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Original Research Article

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

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 *Amazilia amazilia* 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,

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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

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

In practice, to successfully maintain and reproduce captive hummingbirds, the artificial nectar must satisfy all nutritional needs, miming the inputs of a natural feeding. Energy and main nutrients requirements for maintenance, flight and reproduction have been differently studied and indicated (Weathersand Stiles 1989; Peaker 1990; McWhortherand López-Calleja 2000; López-Calleja et al. 2003). From on hand knowledge it appears that hummingbirds do not deliberately forage for fibre, intended as insoluble and indigestible carbohydrates of plants cell wall. Conversely, they prey variable amount and species of arthropods rich of chitin. The role of this insoluble dietary fibre on digestion and absorption of other nutrients and its potential beneficial physiologic and healthy effects received few attentions. If arthropods are not regularly available, other source of dietary fibre might be given in the artificial nectar. Chitin, chitosans and other vegetable insoluble fibre sources are impractical, because insoluble deposit sediments or surfaces in the feeders. Inulin, among the soluble fibrous ingredients, was already used in animal nutrition and appears suitable for the scope (Flickinger et al. 2003; Verdonk et al. 2005). For this reason, we consider to evaluate the effects of the addition of inulin on digestive utilization of the artificial nectar. Beside the nutritional

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facts, when hummingbirds are maintained *ex situ*, also health problems might arise and some aspects as intestinal mycoses, liver steatosis should be monitored and prevented.

Available nutritional data are still incomplete and sketchy and some physiological aspects and behaviours of hummingbirds are undisclosed so that some solution must be attained by trial and error.

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

2. Materials and methods

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

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

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

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
Inulina	95.64	0.18	-	-	0.02	-	99.80	16.30
Nectar (g/l)								**En** (kJ/g)
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

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

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Body weight was recorded throughout the acclimation and experimental period with an analytical balance (accuracy ± 0.001·g). Initial live weights 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.

Preliminarily, based on the existing knowledge the formulation of nectar was devised and progressively adjusted, by checking intake and appetibility and physical and health conditions. 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%, Hydrolysed soy and collagen (Wellesse – Ferndale USA) 1.5% , a mix of essential a.a. 2.2% (Nutricia –Danone), Maize and Soybean oils 2.5%, Pollen 4.6%, 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 ß-configuration of the anomeric C2 in its fructose monomers, resists hydrolysis by intestinal digestive enzymes, which are specific for α-glycosidic bonds (Delzenneand Roberfroid 1994; Roberfroidand Slavin 2000).

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

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

The average daily intakes of hummingbirds are reported in Table 2. When hummingbirds received the nectar containing inulin, the DM intake was significantly increased (P<0.01) as those of the remainder nutrients. Inulin is included in NFE and, as expected, both DM and NFE intakes largely differ between the two nectars; though the intakes and the differences among the intakes of other nutrients between Nt and Ni were lesser, resulted statistically highly significant (Table 2). The daily energy intakes of Nt and Ni, 34.16 and 39.12 kJ/d respectively, were also significantly different (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	5.72B	6.08^{A}	8.45	32

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

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

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

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

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

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

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Table 4 Energy, DM and nutrient metabolizability (%)

	Nt	**Ni**	F	df
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

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.

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

Energy and main nutrients requirements for maintenance, flight and reproduction have been differently studied and indicated (Weathersand Stiles 1989; Peaker 1990; McWhorterand López-Calleja 2000; López-Calleja et al. 2003; Tsahar et al. 2006). Wild hummingbirds meet their energy requirements essentially from nectar sugars while protein needs are independently satisfied by eating a changeable amount of small arthropods (Gassand Montgomerie 1981; Pyke 1980; Hainsworth 1977; Des Granges 1978; Montgomerieand Redsell 1980; Brice 1992), other stuffs and possibly pollen (Wooller et al. 1988, Van Tetsand Nicolson 2000). To the contrary, a diet of only arthropods cannot cover the energy requirements (Brice 1992; López-Calleja et al. 2003).

Flowers habitually visited by hummingbird produce 10-30 ml of nectar having a DM content of 15-40%; the sugar concentration varies between 0.15 M and 1.2 M: most of the nectars contain sucrose, fructose and glucose and rarely are composed by a single sugar (Hainsworthand Wolf 1976; Pykeand Waser 1981). Hummingbirds prefer flowers providing nectars with sucrose, fructose and glucose (Hainsworthand Wolf 1976) and with a higher proportion of sucrose to hexoses (Cruden et al. 1983; Freeman et al. 1984; Gottsberger et al. 1984; Martínez del Rio 1990; Martínez del Rio et al. 1992; Stilesand Freeman 1993; Chalcoff 2001). According to Nicolsonand Fleming (2003), hummingbirds select nectars containing 64.4±18.5% of sucrose. The large range of sugar concentration and composition of nectars in flowers selected by hummingbirds offers them many dietary options for satisfying the energetic demand of distinct lifestyles.

Less univocal is the sugars preference in captive hummingbirds. Environment, cage dimension, colour and shape of feeders and perches, but also imprinting or adaptation, bill length and licking capability look as if influencing sugars preference; the first adaptation to a definite sugar solution or to a frequent variations sugars solution requires several hours or feeding sessions (Hainsworthand

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Wolf 1976; Stiles 1976; Roberts 1996; Schmid 2008). Different results indicate that sucrose is generally preferred alone or when paired with glucose; on the other hand, sucrase activity always proved to be very high in hummingbirds (Martínez del Rio 1990; McWhorter and Martínez del Rio 2000; Schondubeand Martínez del Rio 2004). Captive birds always prefer the more concentrated sugar solution (Hainsworthand Wolf 1976; Schondubeand Martínez del Rio 2003) and notwithstanding the negative relationship ($P<0.02$) between the sucrose concentration and the rate of volumetric nectar intake, the amount of sugar ingested remains nearly the same or increases with the concentration (McWhorterand Martínez del Rio 1999 and 2000). The transit time of a bout increases with sucrose concentration (Tiebout III 1989, Martínez del Rioand Karasov 1990). The preference for solutions of sucrose and hexoses should be compared at the same concentration on equicaloric basis. At high concentration of sugars (≥ 25%), hummingbirds do not discriminate 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

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having moderate sugars' concentration, which appears to favour hexoses preferences and absorption (Schondubeand 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 (McWhorterand 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.

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

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

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

The overall concentration of a.a. in nectar of flowers lies within the range of 30-400 µg/ml and their profile is variable; these amounts are considered insufficient to meet the requirements of nectivorous vertebrates (Baker 1977; Baker and Baker 1973; Martínez del Rio 1994). Scheithauer (1967) and Peaker (1990) report mean values of *Drosophila melanogaster* wet mass between 1.2 and 1.5 mg, with a 60% of water. For Hainsworth (1977), the single DM weight of flies is 1.5 mg. The protein content of arthropods is high, from 54% to 77% on DM, depending upon species¸ the biological value of arthropods body protein, as results from a.a. profile, is variable and usually rather high (Robel et al. 1995; Barker et al. 1998). Remsen et al. (1986) observed that arthropods are extensively digested in the stomach within 3-4 hours, following only brief storage in the crop. Evidently not all parts are equally digested; the components of soft body and some constituents of cuticle (e.g. lipid, waxes and soluble protein) are probably quite digestible and fragments of their body are detectable in faeces. Unfortunately, not all this information is supported by values or measures.

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

The number of fruit flies a hummingbird has to ingest to cover N maintenance requirements is differently quoted as 312 by Peaker (1990), 150 by López-Calleja et al. (2003), 283 by Lotz and 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 *Sephanoides sephaniodes* 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).

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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. 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. 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). More probably, as hummingbirds maintain a high glycaemia and insulin level also in the fasted condition (Beuchat and Chong 1998; McWhorter and Martínez del Rio 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

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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.

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 trough 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; Szczęsna 2007).

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$^{0.75}$ of Nt and Ni nectar

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respectively, corresponding to 121.5 and 134.4 g/kgLW$^{0.75}$ of DM. When inulin was added to the nectar, the hummingbirds increase the average daily intake by 14.3%.

When concentration of sugars is low, as in this experiment, the intakes should be compared at the same molarity or energy or sugar contents. López-Calleja et al (1997), with 0.75, 0.5, 0.25 M sucrose nectars, measured increasing intakes (8.6, 12.6 and 24.4 ml/d). McWhorter and Martínez del Rio (2000), with reference to captive hummingbirds of different species, determined that intake steadily increase when the sucrose molarity of nectar diminishes and, at 584 mmol/l equals 12 ml/d. Practically, higher the sugar molarity lower is the intake but, *ceteris paribus*, the overall daily consumption and the metabolizability are the same. In our experiment (0.5 M, sucrose equivalent), the intake of Nt was 11.5 ml/d and the sugars (NFE) metabolizability resulted 98.4%, within the range of the ordinary values. In fact, sugar assimilation efficiencies measured in hummingbirds using traditional balance methods are higher than 95% and by and large independent from sugar concentration and from *digesta* retention time (McWhorter and Martínez del Rio 2000; Schondube and Martínez del Rio 2003). Hainsworth (1974) measured sucrose digestibility of 98-99%; Karasov (1986), using a double isotope dilution method for measuring crop-emptying kinetics of *Selaphorus rufus* hummingbirds, found an efficiency of sucrose extraction of 97.1±0.3%. Duke (1997) reported that sucrose and hexoses are digested to the same high extent (> 97%).

In our trial the DM intake of Nt nectar averaged 2.05 g/d (128 g/d/kgLW$^{0.75}$) and metabolizability resulted 94.6 %, as a combination of sugars metabolizability (98.4 %) and that of other nutrients which all together results 49.5 %.

In different experiments, where diverse nectars were given to several hummingbird species, the daily intakes of metabolizable energy varied from 29.1 to 138.5 kJ, or from 5.63 to 9.32 kJ/d/g LW (Powers and Nagy 1988; Weathers and Stiles 1989; Powers and Conley 1994; López-Calleja and Bozinovic 2003; López-Calleja et al. 2003). Many factors affect the requirements, the intake and the nutritional efficacy of the diet and the energy balance, practically evaluated by the LW variation, gives an inclusive validation. In all these experiments, the hummingbirds appear to have

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

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

In trial, with S*ephanoides sephaniodes* weighing about 5.5 g, (López-Calleja et al. 2003) compared nectars of different sugars and CP content – from 0 to 11.2%. They observed an increasing DM intake from 93 to 139 g/d/kgLW$^{0.75}$ and a declining energy metabolizabilty (from 0.99 to 0.93); when CP was 4.7%, the DM intake was 119.5 g/d/kgLW$^{0.75}$ and the metabolizabilty 0.97. From an experiment of Brice and Grau (1991), who offered a liquid diet containing a rising percentage of CP - from 0 to 3% in 25% solids – we can easily calculate to some extent an increase of DM consumption from 123 to 130 g/kgLW$^{0.75}$. In another experiment, López-Calleja and Bozinovic (2003), comparing two diets differing for sucrose molarity supplemented with protein, vitamins and 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 (0.75 M); DM metabolizability in both groups was 0.95±0.02. Thus, when nectar contains protein, the intake is adjusted to sugars content and the DM metabolizability decreases. DM intake and metabolizabilty of diets comparable to Nt were similar to those we measured on *Amazilia amazilia*.

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

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

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

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

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

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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 mg N/kg$^{0.75}$/d, being the Total Endogenous Nitrogen Losses (TENL) of 270 mg N/kg$^{0.75}$/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 mg N/kg$^{0.75}$/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·mg/kg$^{0.75}$/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 mg/kg$^{0.75}$/d, and TENL, from 46 to 77.7 mg/kg$^{0.75}$/d; in a previous experiment, on a fourth 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). 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±7% to 48±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 mg N/kg$^{0.76}$/d and TENL of 54.1 mg N/kg$^{0.69}$/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

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

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

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

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

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

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

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

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(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.

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.

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The attempt of simultaneously measuring the metabolizability of main nutrients of a feed open to a different view on the digestive efficiency of hummingbirds and helps in testing other ingredients than the few generally used. In fact, there is a large difference between the sugars metabolizability and that of other nutrients, so that the content of protein, fat and other ingredients in non-useful amount reduces the overall DM metabolizability reducing the sugar intakes. For effective use in metabolism and production, essential nutrients need to be contained in the bird's diet in proportions more - or - less parallel to its needs and must be available for the digestion and the transport. 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 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 only option given captive hummingbirds was that of varying the intake and preying fruit flies. For practical reasons, only one or two recipes could be prepared and offered to hummingbirds kept in an artificial environment.

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, not different from the values reported by many other Authors.

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. From the results of available experiments, it comes out that an increase of nectar's sugar concentration does not significantly modify its daily intake and metabolizability. On the contrary, a higher content of protein, and possibly of other nutrients, result in a greater nectar intake, reducing its metabolizability, by affecting osmolality and feed transit time.

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The metabolizability of protein was similar to that observed in other few experiments when the N source is a mix of a.a. and/or hydrolyzed protein. 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 metabolibazability 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 fat metabolizability was 44% in Nt and 33.6% in Ni, indicating that, as it should likely happen, the fast transit time of a liquid feed is not congruent with an efficient absorption. 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 fiber modified intakes and decreased the metabolizabilities of nutrients. Positive dietetic effects should compensate this negative consequence. The main role of fiber 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.

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