

Running title: Metabolizability of Hummingbirds' nectar

Title

Nutrients Metabolizability of Nectar for Long-Term Maintenance of *Amaziliaamazilia* and
Effect of a Dietary Fibre Source

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Abstract

An artificial nectar was formulated for long-term maintenance of captive hummingbirds. The objective of the experiments is to measure the metabolizability of nutrients in the artificial nectar and to evaluate the effects of dietary fibre addition on its digestive utilization. The fibre (inulin) has been used in replacement of some components that derive from arthropod intake. The trial was conducted on two groups of non-reproductive *Amaziliaamazilia* averagely weighing 4.0 g, giving nectar containing about 179 g/l dry matter (DM). DM content of sucrose and hexoses was 92.3%, (0.75 M), that of crude protein (CP) 4.3% and fat (EE) 2.3%. Inulin was added in the ratio of 7 g/100 g, as fed, of nectar powder. The daily DM intake of hummingbirds was about 2054 mg/d and upraised by 14%, to 2348 mg/d, when inulin was added to the nectar; the energy intake averaged 34 kJ/d. The average metabolizabilities of nectar were about 94% for DM and energy,

28 98.4% for sugars, 57% for CP and 44% for EE. The absorption of DM, sugars, energy and protein
29 were statistically reduced by inulin addition, instead that of ash increased. From the balance trial it
30 results that about one third of inulin should undergo a fermentation in the intestine. Adjustment of
31 sugar and protein contents and of protein quality to hummingbirds' species and rearing environment
32 should be useful.

33 *Keywords:* Captive hummingbirds, *Amazilia amazilia*, Long-term maintenance, Artificial nectar, Inulin, Hummingbird
34 *metabolizability*.

35

36 **1. Introduction**

37 Hummingbirds, according to their feeding habit, are defined as nectivorous. Around 320 species,
38 weighing from 2 up to 20 g have being classified in various environmental and ecological niches.
39 They collect nectar from many flower species, but also eat pollen, ash, sand, tree sap, spiders,
40 insects and other small arthropods (Remsen et al 1986, Stiles 1995). For a very long time in some
41 area of North, Central and South America wild hummingbirds are attracted offering artificial
42 nectars in feeders (Sherman 1913). Many commercial types of nectar, containing sugars and other
43 few ingredients, are offered to attract free-living hummingbirds, e.g. Roudybush, Wombaroo,
44 Nectar+, Perky-Pet.

45 More problematic is to formulate soluble complete diets to raise, maintain and breed hummingbirds
46 in captivity for life. From the available information, it appears that hummingbirds have been kept in
47 captivity since the middle of the 19th century, but the formulation of a complete diet, more or less
48 purified, was only occasionally investigated and made known in the past. Cordier (1953) proposed
49 an artificial nectar containing honey or sugar, meat extract and condensed milk, almond oil and
50 vitamins. Scheithauer (1967) fed hummingbirds various diets and different ingredients recording the
51 results of chemical analysis and the animal response, obtaining successful results in long-term
52 maintenance and in reproduction. Hainsworth and Wolf (1976) offered a sucrose solution 0.5 M
53 (171 g/l solution) added with 5.2 g/l of concentrate supplements containing protein, fat and

54 carbohydrates. Brice and Grau (1989) developed a purified liquid diet containing about 25% of dry
55 matter (DM), 80% sucrose, 3% protein (CP), delivered as commercial isolated soy protein
56 supplemented with DL-methionine, starch (5%), corn oil (3,7%), mineral and vitamins. Zanotto and
57 Bicudo (2005) use a maintenance diet composed by 20% sucrose, 0.5% amino acids (a.a.)
58 (Glicopan P), 3% oil, 0.03% vitamins (Vita Gold) and 1% calcium carbonate. Peaker (1990) dealt
59 with the subject of nutritional exigencies of captive hummingbirds discussing many issues and
60 suggested the formulation of a diet containing 88.5% of sugars, oil 2%, 6.5% of CP and a defined
61 amount of each of 20 a.a., supplemented with a complete array of mineral and vitamins. All these
62 formulations show some common issues and give essential indication on how to devise artificial
63 nectars for maintaining different hummingbird species for long time. It also needed to consider the
64 results of scientific research carried out either on free-living birds, focusing on feeding habit and
65 feeds preference and intake, or on captive individuals, for functional physiology and nutritional
66 requirements.

67 In practice, to successfully maintain and reproduce captive hummingbirds, the artificial nectar must
68 satisfy all nutritional needs, miming the inputs of a natural feeding. Energy and main nutrients
69 requirements for maintenance, flight and reproduction have been differently studied and indicated
70 (Weathers and Stiles 1989; Peaker 1990; McWhorther and López-Calleja 2000; López-Calleja et al.
71 2003). From on hand knowledge, it appears that hummingbirds do not deliberately forage for fibre,
72 intended as insoluble and indigestible carbohydrates of plants cell wall. Conversely, they prey
73 variable amount and species of arthropods rich of chitin, 5 ÷ 20% DM (Paoletti et al. 2009), a
74 polysaccharide which is main constituents of the exoskeletons of many arthropods, classified as
75 added fibre having physiological properties similar to dietary fiber ((NRC 2001; Thomas et al.
76 2013). The digestion of chitin requires chitinases (Barton and Northup 2011). Many bacteria and
77 fungi species, as well as certain invertebrates, fish, birds, terrestrial mammals and humans produce
78 chitinases, which permit the breakdown of polymeric chitin to oligosaccharides and monomeric
79 sugar subunits (Jeuniaux and Cornelius 1978; Jollès and Muzzarelli 1999; Paoletti et al. 2009).

80 Either chitinase production in birds and mammals could occur through a chitinoclastic bacterial gut
81 flora, or by glandular production of chitinases (Jeuniaux 1966; Jeuniaux and Cornelius 1978);
82 instead, according to Eurich et al. (2009), mammals lack chitinases activity. The chitin and chitosan
83 digestibility, mainly measured in marine species, but also in mammals and birds, is variable and
84 affect lipids digestion (Patton et al. 1975; Razdan and Patterson 1994; Hirano et al. 1990;
85 Nakagawa et al. 2007; Hossain and Blair 2007; Deuchi et al. 2014) .This topic appears not yet
86 investigated in hummingbirds as well as the role of this insoluble dietary fibre on digestion and
87 absorption of other nutrients and its potential beneficial physiologic and healthy effects.
88 If arthropods are not regularly available, other source of dietary fibre might be given in the artificial
89 nectar. Chitin, chitosan, produced by alkaline deacetylation of chitin obtained mainly from marine
90 crustacean, and other vegetable insoluble fibre sources are impractical, because insoluble deposit
91 sediments or surfaces in the feeders. Inulin, a heterogeneous assortment of fructose polymers found
92 in a number of vegetables, fruit, and grains, was already used as soluble fibrous ingredients in
93 animal nutrition and appears suitable for the scope (Flickinger et al. 2003; Verdonk et al. 2005). For
94 this reason, we consider to evaluate the effects of the addition of inulin on digestive utilization of
95 the artificial nectar. Beside the nutritional facts, when hummingbirds are maintained *ex situ*, also
96 health problems might arise and some aspects as intestinal mycoses, liver steatosis should be
97 monitored and prevented.
98 Available nutritional data are still incomplete and sketchy and some physiological aspects and
99 behaviours of hummingbirds are undisclosed so that some solution must be attained by trial and
100 error.
101 The objective of our experiments is to measure (i) the metabolizability of the nutrients in a complete
102 diet formulated to maintain in good health captive adult *Amazilia amazilia*, after a critical review of
103 the existing literature, and (ii) the nutritional effect of inulin addition to the nectar.

104

105 2. Materials and methods

106 In Miramare park (Trieste – Italy), at the Centre for the Safeguard of hummingbirds, in fully air
107 conditioned aviaries in a natural mimic environment with tropical plants and where fruit flies
108 (*Drosophila sp.*) were grown in buckets containing fruits, lived about 90 hummingbirds mainly
109 belonging to *Amaziliaamazilia* species. *Amazilia*aremedium-sized trochilid (length 8-11 cm, weight
110 4-7 g) that inhabits arid and semiarid habitats of the tropical Pacific lowlands and adjacent
111 subtropical Andean slopes, eg: edges of deserts scrub and dry forests, of Ecuador and Peru
112 (Koepche 1964, Weller 2000). It is a territorial and nectivorous bird, which by individuals or pairs
113 defends its territories against conspecifics or other nectivorous species (González 1998).

114 The nectar was formulated on the basis of a critical review of results from studies conducted in the
115 wild and in the laboratories, which has been summarized at the beginning of the Discussion (§ 4)
116 and progressively adjusted considering the intake and few vital and health parameters. Finally, we
117 have evaluated the nutrients metabolizability of the complete artificial nectar and the effect of the
118 addition of inulin on intake and metabolizabilities.

119 The experiment was conducted during the non-reproductive period. Adult *Amaziliaamazilia*
120 hummingbirds were randomly assigned to two groups of seven hummingbirds each, 4 males and 3
121 females, fed artificial nectar (Nt) or artificial nectar added with inulin (Ni). A week before the
122 beginning of the trial, each bird was housed separately in an experimental cage (0.80 m x 0.50 m x
123 0.90·m) fitted with a removable tray for *excreta* collection. Cages were allocated in a separate
124 experimental room kept at 25 °C and provided with constant light artificial photoperiod of 12·h L:
125 12·h D and with full spectrum lights bulbs for the “sun bath” (3 times/d for 30-40 min). In the
126 experimental environment, fruit flies were not present. During acclimation and experimental
127 periods, of 7 and 17 days, respectively, the nectar was offered *ad libitum* in a 13 ml glass calibrated
128 tube placed through a hole of the cage. To reduce the fractionation, due to precipitation or
129 surfacing, of less soluble or insoluble ingredientsand the fermentation, nectar was renewed twice
130 daily, at 7 am and at 3 pm; the volumetric nectar intakes were individually recorded at the same
131 time. Water was offered *ad libitum* in 10·ml water feeders and in birdbaths. Nectars were daily

132 diluting in 200 ml of distiller water 40 g, as fed, of Nt powder, or 43 g (inulin 3 g) to prepare Ni. Ni
 133 contains 7.11% of Inulin on DM basis. The volume of Nt solution, equals 210 ml and that of Ni 226
 134 ml. *Excreta* were collected from removable trays twice daily using plastic spatulas, put in plastic
 135 bottles, immediately weighed and frozen at -20°C. Before the chemical analysis, the two pools of
 136 faeces were weighed and thoroughly mixed in 250 ml of distilled water. DM was measured on 15
 137 ml of sample by drying at 60°C to constant weight. On the remaining CP (Nx6.25), Ether Extract
 138 (EE), ash (A.O.A.C., 1990), Neutral Detergent Fibre (NDF) (van Soest, 1982), were determined.
 139 The energy content (kJ) was either measured, using an adiabatic calorimeter (IKA C7000), and
 140 checked using the average energy values of different nutrients proposed by FAO (2003). Water-
 141 soluble carbohydrate contents (NFE) of DM was calculated according to the following: NFE=
 142 100% -% CP-% ash -% EE -% NDF. The results of feeds analysis, which are the average of
 143 duplicate determinations, are reported in Table 1.
 144

Table 1 Dry matter and nutrients content of feeds

Feed (% DM)	DM	CP	EE	CF	Ash	NDF	NFE	En (kJ/g)
Nt	93.76	4.28	2.30	0.28	0.95	0.19	92.28	16.63
Ni	93.89	3.99	2.14	0.26	0.88	0.17	92.81	16.66
Inulin	95.64	0.18	-	-	0.02	-	99.80	16.30
Nectar(g/l)								
Nt (g)	178.56	7.65	4.11	0.50	1.69	0.33	164.78	2970
Ni (g)	178.81	7.14	3.83	0.46	1.58	0.31	165.96	2979

145 DM: Dry Matter; CP: Crude Protein; EE: Ether Extract; CF: Crude Fibre; NDF: Neutral Detergent Fibre; NFE:
 146 Nitrogen Free Extract; En: Energy; Nt: artificial nectar; Ni: artificial nectar with inulin; F: Anova test; df: degree
 147 freedom.
 148

149 The apparent metabolizability of different nutrients was calculated using the following formula:
 150 Metabolizability (%) = [1-Intake (g)/*Excreta* (g)] x 100]. Data were statistically analysed by
 151 ANOVA, and the differences among group means were separated by Dunnett test, setting the
 152 significance levels at P<0.01 (SPSS Statistics 17.0). In the tables F is the ratio between variance of

the group means / mean of the within group variances; the significance level is obtained from the F distribution with numerator and denominator degrees of freedom (df).

The coefficient of variability (CV), calculated as a percentage of average/standard deviation ratio, was used to describe the range of variation of weights (LW), DM intake and excretion among animals.

Body weight was recorded throughout the acclimation and experimental period with an analytical balance (accuracy ± 0.001 g). Initial LW were 4.08 g and 4.14 g for Nt and Ni groups and at the end of the experimental period 3.98 g and 3.80 g, respectively.

The nectar powder (Nt) is prepared once a week using the same ingredients. The nectar contains Sucrose 47.2%, Glucose 19.2%, Fructose 19.2%, Hydrolyzed soy and collagen (Wellesse – Ferndale USA) 1.5%, a mix of essential a.a. 2.2% (Nutricia –Danone), Maize and Soybean oils 2.5%, Flower’s Bio-Pollen 4.6% (Fior di Loto – Torino, Italy), Silimarin (Legalon – Rottapharm-Madaus) 1.1%, Bio-Mos (Alltech) 1.1%, Mineral and Vitamins supplement 1.3%. As for sugar concentration, Nt and Ni were equimolar, 0.75 M or 0.5 M sucrose equivalent. Inulin was added to the nectar powder (Ni) in the ratio of 7 g/100 g, as fed. We used Inulin 90% (Farmalabor - Italy), extracted from *Dahlia variabilis*, *Helianthus tuberosus* and other *Compositae*. This inulin is classified as “soluble non digestible” fibre (solubility 120 g/l) and is composed by fructans which, having a β -configuration of the anomeric C2 in its fructose monomers, resists hydrolysis by intestinal digestive enzymes, which are specific for α -glycosidic bonds (Delzenne and Roberfroid 1994; Roberfroid and Slavin 2000).

173

174 **3. Results**

The chemical composition of nectars and the nutrients’ content of 1 l of solutions are in Table 1. Carbohydrates (NFE) make up the 92.3% DM of Nt, the CP and EE contents are 4.28% and 2.30% of DM, respectively. Sucrose constitutes the 55.1% of added sugars, the balance is equally provided by glucose and fructose, 22.4%; the difference between added sugars and NFE is due to

179 carbohydrates in the additives and other ingredients. The NDF content come from the natural
 180 ingredients used to make up the nectar, as pollen an probiotics, and corresponds to about 0.2% on
 181 DM.

182 The addition of Inulin – 7.1% of total DM - diluted the nutrients content of Ni. The nectar solutions
 183 contained the same amount of DM, 179 g/l, and were equimolar 0.75 M, or 0.5M sucrose
 184 equivalent.

185 The average daily intakes of hummingbirds are reported in Table 2. When hummingbirds received
 186 the nectar containing inulin, the DM intake was significantly increased ($P<0.01$) as those of the
 187 remainder nutrients. Inulin is included in NFE and, as expected, both DM and NFE intakes largely
 188 differ between the two nectars; though the intakes and the differences among the intakes of other
 189 nutrients between Nt and Ni were lesser, resulted statistically highly significant (Table 2). The daily
 190 energy intakes of Nt and Ni, 34.16 and 39.12 kJ/d respectively, were also significantly different
 191 ($P<0.01$).

Table 2 Average daily intake of nectar (ml) and nutrients (mg/d)

	Nt	Ni	F	df
ml	11.5 ^B	13.13 ^A	40.894	32
DM	2053.98 ^B	2348 ^A	41.76	32
CP	87.99 ^B	93.73 ^A	9.36	32
EE	47.3 ^B	50.23 ^A	8.45	32
Ash	19.48 ^B	20.72 ^A	8.92	32
NDF	3.81 ^B	4.05 ^A	8.45	32
NFE	1895.38 ^B	2179.25 ^A	45.42	32
En (kJ)	34.16 ^B	39.12 ^A	45.37	32
CF	5.72 ^B	6.08 ^A	8.45	32

193 DM: Dry Matter; CP: Crude Protein; EE: Ether Extract; CF: Crude Fibre; NDF: Neutral Detergent Fibre; NFE:
 194 Nitrogen Free Extract; En: Energy; Nt: artificial nectar; Ni: artificial nectar with inulin; F: Anova test; df: degree
 195 freedom.

196

197 The DM content of droppings was much lower than that of nectars, but its nutrient concentration
 198 was noticeably higher. The presence of inulin significantly increased the water content of
 199 droppings, which appear more viscous. The bulking effect of dietary fibre was evident: the
 200 excretion of DM and nutrients was significantly increased (Table 3). In consequence, the DM
 201 composition of droppings notably changed. As expected, a bigger quantity of NFE was excreted,
 202 due to the inulin intake (Tables 2, 3). Also the contents of CP and EE were significantly increased,
 203 while that of ash has been reduced. The amounts of NDF excreted are identical, nearly matching the
 204 intakes.

205

Table 3 Average daily excretion and chemical composition of droppings (mg/d)

	Nt	Ni	F	df
Fresh	1110.53 ^B	2890.08 ^A	84.872	32
DM	110.53 ^B	221.68 ^A	83.862	32
CP	37.8 ^B	46 ^A	8.472	32
EE	26.52 ^B	33.25 ^A	11.113	32
Ash	11.49 ^A	9.62 ^B	7.4871	32
NDF	3.70	3.84	0.306	32
NFE	30.39 ^B	128.35 ^A	219.035	32
En (kJ)	2.07 ^B	3.74 ^A	67.783	32
CF	6	6	0.325	32

206 DM: Dry Matter; CP: Crude Protein; EE: Ether Extract; CF: Crude Fibre; NDF: Neutral Detergent Fibre; NFE:
 207 Nitrogen Free Extract; En: Energy; Nt: artificial nectar; Ni: artificial nectar with inulin; F: Anova test; df: degree
 208 freedom.

209 The CV of individual nectar intake within days and animals was similar between the two groups.
 210 The CV was of 6.0%, lower than that observed for the excretion (CV = 19.1%).
 211 Hummingbirds receiving Nt on average maintain their LW (-11 mg), those of the Ni group lost
 212 some LW (-34 mg) during the period of 24 days.
 213 The metabolizabilities of normal nectar DM resulted 94.6% (Table 4).

Table 4 Energy, DM and nutrient metabolizability (%)

Nt	Ni	F	df
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DM	94.62 ^A	90.52 ^B	59.4460	32
CP	57.08 ^a	50.77 ^b	4.3355	32
EE	43.96 ^a	33.59 ^b	6.5733	32
Ash	41.05 ^B	53.43 ^A	14.0682	32
NDF	2.87	4.75	0.0925	32
NFE	98.39 ^A	94.09 ^B	191.0953	32
En (kJ)	93.93 ^A	89.99 ^B	51.2376	32
CF	1.95	3.74	0.0822	32

DM: Dry Matter; CP: Crude Protein; EE: Ether Extract; CF: Crude Fibre; NDF: Neutral Detergent Fibre; NFE:

Nitrogen Free Extract; En: Energy; Nt: artificial nectar; Ni: artificial nectar with inulin; F: Anova test; df: degree freedom.

The sugars metabolizability of Nt was almost complete and those of CP and of fat were 57% and 44%, respectively. The absorption of minerals equals 41.1%. The digestibility of CF and of cell wall components was negligible. The addition of Inulin significantly affected the metabolizability of DM, energy, CP, NFE and ash reflecting the statistically significant change of droppings composition (Table 3). The energy metabolizability of Nt was 94% and 90% that of Ni. Comparing the DM, NFE and energy excretions of the two nectars, we could deduce that, for the most part of Inulin, passed through the digestive tract.

4. Discussion

4.1 Nectar formulation

Many aspects have to be considered for formulating artificial nectar for long-term maintenance of different species of hummingbirds.

Wild hummingbirds meet their energy requirements essentially from nectar sugars, while protein needs are independently satisfied by eating a changeable amount of small arthropods (Gass and Montgomerie 1981; Pyke 1980; Hainsworth 1977; Des Granges 1978; Montgomerie and Redsell 1980; Brice 1992), other stuffs and possibly pollen (Wooller et al. 1988, Van Tets and Nicolson 2000). To the contrary, a diet of only arthropods cannot cover the energy requirements (Brice 1992; López-Calleja et al. 2003).

235 Flowers habitually visited by hummingbird produce 10-30 ml of nectar having a DM content of 15-
 236 40%; the sugar concentration varies between 0.15 M and 1.2 M: most of the nectars contain
 237 sucrose, fructose and glucose and rarely are composed by a single sugar (Hainsworth and Wolf
 238 1976; PykeandWaser 1981). Hummingbirds prefer flowers providing nectars with sucrose, fructose
 239 and glucose (Hainsworth and Wolf 1976) and with a higher proportion of sucrose to hexoses
 240 (Cruden et al. 1983; Freeman et al. 1984; Gottsberger et al. 1984; Martínez del Rio 1990; Martínez
 241 del Rio et al. 1992; Stiles and Freeman 1993; Chalcoff 2001); according to Nicolson & Fleming
 242 (2003), hummingbirds select nectars containing $64.4 \pm 18.5\%$ of sucrose. ~~When foraging, they make~~
 243 ~~up a changeable mixed diet along the day and this modifies continuously transit time, absorption~~
 244 ~~and metabolic adjustment.~~
 245 Less univocal is the sugars preference in captive hummingbirds. Environment, cage dimension,
 246 colour and shape of feeders and perches, but also imprinting or adaptation, bill length and licking
 247 capability look as if influencing sugars preference; the first adaptation to a definite sugar solution or
 248 to a frequent variations sugars solution requires several hours or feeding sessions (Hainsworth and
 249 Wolf 1976; Stiles 1976; Roberts 1996; Schmid 2008). Different results indicate that sucrose is
 250 generally preferred alone or when paired with glucose; on the other hand, sucrase activity always
 251 proved to be very high in hummingbirds (Martínez del Rio 1990; McWhorter and Martínez del Rio
 252 2000; Schondube and Martínez del Rio 2004). Captive birds always prefer the more concentrated
 253 sugar solution (Hainsworth and Wolf 1976; Schondube and Martínez del Rio 2003) and
 254 notwithstanding the negative relationship ($P < 0.02$) between the sucrose concentration and the rate
 255 of volumetric nectar intake, the amount of sugar ingested remains nearly the same or increases with
 256 the concentration (McWhorter and Martínez del Rio 1999 and 2000). The transit time of a bout
 257 increases with sucrose concentration (Tiebout III 1989, Martínez del Rio and Karasov 1990). The
 258 preference for solutions of sucrose and hexoses should be compared at the same concentration on
 259 equicaloric basis. At high concentration of sugars ($\geq 25\%$), hummingbirds do not discriminate
 260 among sugars; instead, at lower concentration, equicaloric, equimolar and equiweight solutions of

diverse combination of sugars rank in a different order of preference (Brown et al. 2008; Schmid 2008; Fleming et al. 2004).

Glucose is more slowly assimilated than other sugars, as birds rested longer between feeding bouts, making a glucose diet less efficient (Martinez Del Rio 1990). The digestion time rather than feed collection limits sugars' assimilation by hummingbirds (Diamond et al. 1986; Karasov 1986). Only at lower substrate concentration the passive absorption, by paracellular diffusion and solvent drag of glucose, significantly contributes to total absorption (Pappenheimer 1993; McWhorter et al. 2005; McWhorter et al. 2009).

According to these suggestions, we consider that the simultaneous presence of sucrose and its hydrolytic products at moderate concentration could represent a more valid starting option for hummingbirds living in a confined artificial environment. Then sucrose, glucose and fructose are added to compose 86% of the nectar DM, where sucrose contributes with the 56% to the mix of three sugars and the rest is equally balanced between the two hexoses. The rest of sugars come from pollen and supplements.

There are some grounds for inclining in the direction of artificial nectar having moderate sugars' concentration, which appears to favour hexoses preferences and absorption (Schondube and Martinez del Rio 2003); firstly, because the sugars' concentration of nectars does not influence the rate of daily energy intake (López-Calleja et al. 1997). The higher water absorption in the intestine is easily disposed by kidney, producing large water loads of diluted urine (McWhorter and Martínez del Rio 1999; Martínez del Rio et al. 2001). All this should favour intake enhancing the number of bouts, fastening the rate of ingestion, by keeping an efficient absorption. For these reasons, being in situation where nectar availability is not limiting and foraging does not require a high-energy cost, we considered preferable to try the option of offering hummingbirds relatively diluted nectar. The drinking solution was prepared to keep DM concentration around 18%, i.e. 0.5 M sucrose equivalent.

287 Energy and main nutrients requirements for maintenance, flight and reproduction have been
288 differently studied and indicated (Weathers and Stiles 1989; Peaker 1990; McWhorter and López-
289 Calleja 2000; López-Calleja et al. 2003; Tsahar et al. 2006). Net energy expenses were evaluated
290 using indirect methods for different conditions or activities, e.g. basal metabolism, torpor, flying,
291 hovering, perching, etc.. Requirements are usually quantified in Watts, as instantaneous
292 requirements. In many other cases, gross or metabolizable energy intakes were measured in feeding
293 trials. More often energy requirement is associated to time budget since different activities have
294 different energetic cost (Hainsworth 1977). Tiebout III (1991) instead of “requirement”, term
295 demoting to an additive evaluation of singly ascertained exigencies, not easy to correctly establish,
296 discern the notion of "energy management" to denote that the changeable behavioural and
297 physiological responses of an individual to specific conditions from time to time determine its total
298 energy budget. Sugars in the nectar provide birds for the most part of energy. Commonly, the daily
299 sugar intake is assumed to cover the energy requirement. The energy requirements of free-living
300 hummingbirds (FMR) has been measured with different methods or estimated in different
301 environmental situations. FMR results high and largely variable, from 29.1 to 81.7 kJ/d (Stiles
302 1971; Hainsworth and Wolf 1972; Tamm 1989; Ewald and Carpenter 1978; Weathers and Stiles
303 1989; Powers and Conley 1994, Suarez and Gass 2002). A frequently reported FMR reference
304 value, measured on *Caliptaeanna* hummingbirds of 4.5 g LW, is 31.8 kJ/d (Powers and Nagy 1988).
305 The experiments with captive animals have shown that the highly adaptive energy metabolism of
306 hummingbirds to thermal changes and dietary conditions can be nevertheless influenced by the
307 natural behaviours and foraging strategies of different species (Pearson 1950; Tiebout III 1991;
308 Garrison 1995; McWhorter and Martínez del Rio 1999). Reduced resting expenditures as night-time
309 torpor or the less severe daytime depression in metabolic rate (day-time hypometabolic
310 compensation) can balance foraging constraint (Bartholomew et al. 1957; Lasiewski 1963;
311 Hainsworth and Wolf 1976; Carpenter 1974; Hainsworth et al. 1977). Typically, the metabolic rate

312 measured in captivity ranges from 7.3 kJ/d to 10 kJ/d per g LW (Scheithauer 1967; Bennett and
313 Harvey 1987; McWhorter et al. 2005).

314 Then, if all these items should be carefully considered to formulate the nectar, its nutritive efficacy
315 could more simply ascertain by checking the energy intake and the body condition of
316 hummingbirds.

317

318 The overall concentration of a.a. in nectar of flowers lies within the range of 30-400 µg/ml and their
319 profile is variable; these amounts are considered insufficient to meet the requirements of
320 nectivorous vertebrates (Baker 1977; Baker and Baker 1973; Martínez del Rio 1994).

321 Scheithauer (1967) and Peaker (1990) report mean values of *Drosophila melanogaster* wet mass
322 between 1.2 and 1.5 mg, with a 60% of water. For Hainsworth (1977), the single DM weight of flies
323 is 1.5 mg. The protein content of arthropods is high, from 54% to 77% on DM, depending upon
324 species, the biological value of arthropods body protein, as results from a.a. profile, is variable and
325 usually rather high (Robel et al. 1995; Barker et al. 1998). Remsen et al. (1986) observed that
326 arthropods are extensively digested in the stomach within 3-4 hours, following only brief storage in
327 the crop. Evidently not all parts are equally digested; the components of soft body and some
328 constituents of cuticle (e.g. lipid, waxes and soluble protein) are probably quite digestible and
329 fragments of their body are detectable in faeces. Unfortunately, not all this information is supported
330 by values or measures.

331 In captive birds, a convenient and easy way to offer arthropods could be that of raising *Drosophila*
332 in the same aviary or in a bottle; *Diptera* are also within the preferred preys in free-living
333 hummingbirds. Brice (1992) and López-Calleja et al. (2003) observed that when nectar and fruit
334 flies are separately offered, their intakes are independently regulated.

335 The number of fruit flies a hummingbird has to ingest to cover N maintenance requirements is
336 differently quoted as 312 by Peaker (1990), 150 by López-Calleja et al. (2003), 283 by Lotz and
337 Martínez del Rio (2004). According to Scheithauer (1967), a hummingbird of 3 g LW ingests 677

flies/d. In contrast, Montgomerie and Redsell (1980) assume that hummingbirds could meet the daily protein requirement by ingesting only 38 flies each containing 2.1 mg of DM, with a protein content of about 60% and a metabolizability of 80%. López-Calleja et al. (2003) observed that *Sephanooidessephaniodes* weighting about 6 g, in addition to a nectar containing sucrose (0.75 mol/l) given *ad libitum*, required approximately 150 fruit flies to maintain body mass, which corresponds to a 4.55 mg/d N, being 78.8±6.4% the N “digestibility” of fruit flies. The CP ingested with *Diptera* equals 36 mg/d.

If the animal preys are not available, a protein substitute must enter the nectar composition in a prearranged fixed amount, as well as alternative sources of fat, ashes, and fibre. Hummingbirds do not specifically necessitate protein but are able to live eating a diet containing free a.a. (Baker and Baker 1979). There are no indications of the effect of the enrichment of the nectar with other nutrients on transit time; e.g. Klasing (1998) reports that dietary fat requires long time for digestion and can have marked impacts on the rate of passage in some species. Much probably, if a.a. are given, instead of *diptera* or of another source of protein, the absorbability and the transit time should be modified. As the prudential criteria was to guarantee an adequate intake of protein to cover synthesis of semi essential and non-essential a.a. and possible metabolizability inefficiencies, we opted for a nectar containing 4.28% on DM or 7.65 g/l of CP, from a balanced mix of a.a. with the addition of an extra amount of organic N (hydrolyzed protein).

On fat utilization by hummingbirds, any specific information has been experimentally obtained on its intake and absorption even if, in some experiments, diets were formulated adding oil (Brice and Grau 1989 and 1991; Peaker 1990; Zanutto and Bicudo 2005). Hummingbirds oxidize carbohydrates preferentially, but when sugar availability is shortened, as after overnight fasting, quickly switch to long chain fatty acids, also sparing the exiguous glycogen reserves (Suarez et al. 1990; Suarez and Gass 2002; Carleton et al. 2006). Body fat content is variable and changes during the day and with season. Highest variation are found in migratory species, which can store more than 40% of body

weight as fat (Odum et al. 1961). The hummingbird's migratory restlessness - *Zugunruhe*- corresponds to a fast fattening sustained by an increased intake of fat and protein following an intensification of insects catching (Hartman and Brownell 1959; Connell et al. 1960; Odum et al. 1961; Hargrove 2005). More probably, as hummingbirds maintain a high glycaemia and insulin level also in the fasted condition (Beuchat and Chong 1998; McWhorter and Martínez del Río 1999), should *de novo* synthesis and lipolysis continuously occur and fattening is the positive result of a short-term balance. Unfortunately, even if fat plays an important role in hummingbirds' metabolism, the fat requirement is unknown and the fat content of diet is indicated in few studies. The reported values for oil inclusion in nectar vary from 2% of crude oil (Peaker 1990), to 3% of corn oil (Zanotto and Bicudo 2005) and 3.7% of corn oil (Brice and Grau 1989 and 1991). The only information on daily lipids intake of about 90 mg can be inferred from the latter Authors' papers, who reported both, fat content of nectar and daily intakes. Arthropods should provide hummingbirds also with essential unsaturated fatty acids, being one of the richest animal feed sources (DeFoliart 1991). *Drosophila sp.*, are a significant source of fat (8-15%) and of mono (13-51%) and poly unsaturated fatty acids (8-31%) (Keith 1966; Green and Geer 1979; Robinson et al. 2000; Overgaard et al. 2005). ~~A valuation from *Drosophila*, made using the previously cited papers and assuming for the fat content of insects sampled in the aviary a value of 11.5% of fat content in insects sampled in the aviary, a variable intake, from 35, and 68 mg/d is obtained. The acidic composition indicates an amount of 51.7% of monounsaturated and 14.8% of polyunsaturated acids.~~ The effective exigencies of unsaturated fatty acids are unknown, but a certain amount of these fatty acids should be available to hummingbirds so that oil has to be added to nectar. This opportunity must be considered especially when captive birds have not the chance of preying live fruit flies. On this basis, it was decided to introduce in the formula 2.5% of a 1:1 mix of olive and soybean oils.

389 The hummingbirds play an important role in the pollination of many species of flowers (Bertin
390 1982; Arita and Martínez del Río1990; Collins et al.1990; Endress 2004),but do not collect pollen
391 and only groom it off their bills (Castellanos M.C. et al. 2003). Bent (1940) found pollen in the
392 hummingbirds' stomach. Pollen contains from 13% to 50% of sugars; free a.a. and protein contents
393 of above 40% have been reported, but the typical range is 7.5% to 35% (Stanley and Linskens 1974;
394 Solberg and Remedios 1980). According to Peaker (1990), the a.a. composition of pollen is similar
395 to that of animals and recommends the use of pollen as protein source in a complete artificial nectar.
396 The potentially digestible useful nutritive substances mainly confined within two walls, intine and
397 exine, highly resistant to degradation. Pollen is definitely a source of dietary fibre as intine , is
398 composed of pectocellulosic material and exine essentially made of sporopollenin, a polymer of
399 carotenes and carotenoid esters highly resistant to degradation (Stanley and Linskens 1974; Franchi
400 et al.1996). Samples of pollen collected or stored by bee indicate a variable content of crude fibre
401 (CF), principally cellulose, from 4.2 to 18.5% on DM (Herbert and Shimanuki 1978;Bell et al.
402 1983;Szcześna 2007). The hummingbirds' attitude for eating pollen and their capability of
403 extracting nutrients from ungerminated pollen is controversial (Wooller et al. 1988; Richardson and
404 Wooller 1990; Van Tets and Nicolson 2000). Brice et al. (1989) feeding Anna's and Costa's
405 hummingbirds 20% pollen on diet DM found that the digested amount was always nil or very low
406 (< 7%), however reported a higher digestibility of pollen in Anna's nestling hummingbirds, but
407 nestling are offered solid and slow passing food, mainly insects (Carpenter and Castronova 1980).
408 Klasing (1998) comments that pollen digestibility is limited by the fast transit trough
409 gastrointestinal tract, but it also depends upon bird's age and species.

410 There are two main issues to look at the proper formulation to satisfy the nutritive requirements in
411 artificial and confined environment and at the best DM concentration of nectar to attain a suitable
412 daily intake of nutrients and energy.

414 **4.2 Nutrients Intake and metabolizability**

415 The LW of hummingbird species largely varies and, to compare values, daily intakes are more often
 416 referred to metabolic weight. Hummingbirds suckled 886 vs. 1004 ml/kg^{0.75} of Nt and Ni nectar
 417 respectively, corresponding to 121.5 and 134.4 g/kgLW^{0.75} of DM. When inulin was added to the
 418 nectar, the hummingbirds increase the average daily intake by 14.3%.
 419 When concentration of sugars is low, as in this experiment, the intakes should be compared at the
 420 same molarity or energy or sugar contents. López-Calleja et al. (1997), with 0.75, 0.5, 0.25 M
 421 sucrose nectars, measured increasing intakes (8.6, 12.6 and 24.4 ml/d). McWhorter and Martínez
 422 del Rio (2000), with reference to captive hummingbirds of different species, determined that intake
 423 steadily increase when the sucrose molarity of nectar diminishes and, at 584 mmol/l equals 12 ml/d.
 424 Practically, higher the sugar molarity lower is the intake but, *ceteris paribus*, the overall daily
 425 consumption and the metabolizability are the same. In our experiment (0.5 M, sucrose equivalent),
 426 the intake of Nt was 11.5 ml/d and the sugars (NFE) metabolizability resulted 98.4%, within the
 427 range of the ordinary values. In fact, sugar assimilation efficiencies measured in hummingbirds
 428 using traditional balance methods are higher than 95% and by and large independent from sugar
 429 concentration and from *digesta* retention time (McWhorter and Martínez del Rio 2000; Schondube
 430 and Martínez del Rio 2003). Hainsworth (1974) measured sucrose digestibility of 98-99%; Karasov
 431 (1986), using a double isotope dilution method for measuring crop-emptying kinetics of
 432 *Selaphorusrufus* hummingbirds, found an efficiency of sucrose extraction of 97.1±0.3%. Duke
 433 (1997) reported that sucrose and hexoses are digested to the same high extent (> 97%).
 434 In our trial the DM intake of Nt nectar averaged 2.05 g/d (128 g/d/kgLW^{0.75}) and metabolizability
 435 resulted 94.6 %, as a combination of sugars metabolizability (98.4 %) and that of other nutrients
 436 which all together results 49.5 %.
 437 In different experiments, where diverse nectars were given to several hummingbird species, the
 438 daily intakes of metabolizable energy varied from 29.1 to 138.5 kJ, or from 5.63 to 9.32 kJ/d/g LW
 439 (Powers and Nagy 1988; Weathers and Stiles 1989; Powers and Conley 1994; López-Calleja and
 440 Bozinovic 2003; López-Calleja et al. 2003). Many factors affect the requirements, the intake and

441 the nutritional efficacy of the diet and the energy balance, practically evaluated by the LW
 442 variation, gives an inclusive validation. In all these experiments, the hummingbirds appear to have
 443 maintained their LW. The indicative average of nine experimental values equals 7.46 ± 1.09 kJ/d/g
 444 LW. The apparent metabolizable energy intake of Nt and Ni were 32.1 and 35.2 kJ/d, which
 445 correspond to 7.97 and 8.87 kJ/d/g LW, values which are consistent with other experimental results.
 446 The Nt group along the 24 days of trial basically maintained their LW and those receiving Ni
 447 nectar, lost a little of LW.
 448 Beside sugars, the Nt nectar contains other ingredients of different palatability and metabolizability,
 449 as protein, fat and minerals, which affect DM intake and metabolizability.
 450 In trial, with *Sephanoidessephaniodes* weighing about 5.5 g, (López-Calleja et al. 2003) compared
 451 nectars of different sugars and CP content – from 0 to 11.2%. They observed an increasing DM
 452 intake from 93 to 139 g/d/kgLW^{0.75} and a declining energy metabolizability (from 0.99 to 0.93);
 453 when CP was 4.7%, the DM intake was 119.5 g/d/kgLW^{0.75} and the metabolizability 0.97. From an
 454 experiment of Brice and Grau (1991), who offered a liquid diet containing a rising percentage of CP
 455 - from 0 to 3% in 25% solids – we can easily calculate to some extent an increase of DM
 456 consumption from 123 to 130 g/kgLW^{0.75}. In another experiment, López-Calleja and Bozinovic
 457 (2003), comparing two diets differing for sucrose molarity supplemented with protein, vitamins and
 458 minerals (8 g/l), measured a DM intake of 2.22 g/d or 110 g/d/kgLW^{0.75} (0.5 M) and 1.87 or 93 g/d
 459 (0.75 M); DM metabolizability in both groups was 0.95 ± 0.02 . Thus, when nectar contains protein,
 460 the intake is adjusted to sugars content and the DM metabolizability decreases. DM intake and
 461 metabolizability of diets comparable to Nt were similar to those we measured on *Amaziliaamazilia*.
 462
 463 The average individual intake of CP was 88 mg/d (Nt) which correspond to 5.5 g CP/d/kgLW^{0.75},
 464 equivalent to 14.1 mg Nitrogen (N)/d or 880 mg N/d/kgLW^{0.75} (Table 2). In the previously reported
 465 experiments, López-Calleja et al. (2003) substituting part of sugars with an increasing amount of
 466 protein (ProMod – Abbott), observed that CP intakes grew up to 313 mg/d (14.1 g CP/d/kgLW^{0.75}),

467 In this experiment and in that of Brice and Grau (1989), the DM intake increases by means of
 468 protein content of DM. Also Zanotto and Bicudo (2005) varying in two diets the protein/sucrose
 469 ratio, measured (in 1.5 hours) a higher nectar and protein intake with high protein-low carbohydrate
 470 diet. López-Calleja and Bozinovic (2003) measured a daily CP intake of 80 mg (0.5M) and 40 mg
 471 (0.75M), corresponding to 3.8 and 1.9 g CP/d/kgLW^{0.75} and observed that the different CP intakes
 472 depend upon sugar molarity. These and other results (McWhorter et al. 2003; Brice and Grau 1991),
 473 lead to assume that, in a complete nectar diet, the frequency of bouts and the DM intakes depend
 474 upon the sugar concentration, but not from that of N. N excretion, when measured, results positively
 475 and, within a “physiological” level, almost positively correlated with the intake.

476 To conclude, when protein is offered in the nectar, hummingbirds are not capable to adjust the N
 477 intake to their exigencies, so that the CP content of the nectar solution must be decided on the basis
 478 the rate of ingestion of DM and the N metabolizability, verifying that the intake correspond to the
 479 requirements.

480 The protein content of nectar DM given hummingbirds to maintain their body condition varies from
 481 2.4 % up to 6.5 %; usually the level protein considered more appropriate seems to be of about 4.5 %
 482 CP on DM (Brice and Grau 1989; Peaker 1990; López-Calleja et al. 2003; López-Calleja and
 483 Bozinovic 2003; McWhorter et al. 2003).

484 In our experiment, giving Nt containing 4.3% CP on DM, the average intake of CP was 88 mg/d or
 485 8 mg/d of apparently metabolizable N (3.1 g/kg^{0.75}/d of metabolizable CP).

486 On protein requirements and feeding of hummingbirds, not much information is available, but is
 487 generally recognized that they have low N requirements compared to non nectarivorous birds (Brice
 488 and Grau 1991; López-Calleja et al. 2003; McWhorter et al. 2003, Tsahar et al. 2006). Robbins
 489 (1993) proposed simple equations, frequently used to weigh up the N requirement of passerines and
 490 non-passerines. The Minimal N Requirement (MNR) is satisfied by 430 mg N/kg^{0.75}/d, being the
 491 Total Endogenous Nitrogen Losses (TENL) of 270 mg N/kg^{0.75}/d. Peaker (1990), on the basis of
 492 this MNR value, suggest a CP content of 6.5 % DM in complete nectar for hummingbirds,

493 assuming a N metabolizability no less than 80%. Presumably, this high metabolizability should be
 494 achieved if the a.a. content of diet is that defined by the Author.
 495 Brice and Grau (1991), in an experiment on *Calypte costae* hummingbirds of 3.8 g LW, measured a
 496 similar MNR and TENL of 77.1 and 77.8 mg N/kg^{0.75}/d. Also López-Calleja et al., 2003, in the
 497 previously cited research on N requirement of *Sebanoides sebanoides*, comparing MNR and
 498 TENL, measured giving also a “Zero N” nectar, obtained an identical value of 67.9·mg/kg^{0.75}/d.
 499 McWhorter et al. (2003), offering three hummingbirds species of different LW a nectar containing
 500 casein acid hydrolyzate (Sigma®), gave evidence of a variable MNR. The values went from 85.5 to
 501 158 mg/kg^{0.75}/d, and TENL, from 46 to 77.7 mg/kg^{0.75}/d; in a previous experiment, on a fourth
 502 species, measured a MNR of 62.5 mg/kg^{0.75}/d and a TENL of 45.5 mg/kg^{0.75}/d (McWhorter 1997).
 503 López-Calleja et al. (2003), shifting the N content in equicaloric nectars from 1.2 up to 11.2%,
 504 registered daily N intakes varying from 5.2 to 50.1 mg (from 32 to 316 mg CP) and only the
 505 hummingbirds receiving at least 4.7% CP did not lose weight. The digestibility coefficients were
 506 inversely related to the protein content and significantly differed among diets, decreasing from
 507 68±7% to 48±3%. Zanutto and Bicudo (2005) also comment that these birds are unable to
 508 efficiently assimilate N in high protein diets, because of a protein overload.
 509 On the basis of these results, Tsahar et al. (2006) in a notable discussion paper on N requirements of
 510 birds, reassessing Robbins’ allometric equation, propose for nectarivorous-frugivorous birds an
 511 average MNR of 152 mg N/kg^{0.76}/d and TENL of 54.1 mg N/kg^{0.69}/d. To compare the reported
 512 MNR and TENL, we recalculated the values according these metabolic weights, always obtaining
 513 lower values than the two averages calculated by Tsahar et al. (2006). Both MNR and TENL varied
 514 giving evidence that this requirement is largely influenced by nectar composition and by
 515 hummingbirds’ species, rearing and experimental conditions.
 516
 517 The relationship between MNR and TENL values measured in all these experiments depends also
 518 on CP metabolizability or retention efficiency, that is the slope of the linear regression between

519 ingested and retained N. Amino acids, if are not quickly deaminated, are used mainly for synthesis
 520 of skeletal muscles and, to a lesser extent, of other protein compounds. The metabolizability
 521 depends on the biological value of protein. In the wild, hummingbirds complemented the nectar
 522 feeding catching arthropods the protein of which is highly metabolizable, $78.8 \pm 6.4\%$ according to
 523 López-Calleja et al. (2003). The few information of N metabolizability experimentally measured
 524 using different protein supplements are always lowers than that from fruit flies. McWhorter et al.
 525 (2003), using casein acid hydrolyzate (SIGMA – St. Louis), measured N metabolizability within 49
 526 and 54 %. López-Calleja et al. (2003), giving different amounts of hydrolyzed collagen (Promod –
 527 Abbott, Columbus, USA), calculated a declining metabolizability from 68% to 48%, increasing with
 528 protein intakes. In our trial, the protein metabolizability was 57 %.
 529 All these N sources solubilised or dispersed in the nectar, other than natural supplies which are
 530 separately ingested, should differently modify osmolarity and transit time and *affect* the intakes.
 531
 532 The average lipids intake of hummingbirds during the trial was of 47 mg/d and the excretion of
 533 26.52 mg/d; the fat concentration in droppings DM was higher than 25%, but we had already
 534 observed that hummingbirds' *excreta* appear "greasy". In our trial, the lipids metabolizability in
 535 hummingbirds receiving normal nectar was 44%, indicating a moderate but effective absorption.
 536 Weight for weight the assimilated lipid was less than 1% of that of digested NFE. Under an
 537 energetic point of view, this value does not appear relevant to captive hummingbirds, but oil brings
 538 also indispensable essential fatty acids.
 539
 540 The intake of fibrous fractions (NDF) is reported in Tables 2. The basal intake was of about 3-4
 541 mg/d. The amount of fibrous fraction of droppings averagely excreted by hummingbirds (Tables 3)
 542 practically matches the intakes (Tables 2 and 3). The digestibility of CF and of NDF, did not
 543 significantly differ between nectars and was always very low and variable.

544 The addition of inulin to the nectar was followed by an increase of DM intake of about 14% (Table
545 2). Apart from significantly modifying the intake, the dietary fibrous source added to normal nectar
546 significantly affected the nutrients' excretion and thus their metabolizability: those calculated for
547 DM, NFE and Energy were reduced by about 4 %; higher differences were measured for CP and
548 EE, instead the absorption of ash was increased. Roberfroid (2007) comments that various
549 hypotheses have been tested to tentatively explain the modifications of the digestion and absorption
550 of macronutrients, especially carbohydrates and triglycerides, and of minerals which may occur in
551 animals and humans, either by delaying gastric emptying and/or shortening small intestinal transit
552 time (Oku et al. 1984). In fact, the increase of daily DM intake and excretion stands for a shortened
553 transit time. The absorption of ashes was significantly higher with Ni (53.4%) than Nt (41.1%).
554 Similar effect of inulin has been observed in poultry, other animal species and in man (Ortiz et. al.
555 2009; Awad et al. 2013). This result also supports the occurrence of an acidification of distal
556 intestine and cloaca. In fact, even if minerals are absorbed along the intestine, a higher assimilation
557 takes place after fermentation as a lower intestinal pH increases mineral solubility and facilitates
558 absorption (Levrat et al. 1991; Lopez et al. 2000).

559 The DM excretion was about doubled by inulin addition, but the difference between the DM and
560 NFE contents of droppings collected from birds fed Nt and Ni nectar was lower than the inulin
561 intake. We have tried to interpret our results, on which there is any specific reference, on the basis
562 of existing knowledge and the rationale which premised this experiment. Crossing data of NFE
563 intakes and excretions in Nt and Ni birds, the inulin disappearance through the digestive tract should
564 concern less than one third of the intake (*i.e.* ~ 55 mg/d). In animals, inulin passes through the
565 stomach and duodenum undigested and is easily available to the gut microorganisms. Preest et al.
566 (2003) report that hummingbirds possess high density of intestinal bacteria and a degradation
567 activity on urinary uric acid, urea and urates, always there, to ammonia has been observed. So that
568 also a fermentation activity on sugars could occur with production of volatile fatty acids (VFA).
569 The viable presence of undigested inulin in the lower gut, where presumably stays longer, could

570 favour the presence of microorganisms producing also different alloenzymes, *e.g.* inulinase, which
571 modify to a certain extent indigestible carbohydrates. The major limit to this potential activity is the
572 swiftness of transit as fluid passes through a hummingbird's digestive tract in about 0.5 h.
573 (Hainsworth and Wolf 1972). In birds, diverse components of the same meal may move at greatly
574 different rates through the different tracts of digestive system (Klasing 1998). Soluble and insoluble
575 fraction of dietary fibre have a high water holding capacity and if not digested they provide bulk to
576 the droppings, differently altering the transit time of nectar in the different sections of the whole
577 digestive tract.

578

579 **5. Conclusions**

580 In this research, we tested a complete diet, which seems to satisfy the major requirement of
581 hummingbirds. In fact, only sugar quality and concentration in the nectar were studied in depth.
582 Few, but consistent information is available on protein, but not with reference to its quality. The
583 knowledge on exigencies of fat and fatty acids, structural, homeostatic and trace elements, vitamins
584 is scarce. Next to that, little information exists on the digestive interaction among nutrients and
585 simple feeds, which are necessary to obtain a relatively complete and balanced nutrients' input.
586 Beside nutritional issues, we also have to deal with dietetic and health aspects. In fact, some deaths,
587 due to infections as aspergillosis and candidiasis, happened and occasionally the necropsy revealed
588 an anamnesis resembling the occurrence of steatosis and/or hepatosis of uncertain origin. This
589 induced to add MOS, Silimarín and inulin to nectar and to consider even the use of a prebiotic as
590 inulin, evaluating their effect on the nectar digestion.

591 The attempt of simultaneously measuring the metabolizability of main nutrients of a feed open to a
592 different view on the digestive efficiency of hummingbirds and helps in testing other ingredients
593 than the few generally used. In fact, there is a large difference between the sugars metabolizability
594 and that of other nutrients, so that the content of protein, fat and other ingredients in non-useful
595 amount reduces the overall DM metabolizability reducing the sugar intakes. There is much to

understand when we consider that typically about 40 single nutrients among a.a., fatty acids, hydro and liposoluble vitamins and minerals are required in the diets of birds.

The only option given captive hummingbirds was that of varying the intake and preying fruit flies. For practical reasons, only one or two nectar recipes could be prepared and offered to hummingbirds kept in an artificial environment. The results of these trials offered some indications that should be considered for a necessary further improvement or adaptation to different rearing conditions and hummingbirds' species.

The average daily intake of DM was 2.05 g/d. The average volumetric consumption of nectar and the DM intakes find a good agreement with other experimental evidences obtained with nectar of similar sucrose molarity. The option of using sucrose and hexoses in a ratio resembling that of floral nectars preferred by hummingbirds seems to guarantee a satisfactory volumetric intake and an overall sugars digestibility. The intake of sugars and energy, which correspond to about 130 g/kg^{0.75}LW/d and 2150 kJ kg^{0.75}LW/d, seem adequate to cover the maintenance requirements.

When intake values are given as metabolizable N per unit of metabolic weight, we measured an intake (about 500 mg N/ kg^{0.75}LW/d) which was about twice the more suitable indication obtained by other Authors for the maintenance (~265 mg/d – López-Calleja et al. 2003). On the basis of existing knowledge and on the result of this trial, assuming a CP metabolizability of 50-60%, the suggestion is to formulate the nectar containing not less than 3.5% of protein or even more if birds have to cover requirement for reproduction and molt.

The addition of dietary fibre significantly reduced the amount of absorbed fat. We try an interpretation of these results considering a possible effect of the inulin on the transit time, but this remains a nutritional aspect which requires more careful evaluation. In fact, the outcome from fat intake and digestibility of fat could give two opposite practical indications. The energy requirements of captive hummingbirds can be easily satisfied by sugars and do not need extra energy source, but a small amount of vegetal is useful to meet essential fatty acids exigency, which is unspecified, but probably not avoidable. On the other hand, if we assume that fat could be

anyhow necessary, the low assimilation suggest to add oil in the amount commonly adopted (2-2.5%) to guarantee a small but significant metabolizable quantity.

The addition of soluble fibre modified intakes and decreased the metabolizabilities of nutrients. Positive dietetic effects should compensate this negative consequence. The main role of fibre was thought to substitute for the indigestible fractions of fruit flies. For inulin a prebiotic effect could also be devised, with some benefit to the health condition of a captive bird living in an aviary. Inulin could be usually added to nectar also in a higher amount, but avoiding undesired effects on nectar intake and nutrients metabolizability.

630

631 **Ethical issues**

The experiment was conducted according the Ethical recommendations adopted by the University of Udine with reference to “Directive. 86/609/EEC concerning the protection of animals used for experimental and other scientific purposes”.

635

636 **Competing Interests**

The authors declare that they have no competing interests.

638

639 **Authors' Contributions**

CF: has made the contribution to design of experimental work, to acquisition, analysis and interpretation of data. She was also involved in the writing of manuscript.

SR: has made the contribution to formulation of the diets and in the revision of the paper.

PAS: has coordinated all the experimental work and has made contribution in writing manuscript and in the critical revision.

Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. All authors read and approved the final manuscript.

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663 **References**

664

- 665 1. A.O.A.C. (1990) Official Methods of Analysis of the Association of Official Analytical
666 Chemist. 16th ed., Publ. Washington, DC, USA. 931-933.
- 667 2. Arita HT, C. Martínez del Río. Interacciones flor-murciélago: un
668 enfoque zoocéntrico. Publicaciones Especiales del Instituto de Biología, UNAM, 1990;4:1-35.
- 669 3. Awad AW, Ghareeb K, Paßlack N, J Zentek. Dietary inulin alters the intestinal absorptive
670 and barrier function of piglet intestine after weaning. Res Vet Sci. 2013;5:249-254.
- 671 4. Baker HG, Baker I. Nectar constitution and pollination – plant coevolution. In Plant Animal
672 Co-Evolution (ed. L. E. Gilbert and P. H. Raven), pp. 100-140. Austin: University of Texas
673 1973.

- 674 5. Baker HG. Non-sugar chemical constituents of nectar. *Apidologie*. 1977;8:349-356.
- 675 6. Baker HG, Baker I. Sugar ratios in nectar. – *Phytochem Bull*. 1979; 2:43-45.
- 676 7. Barker D, Fitzpatrick MP, Dierenfeld ES. Nutrient composition of selected whole
677 invertebrates. *Zoo Biol*.1998;17: 23-134.
- 678 8. Bartholomew GA, Howell TR, Cade TJ. Torpidity in the whitethroated swift, Anna
679 hummingbird, and poor-will. *Condor*. 1957;59:145–155.
- 680 9. Barton LL and Northup DE. *Microbial ecology*. Wiley-Blackwell 2011.
- 681 10. Bell, RR, Thornber EJ, Seet JLL, Groves MT, HO NP, Bell DT. Composition and protein
682 quality of honey bee collected pollen of *Eucalyptus marginata* and *Eucalyptus calophylla*.
683 *Journal of Nutrition* 1983;113(12):2479–2484.
- 684 11. Bennett P, Harvey P. Active and resting metabolism in birds—allometry, phylogeny and
685 ecology. *J Zool*. 1987;213:327–363.
- 686 12. Bent AC. *Life Histories of North American Cuckoos, Goatsuckers, Hummingbirds and
687 Their Allies*. Washington DC: US National Museum Bulletin. 1940.
- 688 13. Bertin RI. Floral biology, hummingbird pollination and fruit production of trumpet creeper
689 (*Campsisradicans*, Bignoniaceae) .*Am J Bot*. 1982;69; 1: 122-134.
- 690 14. Beuchat CA, Chong CR. Hyperglycemia in hummingbirds and its consequences for
691 hemoglobinglycation. *Comp BiochemPhys A*. 1998;120:409–416.
- 692 15. Brice AT, Grau CR. Hummingbird nutrition: development of a purified diet for long-term
693 maintenance. *Zoo Biol*. 1989;8:233-237.
- 694 16. Brice AT, Dahl KH, Grau CR. Pollen digestibility by hummingbird and psittacines. *Condor*.
695 1989;91:681-688.
- 696 17. Brice AT, Grau CR. Protein requirements of Costa's Hummingbirds *Calypste costae*. *Physiol*
697 *Zool*. 1991;64:611–626.
- 698 18. Brice AT. The essentiality of nectar and arthropods in the diet of the Anna's Hummingbird
699 (*Calypsteanna*). *Comp Biochem Phys*. 1992;101:151–155.

19. Brown M, Downs CT, Johnson SD. Sugar preferences of nectar feeding birds—A comparison of experimental techniques. *J Avian Biol.* 2008;39:479-483.
20. Carleton SA, Bakken Hartman B, Martínez del Río C. Metabolic substrate use and the turnover of endogenous energy reserves in broad-tailed hummingbirds (*Selasphorus platycercus*). *J Ex. Biol.* 2006;209:2622–2627.
21. Carpenter FL. Torpor in an Andean hummingbird: its ecological significance. *Science.* 1974;183:545–547.
22. Carpenter FL, Castronova JL. Maternal diet selectivity in *Calypteanna*. *Am Midl Nat.* 1980;103:175-179.
23. Castellanos MA, Wilson P., Thomson JD. Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution.* 2003;57(12): 2742–2752.
24. Chalcoff VR. Composición de azúcares de la flora ornitófila del Bosque Templado de Sudamérica Austral: efectos históricos y preferencias presentes de *Sephanoides sephaniodes*. Undergraduate Thesis, Universidad Nacional del Comahue, Argentina 2001.
25. Collins BG, Grey J, McNee S. Foraging and nectar use in nectarivorous bird communities, p. 110-121. In: M. L. Morrison, C. J. Ralph, J. Verner, and J. R. Jehl, Jr. (eds.) *Avian foraging: theory, methodology, and applications*. Lawrence: Cooper Ornithological Society (Studies in Avian Biology 13) 1990.
26. Connell CE, Odum EP, Kalem H. Fat-free weights of birds. *Auk.* 1960;77: 1-9.
27. Cordier A (1953) A better way to feed hummingbirds. *Avic Mag.* 1960;58(4):143-145.
28. Cruden RW, Hermann S, Peterson S. Patterns of nectar production and plant-pollinator coevolution. In B. Bentley and S. Thomas (Eds.). *The biology of nectaries*, pp. 80-125. Columbia University Press, New York 1983.

- 725 29. DeFoliart GR. Insect fatty acids: similar to those of poultry and fish in their degree of
726 unsaturation, but higher in the poly- unsaturates. Food Insects Newslett. 1991;4:1-4.
- 727 30. Delzenne N, Roberfroid MB. Physiological effects of nondigestible oligosaccharides.
728 Lebensm-Wiss Technol. 1994;27:1–6.
- 729 31. Des Granges JL. Organization of a tropical nectar feeding bird guild in a variable
730 environment. Living Bird. 1978;17:199-236.
- 731 32. Deuchi K, Kanauchi O, Imasato Y, Kobayashi E. Decreasing effect of chitosan on the
732 apparent fat digestibility by rats fed on a high-fat – Bioscience, Biotechnology. 2014; 58(9):
733 1613-1616.
- 734 33. Diamond JM, Karasov WH, Phan D, Carpenter FL. Digestive physiology is a determinant of
735 foraging bout frequency in hummingbirds. Nature. 1986;320:62–63.
- 736 34. Duke GF. Gastrointestinal physiology and nutrition in wild birds. Proc. of the Nutrition
737 Society. 1997;56:1049-1056.
- 738 35. Endress PK. Diversity and evolutionary biology of tropical flowers - Cambridge Univ.
739 Press, 2004.
- 740 36. Eurich K, Segawa M, Toei-Shimizu S, Mizoguchi E. Potential role of chitinase 3-like-1 in
741 inflammation-associated carcinogenic changes of epithelial cells. World J Gastroenterol.
742 2009;14; 15(42):5249-5259.
- 743 37. Ewald PW, Carpenter FL. Territorial responses to energy manipulations in the Anna
744 hummingbird. Oecologia (Berlin) 1978;31:277–292.
- 745 38. FAO - Food and Agricultural Organization. Food energy: methods of analysis and
746 conversion factors. Report of a technical workshop. FAO Food and Nutrition paper No. 77.
747 Rome 2003.
- 748 39. Fleming PA, Bakken BH, Lotz CN, Nicolson SW. Concentration and temperature effects on
749 sugar intake and preferences in a sunbird and a hummingbird. FunctEcol 2004;18:223-232.

40. Flickinger EA, Van Loo J, Fahey GC. Nutritional Responses to the Presence of Inulin and Oligofructose in the Diets of Domesticated Animals: A Review. *Critical Reviews in Food Science and Nutrition*. 2003; Vol. 43, Iss. 1.
41. Franchi GG, Franchi G, Corti P, Pompella A. Microspectrophotometric evaluation of digestibility pollen grains. *Plants Food for Human Nutrition*. 1997;50: 115-126.
42. Freeman CE, Reid WH, Becvar JE, Scogin R. Similarity and Apparent Convergence in the Nectar-Sugar Composition of Some Hummingbird-Pollinated Flowers. *Bot Gaz*. 1984; Vol. 145, 1:132-135.
43. Garrison JSE. Traplining foraging behavior in a tropical hummingbird species *Phaethornissuperciliosus*. M.Sc. Thesis, University of British Columbia 1995.
44. Gass CL, Montgomerie RD. Hummingbird foraging behavior: decision-making and energy regulation. In *Foraging Behavior: Ecological, Ethological and Psychological Approaches* (ed. A. C. Kamil and T. D. Sargent), pp. 159-194. New York: Garland STMP Press 1981.
45. González O. *Coerebaflaveola*, un ave nueva en el ecosistema de losparques de la ciudad de Lima. *Ecología* 1998;1: 79-83.
46. Gottsberger G, Schrauwen J, Linskens HF. Amino acids and sugars in nectar, and their putative evolutionary significance. *Plant SystEvol*. 1984;145:55-77.
47. Grant KA, Grant V. *Hummingbirds and their flowers*. Columbia Univ. Press, New York and London. 1968; 115 p.
48. Green PR, Geer BW. Changes in the fatty acid composition of *Drosophila melanogaster* during development and ageing. *Arch IntPhysiolBiochim* 1979;87:485-491.
49. Hainsworth FR. Food quality and foraging efficiency. *J Comp Phys*. 1974;88:425-431.
50. Hainsworth, F. R., 1977. Foraging efficiency and parental care in *Colibricoruscans*. *Condor* 79:69-75.
51. Hainsworth FR, Wolf LL. Crop volume, nectar concentration and hummingbird energetics. *Comp Biochem Phys*. 1972;42A:359-366.

- 776 52. Hainsworth FR, Wolf , LL. Nectar characteristics and food selection by hummingbirds.
777 Oecologia 1976; 25:101-114.
- 778 53. Hargrove JL. Adipose energy stores, physical work, and the metabolic syndrome: lessons
779 from hummingbirds. Nutr J. 2005;4:36.
- 780 54. Hossain S, Blair R. Chitin utilisation by broilers and its effect on body composition and
781 blood metabolites. British Poultry Science 2007; Vol 48;1.
- 782 55. Hartman FA, Brownell KA Liver lipids in hummingbirds. Condor. 1959;61:273-279.
- 783 56. Herbert EW, Shimanuki H. Chemical composition and nutritive value of bee collected and
784 bee stored pollen. Apidologie. 1978; 9:33-40.
- 785 57. Hirano S, Seino H, Akiyama Y, Nonaka I. Chitosan: a biocompatible material for oral and
786 intravenous administration in: Progress in Biomedical Polymers. Eds. Gebelein CG and
787 Dunn RL - Plenum Press, New York: 283-290.1990.
- 788 58. Jollès P, Muzzarelli RAA. Chitin and Chitinases. EdsBirkhäuserVerlag, Basel 1999. ISBN
789 3-7643-5815-7
- 790 59. Karasov WH. Energetics, physiology and vertebrate ecology. Trends EcolEvol. 1986;1:101-
791 104.
- 792 60. Kay RF,Sheine WS. On the relationship between chitin particle size and digestibility in the
793 primate Galagosenegalensis. Am J PhysAnthropol. 1979;50 (3): 301-308.
- 794 61. Keith AD. Analysis of lipids in Drosophila melanogaster. Com. Biochem Phys.
795 1966;17:1127-1136.
- 796 62. Klasing KC. Comparative Avian Nutrition. CAB International; ISBN: 0851992196 – UK
797 1998.
- 798 63. Koepche, M. Las aves del departamento de Lima. Morson, Lima Perú p. 118 1964.
- 799 64. Lasiewski RC. Oxygen consumption of torpid, resting, active, and flying hummingbirds.
800 Physiol Zool. 1963;36:122-140.

- 801 65. Levrat MA, Rémésy C, Demigné C. High propionic acid fermentations and mineral
802 accumulation in the cecum of rats adapted to different levels of inulin. *J Nutr.*
803 1991;121:1730–1737.
- 804 66. Lopez HW, Coudray C, Levrat MA, Coudray CF, Demigne C, Remesy C. Fructo
805 oligosaccharides enhance mineral apparent absorption and counteract the deleterious effects
806 of phytic acid on mineral homeostasis in rats; *J NutrBiochem.* 2000;11 500–508.
- 807 67. López-Calleja MV, Bozinovic F, Martinez del Rio C. Effects of sugar concentration on
808 hummingbird feeding and energy use. *Comp BiochemPhys.* 1997;118A:1291-1299.
- 809 68. López-Calleja MV, Bozinovic F. Dynamic energy and time budgets in hummingbirds: a
810 study in *Sephanoides sephaniodes*. *Comp BiochemPhys A.* 2003;134(2):283-295.
- 811 69. López-Calleja MV, Fernández MJ, Bozinovic F. The integration of energy and nitrogen
812 balance in the hummingbird *Sephanoides sephaniodes*. *J Exper Biol.* 2003;206:3349-3359.
- 813 70. Lotz CN, Martínez del Rio C. The ability of rufous hummingbirds *Selasphorus rufus* to
814 dilute and concentrate urine. *J Avian Biol.* 2004;35:54-62.
- 815 71. Martínez del Rio C. Sugar preferences in hummingbirds: the influence of subtle chemical
816 differences on food choice. *Condor.* 1990;92:1022-1030.
- 817 72. Martínez del Rio C, Baker HG, Baker I. Ecological and evolutionary implications of
818 digestive processes: bird preferences and the sugar constituents of floral nectar and fruit
819 pulp. *Experientia.* 1992;48:544-551.
- 820 73. Martínez del Rio C. Nutritional ecology of fruit eating and flower visiting birds and bats. In
821 *The Digestive System in Mammals: Food Form and Function* (ed. D. J Chivers and P.
822 Langer), pp. 102-127. Cambridge: Cambridge University Press 1994.
- 823 74. Martínez del Rio C, Karasov WH. Digestion strategies in nectar- and fruit-eating birds and
824 the sugar composition of plant rewards. *Am Nat.* 1990;136:618-637.

- 825 75. Martínez del Rio C, Schondube JE, McWhorter TJ. Intake responses of nectar feeding birds:
826 digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects.
827 Am Zool. 2001;41:902–915.
- 828 76. McWhorter TJ. Energy assimilation, protein balance, and water absorption in broad-tailed
829 hummingbirds, *Selasphorus platycircus*. M.S. Thesis, University of Wyoming, Laramie. vii
830 + 89 pp 1997.
- 831 77. McWhorter TJ, Martínez del Rio C. Food ingestion and water turnover in hummingbirds:
832 how much dietary water is absorbed? J Exper Biol. 1999;202:2851-2858.
- 833 78. McWhorter TJ, López-Calleja MV. The integration of diet, physiology, and ecology of
834 nectar feeding birds. Rev ChilHist Nat. 2000;73:451–460.
- 835 79. McWhorter TJ, Martínez del Rio C. Does gut function limit hummingbird food intake?
836 PhysiolBiochem Zool. 2000;73, 313–324.
- 837 80. McWhorter TJ, Powers D, Martínez del Rio C. Ammonotely, nitrogen excretion and
838 nitrogen requirements in three sympatric hummingbird species. PhysiolBiochem Zool.
839 2003;76:731–743.
- 840 81. McWhorter TJ, Bakken B Hartman, Karasov WH, Martinez del Rio C. Hummingbirds rely
841 on both paracellular and carrier-mediated intestinal glucose absorption to fuel high
842 metabolism. BiolLett. 2005;2:131–134.
- 843 82. McWhorter TJ, Caviedes-Vidal E, Karasov WH. The integration of digestion and
844 osmoregulation in the avian gut. Biol Rev. 2009;84:533-565.
- 845 83. Montgomerie RD, Redsell CA. A nesting hummingbird feeding solely on arthropods.
846 Condor. 1980;82:463-464.
- 847 84. Nakagawa H, Dato M, Gatlin DM. Dietary Supplements for the Health and Quality of
848 Cultured Fish. Eds. – CABI International. 2007 .
- 849 85. NRC. Dietary Reference Intakes: Proposed Definition of Dietary Fiber – National Academy
850 Press. 2001

- 851 86. Nicolson SW, Fleming PA. Nectar as food for birds: The physiological consequences of
852 drinking dilute sugar solutions. *Plant SystEvol.* 2003;238:139–153.
- 853 87. Odum EP, Connell CE, Stoddard HL. Flight energy and estimated flight ranges of some
854 migratory birds. *Auk.* 1961;78:515-527.
- 855 88. Oku T, Tokunaga T, Hosoya N. Non digestibility of a new sweetener “Neosugar” in the rat.
856 *J Nutr.* 1984;114:1574–1581.
- 857 89. Ortiz LT, Rodriguez ML, Alzueta C, Rebole A, Trevino J. Effect of inulin on growth
858 performance, intestinal tract sizes, mineral retention and tibial bone mineralisation in broiler
859 chickens. *Brit Poultry Sci.* 2009;50:325-332.
- 860 90. Overgaard J, Sørensen JG, Peterson SO, Loeschcke V, Holmstrup M. Changes in membrane
861 lipid composition following rapid cold hardening in *Drosophila melanogaster*. *J Insect*
862 *Physiol.* 2005;51:1173-1182.
- 863 91. Paoletti MG, Norberto L, Cozzarini E, Musumeci S. - Role of Chitinases in Human Stomach
864 for Chitin Digestion: AMCase in the Gastric Digestion of Chitin and Chit in Gastric
865 Pathologies. *Binomium Chitin-Chitinase. 2009 Recent Issues* ISBN 978-1-60692-339-9
866 :339-358 - Eds: Salvatore Musumeci and Maurizio G. Paoletti © 2009 Nova Science
867 Publishers, Inc.
- 868 92. Pappenheimer JR. On the coupling of membrane digestion with intestinal absorption of
869 sugars and amino acids. *Am J Physiol.* 1993; 265:409–417.
- 870 93. Patton RS, Chandler PT, Gonzales OG. 1975 Nutritive Value of Crab Meal for Young
871 Ruminating Calves *J. Dairy Sci.* 1975;58;3: 404-409.
- 872 94. Peaker M. Nutritional requirements and diets for hummingbirds and sunbirds. *Int. Zoo Yb.*
873 1990;29:109-118.
- 874 95. Pearson OP. The metabolism of humming- birds. *Condor.* 1950;52:145-152.
- 875 96. Powers DR, Nagy KA. Field metabolic rate and food consumption by free-living Anna's
876 hummingbirds. *Physiol Zool.* 1988;61:500-506.

- 877 97. Powers DR, Conley TM. Field metabolic rate and food consumption of two sympatric
878 hummingbird species in southeastern Arizona. *Condor*. 1994;96:141–150.
- 879 98. Preest MR, Folk DG, Beuchat CA. Decomposition of nitrogenous compounds by intestinal
880 bacteria in hummingbirds. *Auk*. 2003;120:1091–1101.
- 881 99. Pyke GH. Why hummingbirds hover and honeyeaters perch. *Anim. Behav.* 1980;29:861-
882 867.
- 883 100. Pyke GH, Waser NM The production of dilute nectars by hummingbirds flowers.
884 *Biotropica*. 1981;13:260–270.
- 885 101. Razdan A, D. Pettersson. Effect of chitin and chitosan on nutrient digestibility and
886 plasma lipid concentrations in broiler chickens. *British Journal of Nutrition*. 1994;72: 277-
887 288. doi:10.1079/BJN19940029.
- 888 102. Remsen JV, Stiles F, Scott PE. Frequency of arthropods in stomachs of tropical
889 hummingbirds. *Auk*. 1986;103:436-441.
- 890 103. Richardson KC, Wooller RD. Adaptations of the alimentary tracts of some
891 Australian lorikeets to a diet of pollen and nectar. *Aust J Zoo*. 1990;38:581–586.
- 892 104. Robbins CT *Wildlife Feeding and Nutrition* (2nd edition). San Diego: Academic
893 Press. 1993.
- 894 105. Robel RJ, Press BM, Henning BL, Johnson KW, Blocker HD, Kemp KE. Nutrient
895 and energetic characteristics of sweepnet-collected invertebrates. *J Field Ornithol*.
896 1995;66:44–53.
- 897 106. Roberfroid MB, Slavin J. Nondigestible oligosaccharides. *Crit Rev Food SciNutr*.
898 2000;40:461.
- 899 107. Roberfroid M, Buddington RK. Inulin and oligofructose: proven health benefits and
900 claims. *J Nutr*. 2007;137: S2489–S2597.
- 901 108. Roberts WM. Hummingbirds' nectar concentration preferences at low volume: the
902 importance of time scale. *AnimBehav*. 1996;52:361-370.

- 903 109. Robinson SJW, Zwaan B, Partridge L. Starvation resistance and adult body
904 composition in a latitudinal cline of *Drosophila melanogaster*. *Evolution*. 2000;54:1819-
905 1824.
- 906 110. Scheithauer W. Hummingbirds. Thomas Y. Crowell, New York 1967.
- 907 111. Sherman AR. Experiments in feeding Hummingbirds during seven summers. *The*
908 *Wilson's bull.* 1913;86, Vol. xxv December 4:153-166.
- 909 112. Schmid R. Sugar preferences of hummingbirds. 2008.
910 http://www.easterncr.edu/~elliott/documents/Sugar_000.pdf.
- 911 113. Schondube JE, Martínez del Río C. Concentration-dependent sugar preferences in
912 nectar-feeding birds: mechanisms and consequences. *Funct Ecol.* 2003;17:445-453.
- 913 114. Schondube J, Martínez del Río C. Sugar and protein digestion in flowerpiercers and
914 hummingbirds: a comparative test of adaptive convergence. *J Comp Phys B.* 2004;174:263-
915 273.
- 916 115. Solberg, Y, Remedios G. Chemical composition of pure and bee-collected pollen.
917 *Meld NorgLandbruks.* 1980;59 (18):1-13.
- 918 116. Stanley RG, Linskens HF. Pollen Biology Biochemistry Management. Springer-
919 Verlag, Berlin 1974.
- 920 117. Stiles FG. On the field identification of California hummingbirds. *Calif. Birds.*
921 1971;2:41-54.
- 922 118. Stiles, FG. Taste preferences, color preferences, and flower choice in hummingbirds.
923 *Condor.* 1976;78:10-26.
- 924 119. Stiles FG. Geographical aspects of bird-flower coevolution, with particular reference
925 to Central America. *Ann. Missouri Bot. Gard.* 1981;68:323-35.
- 926 120. Stiles FG, Freeman CE. Patterns in floral nectar characteristics of some bird-visited
927 plant species from Costa Rica. *Biotropica.* 1993;25:191-205.

121. Stiles FG. Behavioral, Ecological and Morphological Correlates of Foraging for Arthropods by the Hummingbirds of a Tropical Wet Forest. *The Condor*.1995;97: 853-878.
122. Suarez RK, Lighton JRB, Moyes CD, Brown GS, Gass CL, Hochachka PW. Fuel selection in rufous hummingbirds: ecological implications of metabolic biochemistry. *Proc. of the National Academy of Science USA*. 1990;87:9207–9210.
123. Suarez RK, Gass CL. Hummingbird foraging and the relation between bioenergetics and behaviour. *Comp Biochem Phys*. 2002;133:335-343.
124. Szczęsna T. Study on the sugar composition of honeybee collected pollen. *J Apic Sci*. 2007;51:11-22.
125. Tamm S. Importance of energy costs in central place foraging by hummingbirds. *Ecology*. 1989; 70, 195–205.
126. Tiebout III HM. Tests of a model of food passage rates in hummingbirds. *Auk*. 1989;106:203-208.
127. Tiebout III HM. Daytime energy management by tropical hummingbirds: responses to foraging constraint. *Ecology*. 1991;72:839-851.
128. Thomas S, Durand D, Chassenieux C, Jyotishkumar P. *Handbook of Biopolymer-Based Materials: From Blends and Composites to Gels and Complex Networks* (eds.) - John Wiley & Sons, 2013.
129. Tsahar E, Arad Z, Izhaki I, Martínez del Rio C. Do nectar- and fruit-eating birds have lower nitrogen requirements than omnivores? An allometric test. *Auk*. 2006;123:1004–1012
130. Van Soest PJ. *Nutritional Ecology of the Ruminant: Ruminant metabolism, Nutritional Strategies, the Cellulotic Fermentation and the Chemistry of Forages and Plant Fibres*. O and B Books, Corvallis 1982.
131. Van Tets IG, Nicolson SW. Pollen and the nitrogen requirements of the lesser doublecollared sunbird. *Auk*. 2000;117:826–830.

954 132. Verdonk JM, Shim SB, van Leeuwen P, VerstegenMV. Application of inulin-type
 955 fructans in animal feed and pet food. *Br J Nutr.* 2005;93 Suppl 1:S125-138.

956 133. Weathers WW, Stiles FG. Energetics and water balance in free-living tropical
 957 hummingbirds. *Condor.* 1989;91:324–331.

958 134. Wooller RD, Richardson KC, Pagendham CM. The digestion of pollen by some
 959 Australian birds. *Aus J Zool.* 1988;36:357–362.

960 135. Zanutto FP, Bicudo JEPW. Dietary protein and carbohydrate affect feeding
 961 behaviour and metabolic regulation in hummingbirds (*Melanotrochilus fuscus*). *Rev Chil Hist*
 962 *Nat.* 2005;78:281-294.

963

964