

2

# 4    **Nutrients Metabolizability of Nectar for Long-Term Maintenance of Hummingbirds and** 5    **Effect of a Dietary Fibre Source**

6

8

16

## 17    **Abstract**

18    An artificial nectar was formulated for long-term maintenance of captive hummingbirds.

19    The objective of the experiments is to measure the metabolizability of nutrients in the artificial

20    nectar and to evaluate the effects of dietary fibre addition on its digestive utilization. The fibre

21    (inulin) has been used in replacement of some components that derive from arthropod intake. The

22    trial was conducted on two groups of non-reproductive *Amazilia amazilia* averagely weighing 4.0 g,

23    giving nectar containing about 179 g/l dry matter (DM). DM content of sucrose and hexoses was

24    92.3%, (0.75 M), that of crude protein (CP) 4.3% and fat (EE) 2.3%. Inulin was added in the ratio

25    of 7 g/100 g, as fed, of nectar powder. The daily DM intake of hummingbirds was about 2054 mg/d

26    and upraised by 14%, to 2348 mg/d, when inulin was added to the nectar; the energy intake

27    averaged 34 kJ/d. The average metabolizabilities of nectar were about 94% for DM and energy,

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

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

## 1. Introduction

Hummingbirds, according to their feeding habit, are defined as nectivorous. Around 320 species, weighing from 2 up to 20 g have being classified in various environmental and ecological niches. They collect nectar from many flower species, but also eat pollen, ash, sand, tree sap, spiders, insects and other small arthropods. For a very long time in some area of North, Central and South America wild hummingbirds are attracted offering artificial nectars in feeders (Sherman 1913). Many commercial types of nectar, containing sugars and other few ingredients, are offered to attract free-living hummingbirds. More problematic is to formulate soluble complete diets to raise, maintain and breed hummingbirds in captivity for life. From the available information, it appears that hummingbirds have been kept in captivity since the middle of the 19th century, but the formulation of a complete diet, more or less purified, was only occasionally investigated and made known in the past. Cordier (1953) proposed an artificial nectar containing honey or sugar, meat extract and condensed milk, almond oil and vitamins. Scheithauer (1967) fed hummingbirds various diets and different ingredients recording the results of chemical analysis and the animal response, obtaining successful results in long-term maintenance and in reproduction. Hainsworth & Wolf (1976) offered a sucrose solution 0.5 M (171 g/l solution) added with 5.2 g/l of concentrate supplements containing protein, fat and carbohydrates. Brice and Grau (1989) developed a purified liquid diet containing about 25% of dry

54 matter (DM), 80% sucrose, 3% protein (CP), delivered as commercial isolated soy protein  
 55 supplemented with DL-methionine, starch (5%), corn oil (3,7%), mineral and vitamins. Zanotto and  
 56 Bicudo (2005) use a maintenance diet composed by 20% sucrose, 0.5% amino acids (a.a.)  
 57 (Glicopan P), 3% oil, 0.03% vitamins (Vita Gold) and 1% calcium carbonate. Peaker (1990) dealt  
 58 with the subject of nutritional exigencies of captive hummingbirds discussing many issues and  
 59 suggested the formulation of a diet containing 88.5% of sugars, oil 2%, 6.5% of CP and a defined  
 60 amount of each of 20 a.a., supplemented with a complete array of mineral and vitamins. All these  
 61 formulations show some common issues and give essential indication on how to devise artificial  
 62 nectars for maintaining different hummingbird species for long time. It also needed to consider the  
 63 results of scientific research carried out either on free-living birds, focusing on feeding habit and  
 64 feeds preference and intake, or on captive individuals, for functional physiology and nutritional  
 65 requirements.  
 66 In practice, to successfully maintain and reproduce captive hummingbirds, the artificial nectar must  
 67 satisfy all nutritional needs, miming the inputs of a natural feeding. Energy and main nutrients  
 68 requirements for maintenance, flight and reproduction have been differently studied and indicated  
 69 (Weathers and Stiles 1989; Peaker 1990; McWhorter and López-Calleja 2000; López-Calleja et al.  
 70 2003). From on hand knowledge it appears that hummingbirds do not deliberately forage for fibre,  
 71 intended as insoluble and indigestible carbohydrates of plants cell wall. Conversely, they prey  
 72 variable amount and species of arthropods rich of chitin. ~~The role of this insoluble dietary fibre on~~  
 73 ~~digestion and absorption of other nutrients and its potential beneficial physiologic and healthy~~  
 74 ~~effects received few attentions.~~ If arthropods are not regularly available, other source of dietary  
 75 fibre might be given in the artificial nectar. Chitin, chitosans and other vegetable insoluble fibre  
 76 sources are impractical, because insoluble deposit sediments or surfaces in the feeders. Inulin,  
 77 ~~among the soluble fibrous ingredients, was already used in animal nutrition~~ and appears suitable for  
 78 the scope (Flickinger et al. 2003; Verdonk et al. 2005). For this reason, we consider to evaluate the  
 79 effects of the addition of inulin on digestive utilization of the artificial nectar. Beside the nutritional

80 facts, when hummingbirds are maintained *ex situ*, also health problems might arise and some  
81 aspects as intestinal mycoses, liver steatosis should be monitored and prevented.  
82 Available nutritional data are still incomplete and sketchy and some physiological aspects and  
83 behaviours of hummingbirds are undisclosed so that some solution must be attained by trial and  
84 error.

85 ~~The objective of our experiments is to measure metabolizability of all the nutrients in a complete~~  
86 ~~diet and the nutritional effect of inulin addition, in nectar formulated to maintain in good health~~  
87 ~~captive adult *Amazilia amazilia* and to allow them to breed.~~

88

## 89 2. Materials and methods

90 In Miramare park (Trieste – Italy), at the Centre for the Safeguard of hummingbirds, one  
91 ~~metabolizability trial was carried out with *Amazilia amazilia* hummingbirds. In the fully air~~  
92 ~~conditioned aviaries of the Centre, in a natural mimic environment with tropical plants where fruit~~  
93 ~~flies (*Drosophila sp.*) are grown in buckets containing fruits, lived about 90 hummingbirds,~~  
94 ~~belonging to nine species.~~

95 The experiment was conducted during the non-reproductive period. Adult hummingbirds were  
96 randomly assigned to two groups of seven hummingbirds each, fed artificial nectar (Nt) or artificial  
97 nectar added with inulin (Ni). A week before the beginning of the trial, each bird was housed  
98 separately in an experimental cage (0.80 m x 0.50 m x 0.90·m) fitted with a removable tray for  
99 *excreta* collection. Cages were allocated in a separate experimental room kept at 25 °C and  
100 provided with constant light artificial photoperiod of 12·h L: 12·h D and with full spectrum lights  
101 bulbs for the “sun bath” (3 times/d for 30-40 min). In the experimental environment, fruit flies  
102 were not present. During acclimation and experimental periods, of 7 and 17 days, respectively, the  
103 nectar was offered *ad libitum* in a 13 ml glass calibrated tube placed through a hole of the cage. To  
104 ~~avoid precipitation or surfacing of less soluble ingredients and fermentation,~~ nectar was renewed  
105 twice daily, at 7 am and at 3 pm; the volumetric nectar intakes were individually recorded at the

106 same time. Water was offered *ad libitum* in 10-ml water feeders and in birdbaths. Nectars were  
 107 daily diluting in 200 ml of distiller water 40 g, as fed, of Nt powder, or 43 g (inulin 3 g) to prepare  
 108 Ni. Ni contains 7.11% of Inulin on DM basis. The volume of Nt solution, equals 210 ml and that of  
 109 Ni 226 ml. *Excreta* were collected from removable trays twice daily using plastic spatulas, put in  
 110 plastic bottles, immediately weighed and frozen at -20°C. Before the chemical analysis, the two  
 111 pools of faeces were weighed and thoroughly mixed in 250 ml of distilled water. DM was measured  
 112 on 15 ml of sample by drying at 60°C to constant weight. On the remaining CP (Nx6.25), Ether  
 113 Extract (EE), ash (A.O.A.C., 1990), Neutral Detergent Fibre (NDF) (van Soest, 1982), were  
 114 determined. The energy content (kJ) was either measured, using an adiabatic calorimeter (IKA  
 115 C7000), and checked using the average energy values of different nutrients proposed by FAO  
 116 (2003). Water-soluble carbohydrate contents (NFE) of DM was calculated according to the  
 117 following: NFE= 100% -% CP-% ash -% EE -% NDF. The results of feeds analysis, which are the  
 118 average of duplicate determinations, are reported in Table 1.  
 119

Table 1 Dry matter and nutrients content of feeds

| Feed (% DM)  | DM     | CP   | EE   | CF   | Ash  | NDF  | NFE    | En<br>(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        |
| Inulina      | 95.64  | 0.18 | -    | -    | 0.02 | -    | 99.80  | 16.30        |
|              |        |      |      |      |      |      |        | En<br>(kJ/g) |
| 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         |



120  
 121  
 122 The apparent metabolizability of different nutrients was calculated using the following formula:  
 123 Metabolizability (%) = [1-Intake (g)/*Excreta* (g)] x 100]. Data were statistically analysed by  
 124 ANOVA, and the differences among group means were separated by Dunnett test, setting the  
 125 significance levels at P<0.01 (SPSS Statistics 17.0). 

126 Body weight was recorded throughout the acclimation and experimental period with an analytical  
127 balance (accuracy  $\pm 0.001 \cdot g$ ). Initial ~~live weights~~  were 4.08 g and 4.14 g for Nt and Ni groups and  
128 at the end of the experimental period 3.98 g and 3.80 g, respectively.

~~129 Preliminarily, based on the existing knowledge the formulation of nectar was devised and  
130 progressively adjusted, by checking intake and appetibility and physical and health conditions.~~

131 The nectar powder (Nt) is prepared once a week using the same ingredients. The nectar contains:  
132 Sucrose 47.2%, Glucose 19.2%, Fructose 19.2%, Hydrolysed soy and collagen (Wellesse –  
133 Ferndale USA) 1.5% , a mix of essential a.a. 2.2% (Nutricia –Danone), Maize and Soybean oils  
134 2.5%, Pollen 4.6%, Silimarin (Legalon – Rottapharm-Madaus) 1.1%, Bio-Mos (Alltech) 1.1%,  
135 Mineral and Vitamins supplement 1.3%. As for sugar concentration, Nt and Ni were equimolar,  
136 0.75 M or 0.5 M sucrose equivalent. Inulin was added to the nectar powder (Ni) in the ratio of 7  
137 g/100 g, as fed. We used Inulin 90% (Farmalabor - Italy), extracted from *Dahlia variabilis*,  
138 *Helianthus tuberosus* and other *Compositae*. This inulin is classified as “soluble non digestible”  
139 fibre (solubility 120 g/l) and is composed by fructans which, having a  $\beta$ -configuration of the  
140 anomeric C2 in its fructose monomers, resists hydrolysis by intestinal digestive enzymes, which are  
141 specific for  $\alpha$ -glycosidic bonds (Delzenneand Roberfroid 1994; Roberfroidand Slavin 2000).

142

### 143 3. Results

144 The chemical composition of nectars and the nutrients' content of 1 l of solutions are in Table 1.  
145 Carbohydrates (NFE) make up the 92.3% DM of Nt, the CP and EE contents are 4.28% and 2.30%  
146 of DM, respectively. Sucrose constitutes the 55.1% of added sugars, the balance is equally provided  
147 by glucose and fructose, 22.4%; the difference between added sugars and NFE is due to  
148 carbohydrates in the additives and other ingredients. The NDF content come from the natural  
149 ingredients used to make up the nectar, as pollen an probiotics, and corresponds to about 0.2% on  
150 DM.

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

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

161

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

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

162



163 The DM content of droppings was much lower than that of nectars, but its nutrient concentration  
164 was noticeably higher. The presence of inulin significantly increased the water content of  
165 droppings, which appear more viscous. The bulking effect of dietary fibre was evident: the  
166 excretion of DM and nutrients was significantly increased (Table 3). In consequence, the DM  
167 composition of droppings notably changed. As expected, a bigger quantity of NFE was excreted,  
168 due to the inulin intake (Tables 2, 3). Also the contents of CP and EE were significantly increased,

169 while that of ash has been reduced. The amounts of NDF excreted are identical, nearly matching the  
170 intakes.

171

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

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

172 

173 The ~~coefficient of variability (CV)~~ of individual nectar intake within days and animals was similar  
174 between the two groups. The CV was of 6.0%, lower than that observed for the excretion (CV =  
175 19.1%).

176 Hummingbirds receiving Nt on average maintain their LW (-11 mg), those of the Ni group lost  
177 some LW (-34 mg) during the period of 24 days.

178 The metabolizabilities of normal nectar DM resulted 94.6% (Table 4).

179

180

181

182

183

184

185

186

Table 4 Energy, DM and nutrient metabolizability (%)

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



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. ~~The nectar was formulated on the basis of a critical review of results from studies conducted in the wild and in the laboratories. The scope was that of measuring the nutrients metabolizability of a complete nectar and to evaluate the effect of the addition of inulin on intake and metabolizabilities. For the purpose we start considering that two main feeds constitute their natural nutritional sources, floral nectar and arthropods, but also pollen, which has a controversial importance, was taken into account. When foraging they make up a changeable mixed diet along the day and this modifies continuously transit time, absorption and metabolic adjustment.~~

206 ~~There are two main issues to look at the proper formulation to satisfy the nutritive requirements in~~  
 207 ~~artificial and confined environment and at the best DM concentration of nectar to attain a suitable~~  
 208 ~~daily intake of nutrients and energy.~~  
 209 ~~Energy and main nutrients requirements for maintenance, flight and reproduction have been~~  
 210 ~~differently studied and indicated (Weathersand Stiles 1989; Peaker 1990; McWhorterand López-~~  
 211 ~~Calleja 2000; López-Calleja et al. 2003; Tsahar et al. 2006).~~ Wild hummingbirds meet their energy  
 212 requirements essentially from nectar sugars while protein needs are independently satisfied by  
 213 eating a changeable amount of small arthropods (Gassand Montgomerie 1981; Pyke 1980;  
 214 Hainsworth 1977; Des Granges 1978; Montgomerieand Redsell 1980; Brice 1992), other stuffs and  
 215 possibly pollen (Wooller et al. 1988, Van Tetsand Nicolson 2000). To the contrary, a diet of only  
 216 arthropods cannot cover the energy requirements (Brice 1992; López-Calleja et al. 2003).  
 217  
 218 Flowers habitually visited by hummingbird produce 10-30 ml of nectar having a DM content of 15-  
 219 40%; the sugar concentration varies between 0.15 M and 1.2 M: most of the nectars contain  
 220 sucrose, fructose and glucose and rarely are composed by a single sugar (Hainsworthand Wolf  
 221 1976; Pykeand Waser 1981). Hummingbirds prefer flowers providing nectars with sucrose, fructose  
 222 and glucose (Hainsworthand Wolf 1976) and with a higher proportion of sucrose to hexoses  
 223 (Cruden et al. 1983; Freeman et al. 1984; Gottsberger et al. 1984; Martínez del Rio 1990; Martínez  
 224 del Rio et al. 1992; Stilesand Freeman 1993; Chalcoff 2001). According to Nicolsonand Fleming  
 225 (2003), hummingbirds select nectars containing  $64.4 \pm 18.5\%$  of sucrose. ~~The large range of sugar~~  
 226 ~~concentration and composition of nectars in flowers selected by hummingbirds offers them many~~  
 227 ~~dietary options for satisfying the energetic demand of distinct lifestyles.~~  
 228 Less univocal is the sugars preference in captive hummingbirds. Environment, cage dimension,  
 229 colour and shape of feeders and perches, but also imprinting or adaptation, bill length and licking  
 230 capability look as if influencing sugars preference; the first adaptation to a definite sugar solution or  
 231 to a frequent variations sugars solution requires several hours or feeding sessions (Hainsworthand

232 Wolf 1976; Stiles 1976; Roberts 1996; Schmid 2008). Different results indicate that sucrose is  
 233 generally preferred alone or when paired with glucose; on the other hand, sucrase activity always  
 234 proved to be very high in hummingbirds (Martínez del Rio 1990; McWhorter and Martínez del Rio  
 235 2000; Schondube and Martínez del Rio 2004). Captive birds always prefer the more concentrated  
 236 sugar solution (Hainsworth and Wolf 1976; Schondube and Martínez del Rio 2003) and  
 237 notwithstanding the negative relationship ( $P < 0.02$ ) between the sucrose concentration and the rate  
 238 of volumetric nectar intake, the amount of sugar ingested remains nearly the same or increases with  
 239 the concentration (McWhorter and Martínez del Rio 1999 and 2000). The transit time of a bout  
 240 increases with sucrose concentration (Tiebout III 1989, Martínez del Rio and Karasov 1990). The  
 241 preference for solutions of sucrose and hexoses should be compared at the same concentration on  
 242 equicaloric basis. At high concentration of sugars ( $\geq 25\%$ ), hummingbirds do not discriminate  
 243 among sugars; instead, at lower concentration, equicaloric, equimolar and equiweight solutions of  
 244 diverse combination of sugars rank in a different order of preference (Brown et al. 2008; Schmid  
 245 2008; Fleming et al. 2004).  
 246 Glucose is more slowly assimilated than other sugars, as birds rested longer between feeding bouts,  
 247 making a glucose diet less efficient (Martinez Del Rio 1990). The digestion time rather than feed  
 248 collection limits sugars' assimilation by hummingbirds (Diamond et al. 1986; Karasov 1986). Only  
 249 at lower substrate concentration the passive absorption, by paracellular diffusion and solvent drag of  
 250 glucose, significantly contributes to total absorption (Pappenheimer 1993; McWhorter et al. 2005;  
 251 McWhorter et al. 2009).  
 252 According to these suggestions, we consider that the simultaneous presence of sucrose and its  
 253 hydrolytic products at moderate concentration could represent a more valid starting option for  
 254 hummingbirds living in a confined artificial environment. Then sucrose, glucose and fructose are  
 255 added to compose 86% of the nectar DM, where sucrose contributes with the 56% to the mix of  
 256 three sugars and the rest is equally balanced between the two hexoses. The rest of sugars come from  
 257 pollen and supplements. There are some grounds for inclining in the direction of artificial nectar

258 having moderate sugars' concentration, which appears to favour hexoses preferences and absorption  
 259 (Schondube and Martínez del Rio 2003); firstly, because the sugars' concentration of nectars does  
 260 not influence the rate of daily energy intake (López-Calleja et al. 1997). The higher water  
 261 absorption in the intestine is easily disposed by kidney, producing large water loads of diluted urine  
 262 (McWhorter and Martínez del Rio 1999; Martínez del Rio et al. 2001). All this should favour intake  
 263 enhancing the number of bouts, fastening the rate of ingestion, by keeping an efficient absorption.  
 264 For these reasons, being in situation where nectar availability is not limiting and foraging does not  
 265 require a high-energy cost, we considered preferable to try the option of offering hummingbirds  
 266 relatively diluted nectar. The drinking solution was prepared to keep DM concentration around  
 267 18%, i.e. 0.5 M sucrose equivalent.

268

269  et energy expenses were evaluated using indirect methods for different conditions or activities,  
 270 e.g. basal metabolism, torpor, flying, hovering, perching, etc.. Requirements are usually quantified  
 271 in Watts, as instantaneous requirements. In many other cases, gross or metabolizable energy intakes  
 272 were measured in feeding trials. More often energy requirement is associated to time budget since  
 273 different activities have different energetic cost (Hainsworth 1977). Tiebout III (1991) instead of  
 274 "requirement", term demoting to an additive evaluation of singly ascertained exigencies, not easy to  
 275 correctly establish, discern the notion of "energy management" to denote that the changeable  
 276 behavioural and physiological responses of an individual to specific conditions from time to time  
 277 determine its total energy budget. Sugars in the nectar provide birds for the most part of energy.  
 278 Commonly, the daily sugar intake is assumed to cover the energy requirement. The energy  
 279 requirements of free-living hummingbirds (FMR) has been measured with different methods or  
 280 estimated in different environmental situations. FMR results high and largely variable, from 29.1 to  
 281 81.7 kJ/d (Stiles 1971; Hainsworth and Wolf 1972; Tamm 1989; Ewald and Carpenter 1978;  
 282 Weathers and Stiles 1989; Powers and Conley 1994, Suarez and Gass 2002). A frequently reported  
 283 FMR reference value, measured on *Caliptae anna* hummingbirds of 4.5 g LW, is 31.8 kJ/d (Powers

284 and Nagy 1988). The experiments with captive animals have shown that the highly adaptive energy  
 285 metabolism of hummingbirds to thermal changes and dietary conditions can be nevertheless  
 286 influenced by the natural behaviours and foraging strategies of different species (Pearson 1950;  
 287 Tiebout III 1991; Garrison 1995; McWhorter and Martínez del Rio 1999). Reduced resting  
 288 expenditures as night-time torpor or the less severe daytime depression in metabolic rate (day-time  
 289 hypometabolic compensation) can balance foraging constraint (Bartholomew et al. 1957; Lasiewski  
 290 1963; Hainsworth and Wolf 1976; Carpenter 1974; Hainsworth et al. 1977). Typically, the metabolic  
 291 rate measured in captivity ranges from 7.3 kJ/d to 10 kJ/d per g LW (Scheithauer 1967; Bennett and  
 292 Harvey 1987; McWhorter et al. 2005).

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

296

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

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

310 In captive birds, a convenient and easy way to offer arthropods could be that of raising *Drosophila*  
 311 in the same aviary or in a bottle; *Diptera* are also within the preferred preys in free-living  
 312 hummingbirds. Brice (1992) and López-Calleja et al. (2003) observed that when nectar and fruit  
 313 flies are separately offered, their intakes are independently regulated.  
 314 The number of fruit flies a hummingbird has to ingest to cover N maintenance requirements is  
 315 differently quoted as 312 by Peaker (1990), 150 by López-Calleja et al. (2003), 283 by Lotz and  
 316 Martínez del Rio (2004). According to Scheithauer (1967), a hummingbird of 3 g LW ingests 677  
 317 flies/d. In contrast, Montgomerie and Redsell (1980) assume that hummingbirds could meet the  
 318 daily protein requirement by ingesting only 38 flies each containing 2.1 mg of DM, with a protein  
 319 content of about 60% and a metabolizability of 80%. López-Calleja et al. (2003) observed that  
 320 *Sephaniodes sephaniodes* weighting about 6 g, in addition to a nectar containing sucrose (0.75  
 321 mol/l) given *ad libitum*, required approximately 150 fruit flies to maintain body mass, which  
 322 corresponds to a 4.55 mg/d N, being  $78.8 \pm 6.4\%$  the N “digestibility” of fruit flies. The CP ingested  
 323 with *Diptera* equals 36 mg/d.  
 324 If the animal preys are not available, a protein substitute must enter the nectar composition in a  
 325 prearranged fixed amount, as well as alternative sources of fat, ashes, and fibre. Hummingbirds do  
 326 not specifically necessitate protein but are able to live eating a diet containing free a.a. (Baker and  
 327 Baker 1979). There are no indications of the effect of the enrichment of the nectar with other  
 328 nutrients on transit time; e.g. Klasing (1998) reports that dietary fat requires long time for digestion  
 329 and can have marked impacts on the rate of passage in some species. Much probably, if a.a. are  
 330 given, instead of **diptera** or of another source of protein, the absorbability and the transit time should  
 331 be modified. As the prudential criteria was to guarantee an adequate intake of protein to cover  
 332 synthesis of semi essential and non essential a.a. and possible metabolizability inefficiencies, we  
 333 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  
 334 addition of an extra amount of organic N (hydrolyzed protein).  
 335

336 On fat utilization by hummingbirds, any specific information has been experimentally obtained on  
 337 its intake and absorption even if, in some experiments, diets were formulated adding oil.  
 338 Hummingbirds oxidize carbohydrates preferentially, but when sugar availability is shortened, as  
 339 after overnight fasting, quickly switch to long chain fatty acids, also sparing the exiguous glycogen  
 340 reserves (Suarez et al. 1990; Suarez and Gass 2002; Carleton et al. 2006). Body fat content is  
 341 variable and changes during the day and with season. Highest variation are found in migratory  
 342 species, which can store more than 40% of body weight as fat. The hummingbird's migratory  
 343 restlessness - *Zugunruhe* - corresponds to a fast fattening sustained by an increased intake of fat and  
 344 protein following an intensification of insects catching (Hartman and Brownell 1959; Connell et al.  
 345 1960; Odum et al. 1961). More probably, as hummingbirds maintain a high glycaemia and insulin  
 346 level also in the fasted condition (Beuchat and Chong 1998; McWhorter and Martínez del Rio  
 347 1999), should *de novo* synthesis and lipolysis continuously occur and fattening is the positive result  
 348 of a short-term balance. Unfortunately, even if fat plays an important role in hummingbirds'  
 349 metabolism, the fat requirement is unknown and the fat content of diet is indicated in few studies.  
 350 The reported values for oil inclusion in nectar vary from 2% of crude oil (Peaker 1990), to 3% of  
 351 corn oil (Zanotto and Bicudo 2005) and 3.7% of corn oil (Brice and Grau 1989 and 1991). The only  
 352 information on daily lipids intake of about 90 mg can be inferred from the latter Authors' papers,  
 353 who reported both, fat content of nectar and daily intakes.

354 Arthropods should provide hummingbirds also with essential unsaturated fatty acids, being one of  
 355 the richest animal feed sources (DeFoliart 1991). *Drosophila sp.*, are a significant source of fat (8-  
 356 15%) and of mono (13-51%) and poly unsaturated fatty acids (8-31%) (Keith 1966; Green and Geer  
 357 1979; Robinson et al. 2000; Overgaard et al. 2005). A valuation from *Drosophila*, made using the  
 358 previously cited papers and assuming for the fat content of insects sampled in the aviary a value of  
 359 11.5% of fat content in insects sampled in the aviary, a variable intake, from 35, and 68 mg/d is  
 360 obtained. The acidic composition indicates an amount of 51.7% of monounsaturated and 14.8% of  
 361 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.

366

The hummingbirds play an important role in ~~flower pollination~~. Bent (1940) found pollen in the hummingbirds' stomach. Pollen contains from 13% to 50% of sugars; free a.a. and protein contents of above 40% have been reported, but the typical range is 7.5% to 35% (Stanley and Linskens 1974; Solberg and Remedios 1980). According to Peaker (1990) the a.a. composition of pollen is similar to that of animals and recommends the use of pollen as protein source in a complete artificial nectar.



The hummingbirds' attitude for eating pollen and their capability of extracting nutrients from ungerminated pollen is controversial (Wooller et al. 1988; Richardson and Wooller 1990; Van Tets and Nicolson 2000). Brice et al. (1989) feeding Anna's and Costa's hummingbirds 20% pollen on diet DM found that the digested amount was always nil or very low (< 7%), however reported a higher digestibility of pollen in Anna's nestling hummingbirds, but nestling are offered solid and slow passing food, mainly insects (Carpenter and Castronova 1980). Klasing (1998) comments that pollen digestibility is limited by the fast transit through gastrointestinal tract, but it also depends upon bird's age and species.

~~Pollen is definitely a source of vegetal fibre as exine, composed of cellulose and sporopollenin, highly resistant to degradation (Stanley and Linskens 1974). Samples of pollen collected or stored by bee indicate a variable content of crude fibre (CF), principally cellulose, from 4.2 to 18.5% on DM (Herbert and Shimanuki 1978; Szczesna 2007).~~

384

## 4.2 Nutrients Intake and metabolizability

The LW of hummingbird species largely varies and, to compare values, daily intakes are more often referred to metabolic weight. Hummingbirds suckled 886 vs. 1004 ml/kg<sup>0.75</sup> of Nt and Ni nectar

388 respectively, corresponding to 121.5 and 134.4 g/kgLW<sup>0.75</sup> of DM. When inulin was added to the  
 389 nectar, the hummingbirds increase the average daily intake by 14.3%.  
 390 When concentration of sugars is low, as in this experiment, the intakes should be compared at the  
 391 same molarity or energy or sugar contents. López-Calleja et al (1997), with 0.75, 0.5, 0.25 M  
 392 sucrose nectars, measured increasing intakes (8.6, 12.6 and 24.4 ml/d). McWhorter and Martínez  
 393 del Rio (2000), with reference to captive hummingbirds of different species, determined that intake  
 394 steadily increase when the sucrose molarity of nectar diminishes and, at 584 mmol/l equals 12 ml/d.  
 395 Practically, higher the sugar molarity lower is the intake but, *ceteris paribus*, the overall daily  
 396 consumption and the metabolizability are the same. In our experiment (0.5 M, sucrose equivalent),  
 397 the intake of Nt was 11.5 ml/d and the sugars (NFE) metabolizability resulted 98.4%, within the  
 398 range of the ordinary values. In fact, sugar assimilation efficiencies measured in hummingbirds  
 399 using traditional balance methods are higher than 95% and by and large independent from sugar  
 400 concentration and from *digesta* retention time (McWhorter and Martínez del Rio 2000; Schondube  
 401 and Martínez del Rio 2003). Hainsworth (1974) measured sucrose digestibility of 98-99%; Karasov  
 402 (1986), using a double isotope dilution method for measuring crop-emptying kinetics of *Selaphorus*  
 403 *rufus* hummingbirds, found an efficiency of sucrose extraction of 97.1±0.3%. Duke (1997) reported  
 404 that sucrose and hexoses are digested to the same high extent (> 97%).  
 405 In our trial the DM intake of Nt nectar averaged 2.05 g/d (128 g/d/kgLW<sup>0.75</sup>) and metabolizability  
 406 resulted 94.6 %, as a combination of sugars metabolizability (98.4 %) and that of other nutrients  
 407 which all together results 49.5 %.  
 408 In different experiments, where diverse nectars were given to several hummingbird species, the  
 409 daily intakes of metabolizable energy varied from 29.1 to 138.5 kJ, or from 5.63 to 9.32 kJ/d/g LW  
 410 (Powers and Nagy 1988; Weathers and Stiles 1989; Powers and Conley 1994; López-Calleja and  
 411 Bozinovic 2003; López-Calleja et al. 2003). Many factors affect the requirements, the intake and  
 412 the nutritional efficacy of the diet and the energy balance, practically evaluated by the LW  
 413 variation, gives an inclusive validation. In all these experiments, the hummingbirds appear to have

414 maintained their LW. The indicative average of nine experimental values equals  $7.46 \pm 1.09$  kJ/d/g  
 415 LW. The apparent metabolizable energy intake of Nt and Ni were 32.1 and 35.2 kJ/d, which  
 416 correspond to 7.97 and 8.87 kJ/d/g LW, values which are consistent with other experimental results.  
 417 The Nt group along the 24 days of trial basically maintained their LW and those receiving Ni  
 418 nectar, lost a little of LW. The ~~higher intake of apparent metabolizable energy of hummingbirds fed~~  
 419 ~~Ni to some extent could depend upon a partial fermentation of inulin and on a fading of fat~~  
 420 ~~synthesis. Roberfroid et al (2010) observed that ingredients showing a prebiotic effect are able to~~  
 421 ~~modulate hepatic lipid metabolism in animals, resulting in changes in either triglyceride~~  
 422 ~~accumulation in the liver, and/or serum lipids, and in a decrease in triglyceridaemia without any~~  
 423 ~~protective effect on hepatic triglyceride accumulation and lipogenesis, suggesting a possible~~  
 424 ~~peripheral mode of action.~~

425

426 Beside sugars, the Nt nectar contains other ingredients of different palatability and metabolizability,  
 427 as protein, fat and minerals, which affect DM intake and metabolizability.

428 In trial, with *Sephanoides sephaniodes* weighing about 5.5 g, (López-Calleja et al. 2003) compared  
 429 nectars of different sugars and CP content – from 0 to 11.2%. They observed an increasing DM  
 430 intake from 93 to 139 g/d/kgLW<sup>0.75</sup> and a declining energy metabolizability (from 0.99 to 0.93);  
 431 when CP was 4.7%, the DM intake was 119.5 g/d/kgLW<sup>0.75</sup> and the metabolizability 0.97. From an  
 432 experiment of Brice and Grau (1991), who offered a liquid diet containing a rising percentage of CP  
 433 - from 0 to 3% in 25% solids – we can easily calculate to some extent an increase of DM  
 434 consumption from 123 to 130 g/kgLW<sup>0.75</sup>. In another experiment, López-Calleja and Bozinovic  
 435 (2003), comparing two diets differing for sucrose molarity supplemented with protein, vitamins and  
 436 minerals (8 g/l), measured a DM intake of 2.22 g/d or 110 g/d/kgLW<sup>0.75</sup> (0.5 M) and 1.87 or 93 g/d  
 437 (0.75 M); DM metabolizability in both groups was  $0.95 \pm 0.02$ . Thus, when nectar contains protein,  
 438 the intake is adjusted to sugars content and the DM metabolizability decreases. DM intake and  
 439 metabolizability of diets comparable to Nt were similar to those we measured on *Amazilia amazilia*.

440

441 The average individual intake of CP was 88 mg/d (Nt) which correspond to 5.5 g CP/d/kgLW<sup>0.75</sup>,  
 442 equivalent to 14.1 mg Nitrogen (N)/d or 880 mg N/d/kgLW<sup>0.75</sup> (Table 2). In the previously reported  
 443 experiments, López-Calleja et al. (2003) substituting part of sugars with an increasing amount of  
 444 protein (ProMod – Abbott), observed that CP intakes grew up to 313 mg/d (14.1 g CP/d/kgLW<sup>0.75</sup>),  
 445 In this experiment and in that of Brice and Grau (1989), the DM intake increases by means of  
 446 protein content of DM. Also Zanotto and Bicudo (2005) varying in two diets the protein/sucrose  
 447 ratio, measured (in 1.5 hours) a higher nectar and protein intake with high protein-low carbohydrate  
 448 diet. López-Calleja and Bozinovic (2003) measured a daily CP intake of 80 mg (0.5M) and 40 mg  
 449 (0.75M), corresponding to 3.8 and 1.9 g CP/d/kgLW<sup>0.75</sup> and observed that the different CP intakes  
 450 depend upon sugar molarity. These and other results (McWhorter et al. 2003; Brice and Grau 1991),  
 451 lead to assume that, in a complete nectar diet, the frequency of bouts and the DM intakes depend  
 452 upon the sugar concentration, but not from that of N. N excretion, when measured, results positively  
 453 and, within a “physiological” level, almost positively correlated with the intake.

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

458 The protein content of nectar DM given hummingbirds to maintain their body condition varies from  
 459 2.4 % up to 6.5 %; usually the level protein considered more appropriate seems to be of about 4.5 %  
 460 CP on DM (Brice and Grau 1989; Peaker 1990; López-Calleja et al. 2003; López-Calleja and  
 461 Bozinovic 2003; McWhorter et al. 2003).

462 In our experiment, giving Nt containing 4.3% CP on DM, the average intake of CP was 88 mg/d or  
 463 8 mg/d of apparently metabolizable N (3.1 g/kg<sup>0.75</sup>/d of metabolizable CP).

464 On protein requirements and feeding of hummingbirds, not much information is available, but is  
 465 generally recognized that they have low N requirements compared to non nectarivorous birds (Brice

and Grau 1991; López-Calleja et al. 2003; McWhorter et al. 2003, Tsahar et al. 2006). Robbins (1993) proposed simple equations, frequently used to weigh up the N requirement of passerines and non-passerines. The Minimal N Requirement (MNR) is satisfied by  $430 \text{ mg N/kg}^{0.75}/\text{d}$ , being the Total Endogenous Nitrogen Losses (TENL) of  $270 \text{ mg N/kg}^{0.75}/\text{d}$ . Peaker (1990), on the basis of this MNR value, suggest a CP content of 6.5 % DM in complete nectar for hummingbirds, assuming a N metabolizability no less than 80%. Presumably, this high metabolizability should be achieved if the a.a. content of diet is that defined by the Author.

Brice and Grau (1991), in an experiment on *Calypte costae* hummingbirds of 3.8 g LW, measured a similar MNR and TENL of 77.1 and 77.8  $\text{mg N/kg}^{0.75}/\text{d}$ . Also López-Calleja et al., 2003, in the previously cited research on N requirement of *Sephanoides sephaniodes*, comparing MNR and TENL, measured giving also a “Zero N” nectar, obtained an identical value of  $67.9 \cdot \text{mg/kg}^{0.75}/\text{d}$ .

McWhorter et al. (2003), offering three hummingbirds species of different LW a nectar containing casein acid hydrolyzate (Sigma®), gave evidence of a variable MNR. The values went from 85.5 to  $158 \text{ mg/kg}^{0.75}/\text{d}$ , and TENL, from 46 to  $77.7 \text{ mg/kg}^{0.75}/\text{d}$ ; in a previous experiment, on a fourth species, measured a MNR of  $62.5 \text{ mg/kg}^{0.75}/\text{d}$  and a TENL of  $45.5 \text{ mg/kg}^{0.75}/\text{d}$  (McWhorter 1997).

López-Calleja et al. (2003), shifting the N content in equicaloric nectars from 1.2 up to 11.2%, registered daily N intakes varying from 5.2 to 50.1 mg (from 32 to 316 mg CP) and only the hummingbirds receiving at least 4.7% CP did not lose weight. The digestibility coefficients were inversely related to the protein content and significantly differed among diets, decreasing from  $68 \pm 7\%$  to  $48 \pm 3\%$ . Zanotto and Bicudo (2005) also comment that these birds are unable to efficiently assimilate N in high protein diets, because of a protein overload.

On the basis of these results, Tsahar et al. (2006) in a notable discussion paper on N requirements of birds, reassessing Robbins’ allometric equation, propose for nectarivorous-frugivorous birds an average MNR of  $152 \text{ mg N/kg}^{0.76}/\text{d}$  and TENL of  $54.1 \text{ mg N/kg}^{0.69}/\text{d}$ . To compare the reported MNR and TENL, we recalculated the values according these metabolic weights, always obtaining lower values than the two averages calculated by Tsahar et al. (2006). Both MNR and TENL varied

492 giving evidence that this requirement is largely influenced by nectar composition and by  
493 hummingbirds' species, rearing and experimental conditions.

494

495 The relationship between MNR and TENL values measured in all these experiments depends also  
496 on CP metabolizability or retention efficiency, that is the slope of the linear regression between  
497 ingested and retained N. Amino acids, if are not quickly deaminated, are used mainly for synthesis  
498 of skeletal muscles and, to a lesser extent, of other protein compounds. The metabolizability  
499 depends on the biological value of protein. In the wild, hummingbirds complemented the nectar  
500 feeding catching arthropods the protein of which is highly metabolizable,  $78.8 \pm 6.4\%$  according to  
501 López-Calleja et al. (2003). The few information of N metabolizability experimentally measured  
502 using different protein supplements are always lowers than that from fruit flies. McWhorter et al.  
503 (2003), using casein acid hydrolyzate (SIGMA – St. Louis), measured N metabolizability within 49  
504 and 54 %. López-Calleja et al. (2003), giving different amounts of hydrolysed collagen (Promod –  
505 Abbott, Columbus, USA), calculated a declining metabolizability from 68% to 48%, increasing with  
506 protein intakes. In our trial, the protein metabolizability was 57 %.

507 All these N sources solubilised or dispersed in the nectar, other than natural supplies which are  
508 separately ingested, should differently modify osmolarity and transit time and *affect* the intakes.

509

510 The average lipids intake of hummingbirds during the trial was of 47 mg/d and the excretion of  
511 26.52 mg/d; the fat concentration in droppings DM was higher than 25%, but we had already  
512 observed that hummingbirds' *excreta* appear “greasy”. In our trial, the lipids metabolizability in  
513 hummingbirds receiving normal nectar was 44%, indicating a moderate but effective absorption.  
514 Weight for weight the assimilated lipid was less than 1% of that of digested NFE. Under an  
515 energetic point of view, this value does not appear relevant to captive hummingbirds, but oil brings  
516 also indispensable essential fatty acids.

517

518 The intake of fibrous fractions (NDF) is reported in Tables 2. The basal intake was of about 3-4  
 519 mg/d. The amount of fibrous fraction of droppings averagely excreted by hummingbirds (Tables 3)  
 520 practically matches the intakes (Tables 2 and 3). The digestibility of CF and of NDF, did not  
 521 significantly differ between nectars and was always very low and variable.

522 The addition of inulin to the nectar was followed by an increase of DM intake of about 14% (Table  
 523 2). Apart from significantly modifying the intake, the dietary fibrous source added to normal nectar  
 524 significantly affected the nutrients' excretion and thus their metabolizability: those calculated for  
 525 DM, NFE and Energy were reduced by about 4 %; higher differences were measured for CP and  
 526 EE, instead the absorption of ash was increased. Roberfroid (2007) comments that various  
 527 hypotheses have been tested to tentatively explain the modifications of the digestion and absorption  
 528 of macronutrients, especially carbohydrates and triglycerides, and of minerals which may occur in  
 529 animals and humans, either by delaying gastric emptying and/or shortening small intestinal transit  
 530 time (Oku et al. 1984). In fact, the increase of daily DM intake and excretion stands for a shortened  
 531 transit time. The absorption of ashes was significantly higher with Ni (53.4%) than Nt (41.1%).  
 532 Similar effect of inulin has been observed in poultry, other animal species and in man (Ortiz et. al.  
 533 2009; Awad et al. 2013). This result also supports the occurrence of an acidification of distal  
 534 intestine and cloaca. In fact, even if minerals are absorbed along the intestine, a higher assimilation  
 535 takes place after fermentation as a lower intestinal pH increases mineral solubility and facilitates  
 536 absorption (Levrat et al. 1991; Lopez et al. 2000).

537 The DM excretion was about doubled by inulin addition, but the difference between the DM and  
 538 NFE contents of droppings collected from birds fed Nt and Ni nectar was lower than the inulin  
 539 intake. We have tried to interpret our results, on which there is any specific reference, on the basis  
 540 of existing knowledge and the rationale which premised this experiment. Crossing data of NFE  
 541 intakes and excretions in Nt and Ni birds, the inulin disappearance trough the digestive tract should  
 542 concern less than one third of the intake (*i.e.* ~ 55 mg/d). In animals, inulin passes through the  
 543 stomach and duodenum undigested and is easily available to the gut microorganisms. Preest et al.

(2003) report that hummingbirds possess high density of intestinal bacteria and a degradation activity on urinary uric acid, urea and urates, always there, to ammonia has been observed. So that also a fermentation activity on sugars could occur with production of volatile fatty acids (VFA). The viable presence of undigested inulin in the lower gut, where presumably stays longer, could favour the presence of microorganisms producing also different alloenzymes, *e.g.* inulinase, which modify to a certain extent indigestible carbohydrates. The major limit to this potential activity is the swiftness of transit as fluid passes through a hummingbird's digestive tract in about 0.5 h. (Hainsworth and Wolf 1972). In birds, diverse components of the same meal may move at greatly different rates through the different tracts of digestive system (Klasing 1998). Soluble and insoluble fraction of dietary fibre have a high water holding capacity and if not digested they provide bulk to the droppings, differently altering the transit time of nectar in the different sections of the whole digestive tract.

556

## 5. Conclusions

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

569 The attempt of simultaneously measuring the metabolizability of main nutrients of a feed open to a  
 570 different view on the digestive efficiency of hummingbirds and helps in testing other ingredients  
 571 than the few generally used. In fact, there is a large difference between the sugars metabolizability  
 572 and that of other nutrients, so that the content of protein, fat and other ingredients in non-useful  
 573 amount reduces the overall DM metabolizability reducing the sugar intakes. ~~For effective use in~~  
 574 ~~metabolism and production, essential nutrients need to be contained in the bird's diet in proportions~~  
 575 ~~more - or - less parallel to its needs and must be available for the digestion and the transport.~~ There  
 576 is much to understand when we consider that typically about 40 single nutrients among a.a., fatty  
 577 acids, hydro and liposoluble vitamins and minerals are required in the diets of birds.  
 578 ~~The results of these trials offered some indications that should be considered for a necessary further~~  
 579 ~~improvement or adaptation to different rearing conditions and hummingbirds' species.~~ The only  
 580 option given captive hummingbirds was that of varying the intake and preying fruit flies. For  
 581 practical reasons, only one or two  recipes could be prepared and offered to hummingbirds kept in an  
 582 artificial environment.   
 583 The average daily intake of DM was 2.05 g/d. The average volumetric consumption of nectar and  
 584 the DM intakes find a good agreement with other experimental evidences obtained with nectar of  
 585 similar sucrose molarity. The option of using sucrose and hexoses in a ratio resembling that of floral  
 586 nectars preferred by hummingbirds seems to guarantee a satisfactory volumetric intake and an  
 587 overall sugars digestibility, ~~not different from the values reported by many other Authors.~~   
 588 The intake of sugars and energy, which correspond to about  $130 \text{ g/kg}^{0.75} \text{ LW/d}$  and  $2150 \text{ kJ}$   
 589  $\text{kg}^{0.75} \text{ LW/d}$ , seem adequate to cover the maintenance requirements. From the results of available  
 590 experiments, it comes out that an increase of nectar's sugar concentration does not significantly  
 591 modify its daily intake and metabolizability. On the contrary, a higher content of protein, and  
 592 possibly of other nutrients, result in a greater nectar intake, reducing its metabolizability, by  
 593 affecting osmolality and feed transit time.

594 ~~The metabolizability of protein was similar to that observed in other few experiments when the N~~  
 595 ~~source is a mix of a.a. and/or hydrolyzed protein.~~ When intake values are given as metabolizable N  
 596 per unit of metabolic weight, we measured an intake (about 500 mg N/ kg<sup>0.75</sup>LW/d) which was  
 597 about twice the more suitable indication obtained by other Authors for the maintenance (~265 mg/d  
 598 – López-Calleja et al. 2003). On the basis of existing knowledge and on the result of this trial,  
 599 assuming a CP metabolizability of 50-60%, the suggestion is to formulate the nectar containing  
 600 not less than 3.5% of protein or even more if birds have to cover requirement for reproduction and  
 601 molt.

602 ~~The fat metabolizability was 44% in Nt and 33.6% in Ni, indicating that, as it should likely happen,~~  
 603 ~~the fast transit time of a liquid feed is not congruent with an efficient absorption.~~ The addition of  
 604 dietary fibre significantly reduced the amount of absorbed fat. We try an interpretation of these  
 605 results considering a possible effect of the inulin on the transit time, but this remains a nutritional  
 606 aspect which requires more careful evaluation. In fact, the outcome from fat intake and digestibility  
 607 of fat could give two opposite practical indications. The energy requirements of captive  
 608 hummingbirds can be easily satisfied by sugars and do not need extra energy source, but a small  
 609 amount of vegetal is useful to meet essential fatty acids exigency, which is unspecified, but  
 610 probably not avoidable. On the other hand, if we assume that fat could be anyhow necessary, the  
 611 low assimilation suggest to add oil in the amount commonly adopted (2-2.5%) to guarantee a small  
 612 but significant metabolizable quantity.

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

619



## References

1. A.O.A.C. (1990) Official Methods of Analysis of the Association of Official Analytical Chemist. 16th ed., Publ. Washington, DC, USA. 931-933.
2. [Awad AW](#), Ghareeb K, Paßlack N , J Zentek. Dietary inulin alters the intestinal absorptive and barrier function of piglet intestine after weaning. Res Vet Sci. 2013;5:249-254.

3. Baker HG, Baker I. Nectar constitution and pollination – plant coevolution. In Plant Animal Co-Evolution (ed. L. E. Gilbert and P. H. Raven), pp. 100-140. Austin: University of Texas 1973.
4. Baker HG. Non-sugar chemical constituents of nectar. *Apidologie*. 1977;8:349-356.
5. Baker HG, Baker I. Sugar ratios in nectar. – *Phytochem Bull*. 1979; 2:43-45.
6. Barker D, Fitzpatrick MP, Dierenfeld ES. Nutrient composition of selected whole invertebrates. *Zoo Biol*.1998;17: 23-134.
7. Bartholomew GA, Howell TR, Cade TJ. Torpidity in the whitethroated swift, Anna hummingbird, and poor-will. *Condor*. 1957;59:145–155.
8.  Bennett P, Harvey P. Active and resting metabolism in birds—allometry, phylogeny and ecology. *J Zool*. 1987;213:327–363
9. Bent AC. Life Histories of North American Cuckoos, Goatsuckers, Hummingbirds and Their Allies. Washington DC: US National Museum Bulletin. 1940.
10. Beuchat CA, Chong CR. Hyperglycemia in hummingbirds and its consequences for hemoglobin glycation. *Comp Biochem Phys A*. 1998;120:409–416.
11. Brice AT, Grau CR. Hummingbird nutrition: development of a purified diet for long-term maintenance. *Zoo Biol*. 1989;8:233-237.
12. Brice AT, Dahl KH, Grau CR. Pollen digestibility by hummingbird and psittacines. *Condor*. 1989;91:681-688.
13. Brice AT, Grau CR. Protein requirements of Costa's Hummingbirds *Calypte costae*. *Physiol Zool*. 1991;64:611–626.
14. Brice AT. The essentiality of nectar and arthropods in the diet of the Anna's Hummingbird (*Calypte anna*). *Comp Biochem Phys*. 1992;101:151–155.
15. Brown M, Downs CT, Johnson SD. Sugar preferences of nectar feeding birds—A comparison of experimental techniques. *J Avian Biol*. 2008;39:479-483.

16. Carleton SA, Bakken Hartman B, Martínez del Rio C. Metabolic substrate use and the turnover of endogenous energy reserves in broad-tailed hummingbirds (*Selasphorus platycercus*). J Ex. Biol. 2006;209:2622–2627.
17. Carpenter FL. Torpor in an Andean hummingbird: its ecological significance. Science. 1974;183:545–547.
18. Carpenter FL, Castronova JL. Maternal diet selectivity in *Calypte anna*. Am Midl Nat. 1980;103:175-179.
19. 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.
20. Connell CE, Odum EP, Kalem H. Fat-free weights of birds. Auk, 77: 1-9.
21. Cordier A (1953) A better way to feed hummingbirds. Avic Mag. 1960;58(4):143-145.
22. 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.
23. DeFoliart GR. Insect fatty acids: similar to those of poultry and fish in their degree of unsaturation, but higher in the poly- unsaturates. Food Insects Newslett. 1991;4:1-4.
24. Delzenne N, Roberfroid MB. Physiological effects of nondigestible oligosaccharides. Lebensm-Wiss Technol. 1994;27:1–6.
25. Des Granges JL. Organization of a tropical nectar feeding bird guild in a variable environment. Living Bird. 1978;17:199-236.
26. Diamond JM, Karasov WH, Phan D, Carpenter FL. Digestive physiology is a determinant of foraging bout frequency in hummingbirds. Nature. 1986;320:62–63.
27. Duke GF. Gastrointestinal physiology and nutrition in wild birds. Proc. of the Nutrition Society. 1997;56:1049-1056.



- 702 28. Ewald PW, Carpenter FL. Territorial responses to energy manipulations in the Anna  
703 hummingbird. *Oecologia* (Berlin) 1978;31:277–292.
- 704 29. FAO) Food and Agricultural Organization. Food energy: methods of analysis and  
705 conversion factors. Report of a technical workshop. FAO Food and Nutrition paper No.  
706 77. Rome 2003.
- 707 30. Fleming PA, Bakken BH, Lotz CN, Nicolson SW. Concentration and temperature effects  
708 on sugar intake and preferences in a sunbird and a hummingbird. *Funct Ecol*  
709 2004;18:223-232.
- 710 31. Flickinger EA, Van Loo J, Fahey GC. Nutritional Responses to the Presence of Inulin  
711 and Oligofructose in the Diets of Domesticated Animals: A Review. *Critical Reviews in*  
712 *Food Science and Nutrition*. 2003; Vol. 43, Iss. 1.
- 713 32. Freeman CE, Reid WH, Becvar JE, Scogin R. Similarity and Apparent Convergence in  
714 the Nectar-Sugar Composition of Some Hummingbird-Pollinated Flowers. *Bot Gaz*.  
715 1984; Vol. 145, 1:132-135.
- 716 33. Garrison JSE. Traplining foraging behavior in a tropical hummingbird species  
717 *Phaethornis superciliosus*. M.Sc. Thesis, University of British Columbia 1995.
- 718 34. Gass CL, Montgomerie RD. Hummingbird foraging behavior: decision-making and  
719 energy regulation. In *Foraging Behavior: Ecological, Ethological and Psychological*  
720 *Approaches* (ed. A. C. Kamil and T. D. Sargent), pp. 159-194. New York: Garland  
721 STMP Press 1981.
- 722 35. Gottsberger G, Schrauwen J, Linskens HF. Amino acids and sugars in nectar, and their  
723 putative evolutionary significance. *Plant Syst Evol*. 1984;145:55–77.
- 724 36. Green PR, Geer BW. Changes in the fatty acid composition of *Drosophila*  
725 *melanogaster* during development and ageing. *Arch Int Physiol Biochim* 1979;87:485-  
726 491.
- 727 37. Hainsworth FR. Food quality and foraging efficiency. *J Comp Phys*. 1974;88:425–431.

38. Hainsworth, F. R., 1977. Foraging efficiency and parental care in *Colibri coruscans*.  
Condor 79:69-75.
39. Hainsworth FR, Wolf LL. Crop volume, nectar concentration and hummingbird  
energetics. Comp Biochem Phys. 1972;42A:359-366.
40. Hainsworth FR, Wolf LL. Nectar characteristics and food selection by hummingbirds.  
Oecologia 1976; 25:101-114.
41. Hartman FA, Brownell KA Liver lipids in hummingbirds. Condor. 1959;61:273-279.
42. Herbert EW, Shimanuki H. Chemical composition and nutritive value of bee collected  
and bee stored pollen. Apidologie. 1978; 9:33-40.
43. Karasov WH. Energetics, physiology and vertebrate ecology. Trends Ecol Evol.  
1986;1:101-104.
44. Keith AD. Analysis of lipids in *Drosophila melanogaster*. Com. Biochem Phys.  
1966;17:1127-1136.
45. Klasing KC. Comparative Avian Nutrition. CAB International; ISBN: 0851992196 –  
UK 1998.
46. Lasiewski RC. Oxygen consumption of torpid, resting, active, and flying hummingbirds.  
Physiol Zool. 1963;36:122-140.
47. Levrat MA, Rémésy C, Demigné C. High propionic acid fermentations and mineral  
accumulation in the cecum of rats adapted to different levels of inulin. J Nutr.  
1991;121:1730–1737.
48. Lopez HW, Coudray C, Levrat MA, Coudray CF, Demigne C, Remesy C. Fructo  
oligosaccharides enhance mineral apparent absorption and counteract the deleterious  
effects of phytic acid on mineral homeostasis in rats; J Nutr Biochem. 2000;11 500–508.
49. López–Calleja MV, Bozinovic F, Martinez del Rio C Effects of sugar concentration on  
hummingbird feeding and energy use. Comp Biochem Phys .1997;118A:1291-1299.

50. López-Calleja MV, Bozinovic F. Dynamic energy and time budgets in hummingbirds: a study in *Sephanoides sephaniodes*. *Comp Biochem Phys A*. 2003;134(2):283-295.
51. López-Calleja MV, Fernández MJ, Bozinovic F. The integration of energy and nitrogen balance in the hummingbird *Sephanoides sephaniodes*. *J Exper Biol*. 2003;206:3349-3359.
52. Lotz CN, Martínez del Rio C. The ability of rufous hummingbirds *Selasphorus rufus* to dilute and concentrate urine. *J Avian Biol*. 2004;35:54-62.
53. Martínez del Rio C. Sugar preferences in hummingbirds: the influence of subtle chemical differences on food choice. *Condor*. 1990;92:1022-1030.
54. Martínez del Rio C, Baker HG, Baker I. Ecological and evolutionary implications of digestive processes: bird preferences and the sugar constituents of floral nectar and fruit pulp. *Experientia*. 1992;48:544-551.
55. Martínez del Rio C. Nutritional ecology of fruit eating and flower visiting birds and bats. In *The Digestive System in Mammals: Food Form and Function* (ed. D. J Chivers and P. Langer), pp. 102-127. Cambridge: Cambridge University Press 1994.
56. Martínez del Rio C, Karasov WH. Digestion strategies in nectar- and fruit-eating birds and the sugar composition of plant rewards. *Am Nat*. 1990;136:618-637.
57. Martínez del Rio C, Schondube JE, McWhorter TJ. Intake responses of nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. *Am Zool*. 2001;41:902–915.
58. McWhorter TJ. Energy assimilation, protein balance, and water absorption in broad-tailed hummingbirds, *Selasphorus platycircus*. M.S. Thesis, University of Wyoming, Laramie. vii + 89 pp 1997.
59. McWhorter TJ, Martínez del Rio C. Food ingestion and water turnover in hummingbirds: how much dietary water is absorbed? *J Exper Biol*. 1999;202:2851-2858.

- 779 60. McWhorter TJ, López-Calleja MV. The integration of diet, physiology, and ecology of  
780 nectar feeding birds. *Rev Chil Hist Nat.* 2000;73:451–460.
- 781 61. McWhorter TJ, Martínez del Rio C. Does gut function limit hummingbird food intake?  
782 *Physiol Biochem Zool.* 2000;73, 313–324.
- 783 62. McWhorter TJ, Powers D, Martínez del Rio C. Ammonotely, nitrogen excretion and  
784 nitrogen requirements in three sympatric hummingbird species. *Physiol Biochem Zool.*  
785 2003;76:731–743.
- 786 63. McWhorter TJ, Bakken B Hartman, Karasov WH, Martinez del Rio C. Hummingbirds  
787 rely on both paracellular and carrier-mediated intestinal glucose absorption to fuel high  
788 metabolism. *Biol Lett.* 2005;2:131–134.
- 789 64. McWhorter TJ, Caviedes-Vidal E, Karasov WH. The integration of digestion and  
790 osmoregulation in the avian gut. *Biol Rev.* 2009;84:533-565.
- 791 65. Montgomerie RD, Redsell CA. A nesting hummingbird feeding solely on arthropods.  
792 *Condor.* 1980;82:463-464.
- 793 66. [Nicolson](#) SW, Fleming PA. Nectar as food for birds: The physiological consequences of  
794 drinking dilute sugar solutions. *Plant Syst Evol.* 2003;238:139–153.
- 795 67. [Odum](#) EP, Connell CE, Stoddard HL. Flight energy and estimated flight ranges of some  
796 migratory birds. *Auk.* 1961;78:515-527.
- 797 68. Oku T, Tokunaga T, Hosoya N. Non digestibility of a new sweetener “Neosugar” in the  
798 rat. *J Nutr.* 1984;114:1574–1581.
- 799 69. Ortiz LT, Rodriguez ML, Alzueta C, Rebole A, Trevino J. Effect of inulin on growth  
800 performance, intestinal tract sizes, mineral retention and tibial bone mineralisation in  
801 broiler chickens. *Brit Poultry Sci.* 2009;50:325-332.
- 802 70. Overgaard J, Sørensen JG, Peterson SO, Loeschcke V, Holmstrup M. Changes in  
803 membrane lipid composition following rapid cold hardening in *Drosophila*  
804 *melanogaster*. *J Insect Physiol.* 2005;51:1173-1182.

- 805 71. Pappenheimer JR. On the coupling of membrane digestion with intestinal absorption of  
806 sugars and amino acids. *Am J Physiol.* 1993; 265:409–417.
- 807 72. Peaker M. Nutritional requirements and diets for hummingbirds and sunbirds. *Int. Zoo*  
808 *Yb.* 1990;29:109-118.
- 809 73. Pearson OP. The metabolism of humming- birds. *Condor.* 1950;52:145-152.
- 810 74. Powers DR, Nagy KA. Field metabolic rate and food consumption by free-living Anna's  
811 hummingbirds. *Physiol Zool.* 1988;61:500-506.
- 812 75. Powers DR, Conley TM. Field metabolic rate and food consumption of two sympatric  
813 hummingbird species in southeastern Arizona. *Condor.* 1994;96:141–150.
- 814 76. Preest MR, Folk DG, Beuchat CA. Decomposition of nitrogenous compounds by  
815 intestinal bacteria in hummingbirds. *Auk.* 2003;120:1091–1101.
- 816 77. Pyke GH. Why hummingbirds hover and honeyeaters perch. *Anim. Behav.* 1980;29:861-  
817 867.
- 818 78. Pyke GH, Waser NM The production of dilute nectars by hummingbirds flowers.  
819 *Biotropica.* 1981;13:260–270.
- 820 79. Remsen JV, Stiles F, Scott PE. Frequency of arthropods in stomachs of tropical  
821 hummingbirds. *Auk.* 1986;103:436-441.
- 822 80. Richardson KC, Wooller RD. Adaptations of the alimentary tracts of some Australian  
823 lorikeets to a diet of pollen and nectar. *Aust J Zoo.* 1990;38:581–586.
- 824 81. Robbins CT *Wildlife Feeding and Nutrition* (2nd edition). San Diego: Academic Press.  
825 1993.
- 826 82. Robel RJ, Press BM, Henning BL, Johnson KW, Blocker HD, Kemp KE. Nutrient and  
827 energetic characteristics of sweepnet-collected invertebrates. *J Field Ornithol.*  
828 1995;66:44–53.
- 829 83. Roberfroid MB, Slavin J. Nondigestible oligosaccharides. *Crit Rev Food Sci Nutr.*  
830 2000;40:461.

- 831 84. Roberfroid M, Buddington RK. Inulin and oligofructose: proven health benefits and  
832 claims. J Nutr. 2007;137: S2489–S2597.
- 833 85. ~~Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, Wolvers D,~~  
834 ~~Watz B, Szajewska H, Stahl B, Guarner F, Respondek F, Whelan K, Coxam V, MJ~~  
835 ~~Davicco, L Léotoing, Y Wittrant, NM Delzenne, PD Cani, AM Neyrinck, A Meheust.~~  
836 ~~Probiotic Effects: Metabolic and Health. Brit J Nutr 2010.~~  
837 ~~<http://centaur.reading.ac.uk/8179/>.~~
- 838 86. Roberts WM. Hummingbirds' nectar concentration preferences at low volume: the  
839 importance of time scale. Anim Behav. 1996;52:361-370.
- 840 87. Robinson SJW, Zwaan B, Partridge L. Starvation resistance and adult body composition  
841 in a latitudinal cline of *Drosophila melanogaster*. Evolution. 2000;54:1819-1824.
- 842 88. Scheithauer W. Hummingbirds. Thomas Y. Crowell, New York 1967.
- 843 89. Sherman AR. Experiments in feeding Hummingbirds during seven summers. The  
844 Wilsons bull. 1913;86, Vol. xxv December 4:153-166.
- 845 90. Schmid R. Sugar preferences of hummingbirds. 2008.  
846 [http://www.easternct.edu/~elliott/documents/Sugar\\_000.pdf](http://www.easternct.edu/~elliott/documents/Sugar_000.pdf).
- 847 91. Schondube JE, Martínez del Rio C. Concentration-dependent sugar preferences in  
848 nectar-feeding birds: mechanisms and consequences. Funct Ecol. 2003;17:445-453.
- 849 92. Schondube J, Martínez del Rio C. Sugar and protein digestion in flowerpiercers and  
850 hummingbirds: a comparative test of adaptive convergence. J Comp Phys B.  
851 2004;174:263-273.
- 852 93. Solberg, Y, Remedios G. Chemical composition of pure and bee-collected pollen. Meld  
853 Norg Landbruks. 1980;59 (18):1-13.
- 854 94. Stanley RG, Linskens HF. Pollen Biology Biochemistry Management. Springer-Verlag,  
855 Berlin 1974.

- 856 95. Stiles FG. On the field identification of California hummingbirds. Calif. Birds.  
857 1971;2:41-54.
- 858 96. Stiles, FG. Taste preferences, color preferences, and flower choice in hummingbirds.  
859 Condor. 1976;78:10–26.
- 860 97. Stiles FG, Freeman CE. Patterns in floral nectar characteristics of some bird-visited plant  
861 species from Costa Rica. Biotropica. 1993;25:191–205.
- 862 98. Suarez RK, Lighton JRB, Moyes CD, Brown GS, Gass CL, Hochachka PW. Fuel  
863 selection in rufous hummingbirds: ecological implications of metabolic biochemistry.  
864 Proc. of the National Academy of Science USA. 1990;87:9207–9210.
- 865 99. Suarez RK, Gass CL. Hummingbird foraging and the relation between bioenergetics and  
866 behaviour. Comp Biochem Phys. 2002;133:335-343.
- 867 100. Szczęsna T. Study on the sugar composition of honeybee collected pollen. J Apic  
868 Sci. 2007;51:11-22.
- 869 101. Tamm S. Importance of energy costs in central place foraging by hummingbirds.  
870 Ecology. 1989; 70, 195–205.
- 871 102. Tiebout III HM. Tests of a model of food passage rates in hummingbirds. Auk.  
872 1989;106:203-208.
- 873 103. Tiebout III HM. Daytime energy management by tropical hummingbirds: responses  
874 to foraging constraint. Ecology. 1991;72:839-851.
- 875 104. Tsahar E, Arad Z, Izhaki I, Martínez del Rio C. Do nectar- and fruit-eating birds  
876 have lower nitrogen requirements than omnivores? An allometric test. Auk.  
877 2006;123:1004–1012
- 878 105. Van Soest PJ. Nutritional Ecology of the Ruminant: Ruminant metabolism,  
879 Nutritional Strategies, the Cellulotic Fermentation and the Chemistry of Forages and  
880 Plant Fibres. O and B Books, Corvallis 1982.

- 881 106. Van Tets IG, Nicolson SW. Pollen and the nitrogen requirements of the lesser  
882 doublecollared sunbird. *Auk*. 2000;117:826–830.
- 883 107. Verdonk JM, Shim SB, van Leeuwen P, Verstegen MV. Application of inulin-type  
884 fructans in animal feed and pet food. *Br J Nutr*. 2005;93 Suppl 1:S125-138.
- 885 108. Weathers WW, Stiles FG. Energetics and water balance in free-living tropical  
886 hummingbirds. *Condor*. 1989;91:324–331.
- 887 109. Wooller RD, Richardson KC, Pagendham CM. The digestion of pollen by some  
888 Australian birds. *Aus J Zool*. 1988;36:357–362.
- 889 110. Zanutto FP, Bicudo JEPW. Dietary protein and carbohydrate affect feeding  
890 behaviour and metabolic regulation in hummingbirds (*Melanotrochilus fuscus*). *Rev*  
891 *Chil Hist Nat*. 2005;78:281-294.