$; Fig. 1). In trial 2 the sugar-rich food was also selected ($\bar{X} \pm SE = 0.72 \pm 0.06$) over a food rich in protein (-0.72 ± 0.06 ; GH: $N=4$; SH: $N=3$; BD: $N=5$, all $P_s < 0.0001$; Fig. 1).

Sugar Concentrations

The three nectarivorous species of the Dacninae did not discriminate between different types of sugar. Glucose ($\bar{X} \pm SE = -0.08 \pm 0.77$) was not preferred over sucrose (0.08 ± 0.78) at 20% concentrations of each (GH: $N=4$; SH: $N=3$; BD: $N=5$; all NS). We tested all four species for choice of sugar concentration (range 2.5–12%). When offered a choice between foods containing 2.5 or 12% sugar concentration (trial 3), birds chose the 12% ($\bar{X} \pm SE = 1.02 \pm 0.09$) over the 2.5% one (-1.02 ± 0.08 ; GH: $N=4$; SH: $N=4$; BD: $N=4$; all $P_s < 0.0001$; Fig. 2). Similarly, with a choice between 8 and 12% sugar (trial 4, Fig. 2), all three species selected the 12% one (12%: $\bar{X} \pm SE = 0.79 \pm 0.06$; 8%: -0.79 ± 0.09 ; GH: $N=6$; BD: $N=10$; SH: $N=8$; all $P_s < 0.001$). As a next step (trial 5), we tested the birds' choice between 8 and 6% sugar foods (Fig. 3a); three species consumed more of the 8% food ($\bar{X} \pm SE = 0.93 \pm 0.11$) than of the 6% food (-0.93 ± 0.09 ; GH and BD: $N=6$; flame-crested tanager, FT: $N=5$; all $P_s < 0.0001$; SH: $N=4$, $P = 0.074$). When the difference between foods was reduced to 1% (trial 6), three species

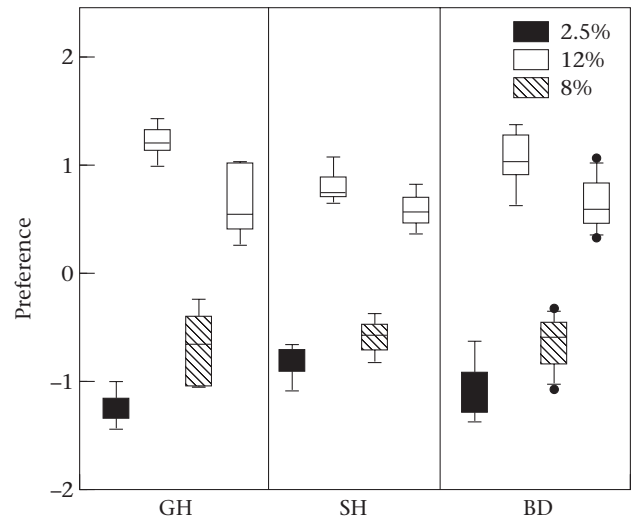


Figure 2. Sugar preferences of three tanager species for diets differing in sugar concentrations. Two low sugar diets (2.5 and 8%) were tested against a diet containing 12% sugar. Indicated are median, 2nd and 3rd interquartiles and 5th and 95th percentiles; dots represent outliers. GH: Green honeycreeper; SH: short-billed honeycreeper; BD: blue dacnis.

chose the 6% food ($\bar{X} \pm SE = 0.43 \pm 0.08$) over the 5% food (-0.43 ± 0.09 ; GH: $N=6$; FT: $N=5$; both $P < 0.05$; BD: $N=10$, $P < 0.0001$; SH: $N=8$, NS; Fig. 3b). The birds' preferences for a food containing 1% more sugar than the other food depended on the absolute amount of sugar in both foods. When birds were offered a choice between one food containing 12% sugar and the other 13%, birds showed no preference (12%: $\bar{X} \pm SE = -0.23 \pm 0.16$; 13%: 0.23 ± 0.17 ; GH, SH and BD: $N=4$, all NS). The mean intake for the 12% food $\pm SE$ was 2.4 ± 0.3 g and for the 13% food 2.6 ± 0.3 g. We compared the food intake of the similar-sized blue dacnis and green honeycreeper at different sugar concentrations. The volumetric food intake depended on the sugar concentration used in the experimental foods. While there was no difference in food intake between the trials of 2.5–12% and 8–12% sugar and between the trials with low-sugar foods (5–6% and 6–8%), birds of both species consumed less food in trials with the high-sugar food (12%; Table 2).

Lipid Concentrations

In trials 7–9 we offered a basic food containing 4% lipids and tested selection for lipid concentrations with foods that had a higher lipid content. We reduced the lipid content of the lipid-rich food from 12 to 7% and then to 6%. Blue dacnis and green honeycreeper always chose foods with a higher lipid content (12%: $\bar{X} \pm SE = 1.18 \pm 0.07$; 7%: 0.65 ± 0.15 ; 6%: 0.73 ± 0.14) over the food containing 4% lipids (GH: $N=6$; BD: $N=5$; all $P_s < 0.0001$, except for difference in 4–6%, BD: $N=10$, $P < 0.01$; Fig. 4). Short-billed honeycreeper selected the food with a higher lipid content only at 12% ($N=4$, $P < 0.001$; 4 versus 7%: $P = 0.058$; 6%: $N=8$, NS).

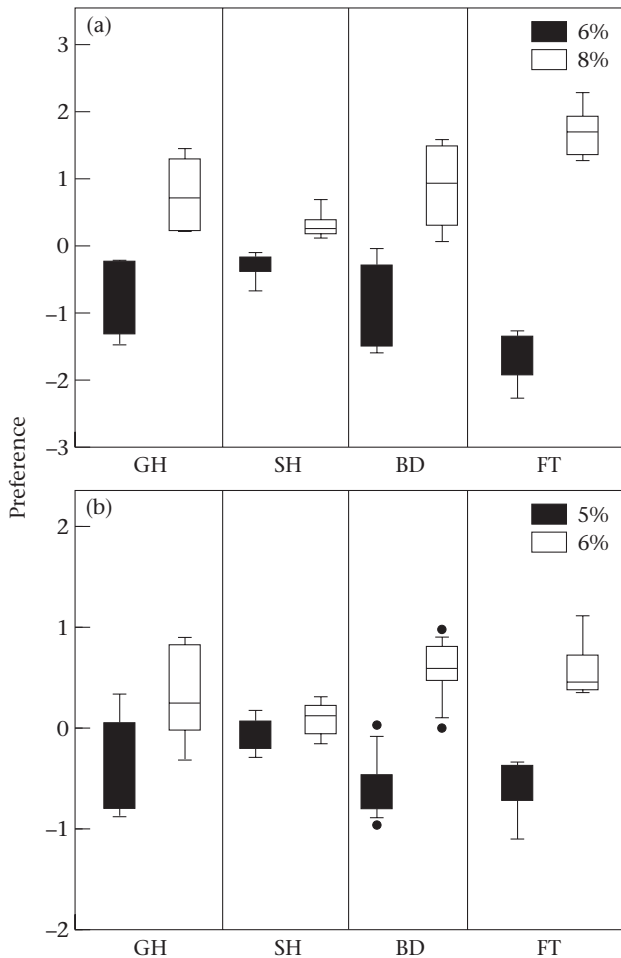


Figure 3. Sugar preferences for diets differing by (a) 2% and (b) 1% sugar concentration. Preference values are centred at zero. Indicated are median, 2nd and 3rd interquartiles and 5th and 95th percentiles; dots represent outliers. GH: Green honeycreeper; SH: short-billed honeycreeper; BD: blue dacnis; FT: flame-crested tanager.

Protein Discrimination

When nutrient concentrations were kept constant, and foods differed only in the type of protein, birds preferred BSA ($\bar{X} \pm \text{SE} = 1.07 \pm 0.21$) to casein (-1.07 ± 0.15 ; GH: $N=6$; BD: $N=5$; both $P < 0.0001$; Fig. 5). The replicate experiment similarly showed a BSA preference. When we supplemented the casein food with 4% cysteine to match the cysteine concentrations of BSA, the birds then selected pure casein ($\bar{X} \pm \text{SE} = 0.40 \pm 0.14$) over the cysteine-supplemented casein food (-0.40 ± 0.10 ; GH: $N=6$, $P < 0.001$; BD: $N=5$, $P < 0.05$; Fig. 5).

Nutrient Regulation

Carbohydrates

The nutrient ratios of foods differed in the experiments on sugar, lipid and overall nutrient choice (trials 1–9, Table 3). The nutrient ratios of each food can be shown as food rails in nutritional space (Fig. 6a). Foods in trial 2 had the greatest differences in protein and carbohydrate

content enabling birds to move in nutrient space to the greatest extent. In this trial, birds selected food to obtain a low protein and a high lipid and carbohydrate intake (protein/carbohydrate (p/c) ratio of total food intake = 1:1.4; Table 4). In the following trials (3–6), birds were restricted to foods that did not allow them to reach the self-selected nutrient intake of trial 2. They consumed less carbohydrate and more protein as a result. Birds regulated their intake of lipids (l) and carbohydrates (c) to obtain an l/c ratio of 1:2.5 in trial 1 and 1 in trial 2 (Fig. 6a). This intake was also accessible in trials 3 and 4 (with foods of 12% sugar) but not in subsequent trials (5 and 6) because of lower carbohydrate contents and smaller differences in nutrient ratio between alternative foods. In trials 3 and 4, birds regulated their intake and ingested food at the same l/c ratio (1) as in trial 2, and the total intake in these trials is aligned on one line with a positive slope (Fig. 6a). When this l/c ratio was not accessible in trials 5 and 6, birds ingested more lipids. The intake in these trials is aligned with the intake of trial 2 on a line with a slope near -2 , which is consistent with lipids and carbohydrates being interchangeable as energy sources but only at low carbohydrate concentrations. Protein intake in these trials was not tightly controlled, as illustrated by the large standard error in protein intake. Birds ingested a maximum of 0.96 g of protein per day (Fig. 6b). Because of a higher overall food intake in trials 5 and 6 with low carbohydrate concentrations, birds ingested more protein in these trials than in trials 3 and 4 with higher sugar concentrations (Mann–Whitney U test: $U=31.5$, $N_1=N_2=12$, $P < 0.05$).

Lipids

When presented with alternative foods of distinct l/p and l/c ratios in trials 1 and 7, birds did not defend a consistent lipid intake, because of their preference for carbohydrates. Consequently, birds ingested different amounts of lipids in both trials (Fig. 7a). Although lipid intake varied, protein intake was tightly controlled between 0.75 and 0.8 g/day. The implied constraint on protein intake would account for the low lipid and carbohydrate intake in these trials (Table 4), and is also apparent when carbohydrate intake is plotted versus protein intake. Birds ingested the same amount of sugar in the first two trials on general nutrient preference (Fig. 7b). In trials 7–9, birds were confined to a fixed p/c ratio. They moved their p/c intake on this line until reaching the same daily protein intake as in trial 1. When carbohydrate intake is plotted against lipid intake, birds ingested the same amount of carbohydrates in all lipid trials (Fig. 7c).

DISCUSSION

Discrimination Abilities

Birds discriminated precisely between foods differing by 1% carbohydrate concentration, 2% lipid content and 4% cysteine content. We thus falsified the hypothesis that birds do not possess fine-tuned discrimination abilities (Poston & Middendorf 1988). Within

Table 2. Food and energy intake of blue dacnis and green honeycreeper during four trials lasting 7 h with foods of different sugar concentration

Sugar concentration (%)	Food intake (g)	Energy density of intake (kJ/g)	Energy intake (kJ/7 h)	N	Difference in volumetric intake (Mann–Whitney <i>U</i>)		
					8 versus 12	2.5 versus 12	6 versus 8
5 versus 6	5.90	7.34	43.32	16	23**	15***	23
6 versus 8	6.48	7.64	49.46	12	31*	21**	
2.5 versus 12	4.45	8.05	35.80	8	62		
8 versus 12	4.52	9.11	41.16	16			

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

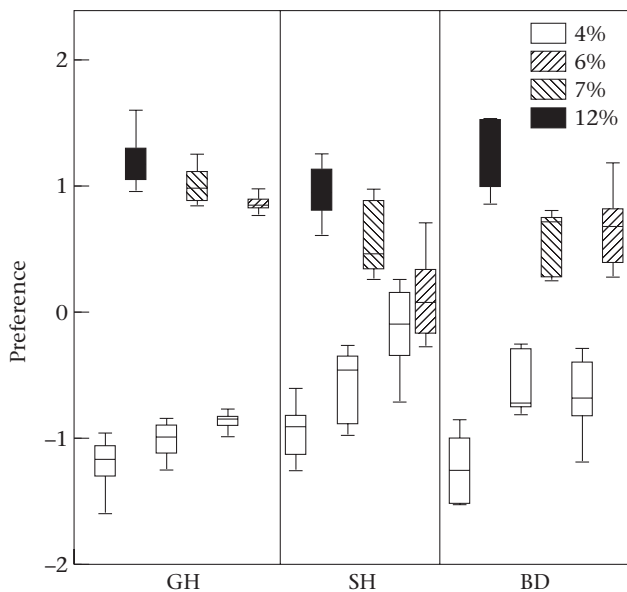


Figure 4. Lipid selection of three tanager species in three trials with diet differing in lipid content. Food choice was tested by comparing intake of a basic food of 4% lipids against diets with lipid contents of 6, 7 and 12%. Preference values are centred at zero. Indicated are median, 2nd and 3rd interquartiles and 5th and 95th percentiles. GH: Green honeycreeper; SH: short-billed honeycreeper; BD: blue dacnis.

the experimental time span of 7 h the birds' sensory preferences for nutrients might have been biased by metabolic feedbacks but this time span may be too short to measure nutrient regulation strategies accurately. Nevertheless, the results of these experiments with foods of different energy density are interpretable by both ODT and nutrient regulation theory. However, only the latter accounts for the birds' consistent choices of isocaloric foods differing in macro- or micronutritional contents.

Carbohydrates

Sugar discrimination abilities are well studied in nectarivores (e.g. Lotz & Nicolson 1996; Jackson et al. 1998; Blem et al. 2000). Like hummingbirds (Blem et al. 2000), tanagers were able to discriminate differences in sugar concentrations of 1%. Levey (1987) reported that three of four tanager species discriminated differences of 2% and argued that olive-backed tanagers, *Tachyphonus*

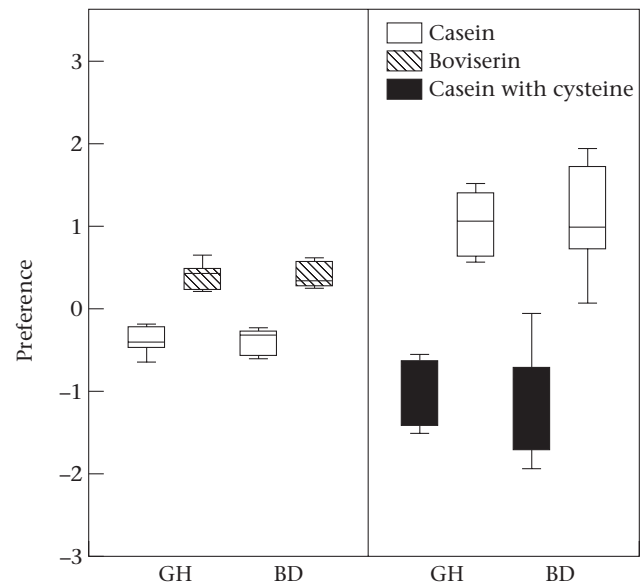


Figure 5. Protein selection by two tanager species. Birds discriminated between two types of protein: (a) casein versus albumin and (b) casein versus casein supplemented with 4% cysteine. Preference values are centred at zero. Indicated are median, 2nd and 3rd interquartiles and 5th and 95th percentiles. GH: Green honeycreeper; BD: blue dacnis.

delatrii ($N=2$) were unable to discriminate sugar concentrations at such a fine scale because of their insectivorous habits. However, different feeding habits did not result in different discrimination ability between the insectivorous flame-crested tanagers ($N=5$) and the more nectarivorous blue dacnis and green honeycreeper. While the observed difference in sugar preference by Levey (1987) might be due to different choices of single individuals (Jung 1992), this does not account for the less developed discrimination abilities of short-billed honeycreepers ($N=8$).

Apart from relative differences in concentration levels, the absolute concentration of carbohydrates influences food choice. Differences of 1% sugar at high concentration levels (12 or 13%) either are not detectable for tanagers, or both concentration levels are high enough to meet their energy requirements. Similarly, hummingbirds discriminated differences of 1% sucrose concentrations

Table 3. Nutrient ratios of foods used in trials on lipid (l) and carbohydrate (c) choices

Trial	Diet type	l/c ratio	l/p ratio	p/c ratio
1	Sugar food	0.18	0.18	0.99
1	Lipid food	4.00	0.57	7.05
2	Sugar food	0.71	4.76	0.15
2	Protein food	4.76	0.71	6.71
3,4	12% sugar	0.83	0.71	1.18
3	2.5% sugar	4.00	0.71	5.64
4,5	8% sugar	1.25	0.71	1.76
5,6	6% sugar	1.67	0.71	2.35
6	5% sugar	2.00	0.71	2.82
7-9	4% lipid	0.80	0.28	2.82
7	12% lipid	2.40	0.85	2.82
8	7% lipid	1.40	0.50	2.82
9	6% lipid	1.20	0.43	2.82
	Maintenance food	2.00	0.71	2.82

p=protein.

only at a concentration level of 20%, which matches sucrose concentrations of hummingbird-dispersed flowers (Blem et al. 2000). When concentration levels were raised, the differences necessary to cause a preference increased in curvilinear fashion (Blem et al. 2000).

Hexoses are the dominant sugars in most bird-dispersed fruits, whereas nectar of flowers pollinated by hummingbirds is rich in sucrose (Martinez del Rio et al. 1992; Baker et al. 1998). The lack of sucrase in the intestinal membrane of some species explains the sucrose avoidance of a number of species from different passerine families (Martinez del Rio et al. 1988, 1992; Martinez del Rio & Stevens 1989; Brugger 1992; Malcarney et al. 1994). Martinez del Rio & Karasov (1990) thus postulated a general hexose preference for passerines in contrast to specialized nonpasserine nectar feeders. Avery et al. (1999) provided evidence supporting this hypothesis for an opportunistic frugivore. However, studies on two specialized nectarivorous passerines showed that they behaved similarly to hummingbirds: one species preferred sucrose and the other was indifferent to the type of sugar presented at 20% concentrations (Lotz & Nicolson 1996; Jackson et al. 1998). Our results on omnivorous passerine species also do not support the hypothesis of a general hexose preference in passerines.

Tanagers had lower volumetric food intake rates when fed with sugar-rich than low-sugar foods. The inverse relation between volumetric intake and sugar concentration has been noted for other nectar-feeding bird species (Downs 1997) and has been attributed to a compensatory feeding strategy of maintaining constant sugar intake rates (Lopez-Calleja et al. 1997). The inverse relation might also be due to physiological constraints such as intestinal sucrose hydrolysis rates in hummingbirds (McWhorter & Martinez del Rio 2000). At high sugar concentrations, cinnamon flowerpiercer, *Diglossa baritula*, and four species of hummingbirds had a higher sugar intake, despite a reduced volumetric intake compared with low sugar concentrations (Martinez del Rio et al., in press).

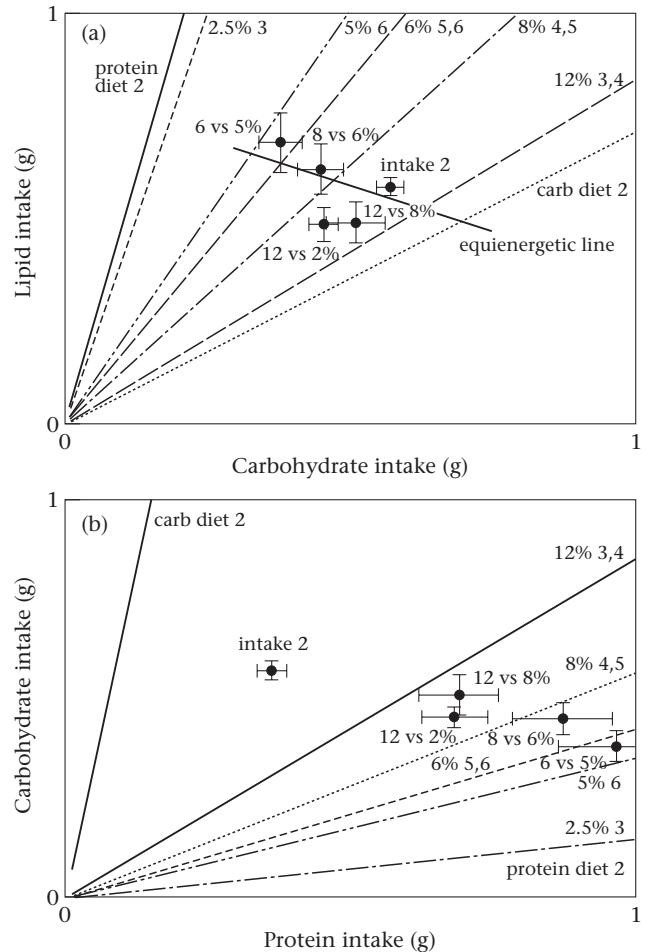


Figure 6. Nutrient ratios of diets with different carbohydrate (carb) contents (%) represented as lines in the nutritional space of macronutrients. Numbers refer to the trials. The total food intake of trials is represented by bicoordinate error plots illustrating the standard error. (a) Lipid and carbohydrate food intake. (b) Protein and carbohydrate food intake. The equienergetic line denotes birds' equalcaloric intake when lipids and carbohydrates were combined in trials 2, 5 and 6.

Table 4. Total protein (p) and total combined intake (g) of lipids (l) and carbohydrates (c) in trials with different nutrient ratios

Trial	p intake	l+c intake
1	0.74	0.80
2	0.39	1.16
3	0.65	0.92
4	0.69	0.93
5	0.87	1.01
6	0.96	0.97
7	0.77	0.81
8	0.80	0.62
9	0.77	0.52

Lipids

Because birds prefer carbohydrates over lipids, Lepzyk et al. (2000) hypothesized that they use sweetness as a primary indicator and are thus not influenced by lipid

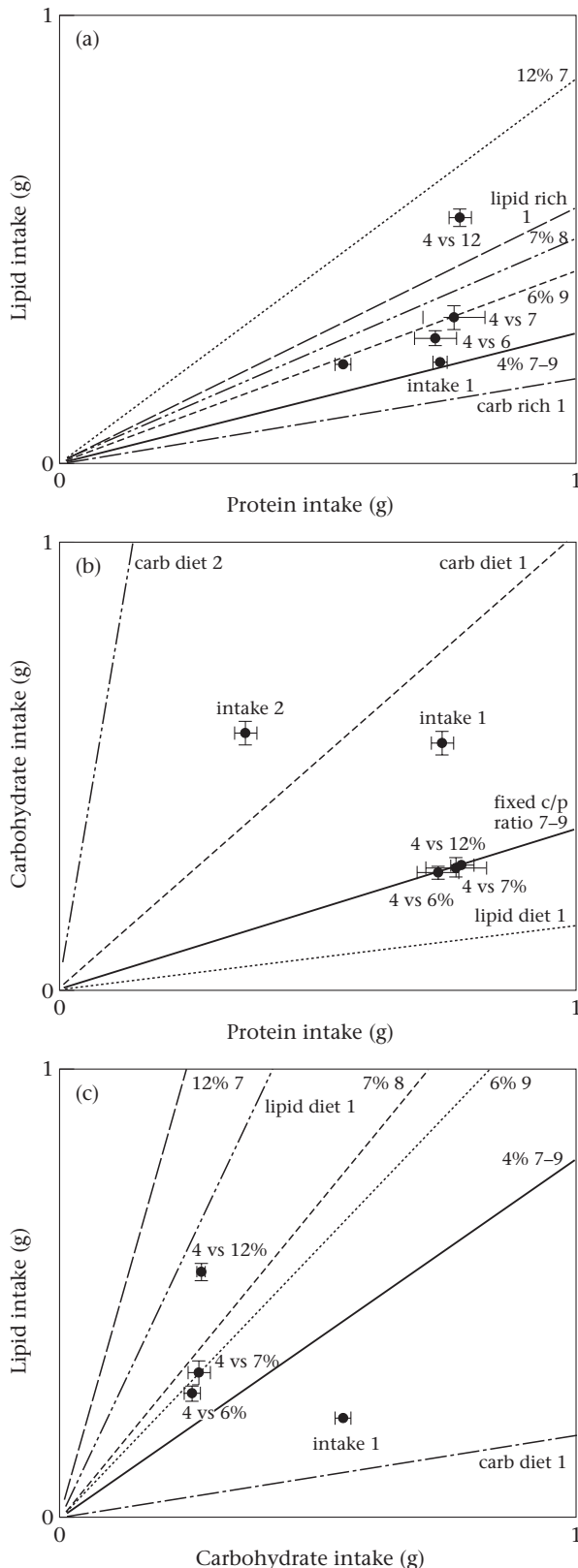


Figure 7. Nutrient ratios of foods with different lipid contents (%) represented by lines. Numbers refer to the trials. The total nutritional intake is represented by bicoordinate error plots illustrating the standard error. (a) Lipid and protein intake. (b) Carbohydrate and protein intake. (c) Lipid and carbohydrate intake.

content. However, all three tanager species discriminated between foods differing in lipid content and two detected 2% differences in lipid content. This result corroborates studies on diets of frugivores reporting preferences for lipid-rich fruits (Fuentes 1994; Herrera 1998). In contrast, most feeding trials using fruit species with different lipid content (Johnson et al. 1985; Borowicz 1988; Whelan & Willson 1994) and artificial fruits (Lepzyk et al. 2000) failed to find a preference for lipid-rich fruits. Choice experiments using a limited number of fruits are not well suited to detect nutrient preferences because of the many covarying fruit compounds. Contrasting results might be due to differences in secondary compounds which may act as deterrents (Izhaki & Safriel 1989; Cipollini & Levey 1997; Schaefer et al., in press) or stimulate food intake (Bairlein & Gwinner 1994). For instance, lipid preference of garden warblers, *Sylvia borin*, was recorded with semi-synthetic food but not when birds were simultaneously fed five fruit species (Bairlein 1991). In future studies on nutrient preferences we therefore suggest using either semisynthetic foods or many fruit species.

Proteins

Data on protein discrimination in passerines are limited to the white-crowned sparrow, *Zonotrichia leucophrys*. The amino acid requirements of this granivorous species are well documented (Murphy 1993). White-crowned sparrows discriminated foods differing only in lysine and valine concentrations, and also preferred sulphur amino acids (cysteine and methionine) during moult when large amounts of these amino acids are needed to make keratin (Murphy & King 1987, 1989). Methionine and cysteine are scarce in fruits which are deficient in several essential amino acids (Izhaki 1998). Of the essential amino acids, BSA and casein differ mainly in the amount of cysteine (Murphy 1993; Friedli 1996). In the present study, BSA was selected over casein in two trials regardless of moulting stage. We first thought this might be a result of the relative cysteine deficiency of casein. When foods differed only in cysteine levels, birds did not prefer the high-cysteine food. They possibly rejected this food because on the balanced maintenance diet they did not experience a deficiency in cysteine or because of an unbalanced amino acid ratio in the cysteine-supplemented food. However, in avoiding cysteine-rich food, tanagers differed from white-crowned sparrows, which were indifferent to this food before and after moult (Murphy & King 1987).

Owing to low proportions of digestible protein and the lack of essential amino acids in fruits (Izhaki & Safriel 1989; Izhaki 1998), the adaptive significance of the fine-tuned food discrimination abilities in birds is apparently a strategy of active dietary amino acid complementation (Witmer 2001). Diet complementation has been proposed as a foraging strategy for granivores and frugivores (Murphy & Pearcy 1993; Whelan et al. 1998) but birds might also passively achieve it by feeding on different food items in response to other factors, especially the generalist feeders such as the tanagers that we studied. The ability to discriminate nutrient concentration differences of 1 or 2% allows birds to choose not only interspecifically but also intraspecifically between fruits of

different plant individuals (Levey 1987) and between different ripening stages (Schaefer & Schmidt 2002).

Mechanisms of Food Choice

ODT and the theory of nutrient regulation are alternative, often mutually nonexclusive, hypotheses about the mechanisms underlying food choice. ODT predicts that foragers prefer energetically profitable prey items at the attack stage (Sih & Christensen 2001), while nutrient regulation considers the multiple nutritional requirements of consumers, assuming that those food items are chosen that represent the direct link between the consumers' current state and their nutritional requirements because of their nutrient ratio (Raubenheimer & Simpson 1993; Simpson & Raubenheimer 2001). In our experimental design, the complexity of food choice was reduced to the difference in nutritional composition between alternative foods. In trials with foods of different energetic value, birds' choices were consistent with ODT. This result corroborates earlier studies with semisynthetic foods on single nutrients or studies on diets of free-ranging birds (Levey 1987; Bairlein 1990; Fuentes 1994; Herrera 1998). A common critique of ODT is its focus on energy and single nutrients as sole currency, neglecting the inherent complexities of consumers' digestion (Bozinovic & Martinez del Rio 1996; Levey & Martinez del Rio 2001). For instance, our birds' consistent choices of isocaloric foods that differed in macro- (trials 1, 2) or micronutritional content (protein trials) are not related to univariate optimality theory but rather to physiological constraints (Martinez del Rio & Stevens 1989) or to nutrient regulation to meet specific consumer requirements.

Others have shown that nutrient regulation determines food selection in vertebrates and insects (Simpson & Raubenheimer 1997; Behmer et al. 2001). For instance, rats, *Rattus norvegicus*, and hens, *Gallus gallus*, regulated food choice to obtain a 1:3 p/c ratio (Simpson & Raubenheimer 1999) but the concept of nutrient regulation has been rigorously tested only for insects. When given the choice in trial 2, birds chose a 1:1.4 p/c ratio, and had the lowest daily protein intake and the highest carbohydrate and lipid intake. To establish whether these were truly regulated intake ratios (targets) would require evidence that birds 'defend' these ratios when given various food choices, nutrient dilutions or food frequencies (Simpson & Raubenheimer 1999; Behmer et al. 2001). If we assume that the 1:1.4 p/c ratio does represent the intake target ratio, our birds selected food at a higher p/c ratio than hens despite the comparable low protein requirements of frugivorous birds (Witmer & Van Soest 1998; Pryor et al. 2001). In the other trials, birds were restricted to diets with higher p/c ratios than selected in trial 2, and were thus forced to undereat some nutrients and to overeate others relative to the trial 2 diet. It is in such situations that nutritional balancing strategies become apparent (Simpson & Raubenheimer 2000). Overall, tanagers consumed more carbohydrates than lipids and proteins, and maintained a similar carbohydrate intake in trials 1 and 2 on macronutritional

food choices with varying protein and lipid intake. However, nutritional constraints apparently limited food intake in lipid trials. The data were consistent with food intake of a suboptimal diet with a high p/c ratio controlled by the protein intake. This constraint is indicated by the total food intake in all lipid trials which align vertically with the intake of trial 1 at ca. 0.8 g of protein (Fig. 7a). Because we held the p/c ratio in the lipid trials constant, such an upper limit for protein intake would also account for the vertical line that connects the intake in these trials when lipid and carbohydrates are plotted (Fig. 7c).

However, the nutritional constraint of protein intake apparently depends on the geometry of nutrients. Owing to the inverse relation between volumetric food intake and carbohydrate concentration, the daily protein intake was higher in trials with a low sugar content but intermediate lipid content (trials 5 and 6) than in trials with a high sugar content (trials 3 and 4) and in lipid trials. Furthermore, the degree to which lipids and carbohydrates were interchangeable depended on the carbohydrate concentration of diets. When feeding on high-sugar foods, birds preferred carbohydrates over lipids, but increased their lipid intake at low sugar concentrations (see negative slope Fig. 6a). There seemed to be an upper limit for the combined carbohydrate and lipid intake of 1.1 g/7 h. These different results show that nutritional constraints on feeding are nonlinear depending on the nutritional composition of the food. Interactions between nutrients might be modulated by physiological constraints or by postingestive mechanisms of differential use of nutrients (Zanotto et al. 1993, 1997). The nonlinearity of nutritional constraints on feeding has also been documented in the interactions between allelochemicals and macronutrients (Simpson & Raubenheimer 2001).

Conclusions

Tanagers were able to make fine-scale decisions about choosing food regardless of whether the energy source consisted of lipids or sugars. Their fine-tuned discrimination abilities enabled them to use strategies of complementary feeding. The consistent preferences for macronutrients, different proteins and amino acid concentrations of isocaloric foods indicate that mechanisms other than ODT explain food selection and that energy as sole currency is an inappropriate generalization of foraging behaviour. We also showed that nutritional composition and consequently nutrient regulation exert a strong influence on food intake in birds. Feeding constraints apparently depend on the geometry of nutrients. Because nutrient regulation has not yet been tested in detail for vertebrates, there is little indication of how nonlinear constraints affect food selection in different prey and consumer species. Hence, there is a clear need for studies to address both micronutritional differences in food and multinutritional requirements of consumers to obtain new insights into the complex foraging strategies of birds.

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