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Nutrients Metabolizability of Nectar for Long-Term Maintenance of *Amazilia amazilia* and Effect of a Dietary Fiber Source

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Authors' contributions

This work was carried out in collaboration between all authors. Author CF has made the contribution to design of experimental work, to acquisition, analysis and interpretation of data. She was also involved in the writing of manuscript. Author SR has made the contribution to formulation of the diets and in the revision of the paper. Author PAS has coordinated all the experimental work and has made contribution in writing manuscript and in the critical revision. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To formulate an artificial nectar for long-term maintenance of captive hummingbird, to measure the metabolizability of nutrients in the artificial nectar and to evaluate the effects of dietary fiber (inulin) addition on its digestive utilization.
Study Design: Two groups of seven hummingbirds randomly distributed in two diets with different levels of inulin (0-7% DM).
Methodology: The trial was conducted on two groups of seven 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 experiment lasted 24 days; the

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birds were separately housed in experimental cages.
Results: 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, 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 [1,2]. For a very long time in some area of North, Central and South America wild hummingbirds are attracted offering artificial nectars in feeders [3]. Many commercial types of nectar, containing sugars and other few ingredients, are offered to attract free-living hummingbirds, e.g. Roudybush, Wombaroo, Nectar+, Perky-Pet.

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. [4] proposed an artificial nectar containing honey or sugar, meat extract and condensed milk, almond oil and vitamins. [5] 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. [6] 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. [7] developed a purified liquid diet containing about 25% of dry 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. [8] use a maintenance diet composed by 20% sucrose, 0.5% amino acids (Glicopan P), 3% oil, 0.03% vitamins (Vita Gold) and 1% calcium carbonate. [9] 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 amino acids, 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 [9-12]. From on hand knowledge, it appears that hummingbirds do not deliberately forage for fiber, intended as insoluble and indigestible carbohydrates of plants cell wall. Conversely, they prey variable amount and species of arthropods rich of chitin, 5÷20% DM [13], a polysaccharide which is main constituents of the exoskeletons of many arthropods, classified as added fiber having physiological properties similar to dietary fiber [14,15]. The digestion of chitin requires chitinases [16]. Many bacteria and fungi species, as well as certain invertebrates, fishes, birds, terrestrial mammals and humans produce chitinases, which permit the breakdown of polymeric chitin to oligosaccharides and monomeric sugar subunits [13]. Either chitinase production in birds and mammals could occur through a chitinoclastic bacterial gut flora, or by glandular production of chitinases [17] instead, according to [18], mammals lack chitinases activity. The chitin and chitosan digestibility, mainly measured in marine species, but also in mammals and birds, is variable and affect lipids digestion [19-22]. This topic appears not yet investigated in hummingbirds as well as the role of this insoluble dietary fiber on digestion and absorption of other nutrients and its potential beneficial physiologic and healthy effects.

If arthropods are not regularly available, other source of dietary fiber might be given in the artificial nectar. Chitin, chitosan, produced by alkaline deacetylation of chitin obtained mainly from marine crustacean, and other vegetable insoluble fiber sources are impractical, because insoluble deposit sediments or surfaces in the feeders. Inulin, a heterogeneous assortment of fructose polymers found in a number of vegetables, fruit, and grains, was already used as soluble fibrous ingredients in animal nutrition and appears suitable for the scope [23,24]. For this reason, we consider to evaluate the effects of the addition of inulin on digestive utilization of the artificial nectar. Beside the nutritional 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 behaviors of hummingbirds are undisclosed so that some solution must be attained by trial and error.

The objective of our experiments is to measure

i. the metabolizability of the nutrients in a complete diet formulated to maintain in good health captive adult *Amazilia amazilia,* after a critical review of the existing literature, and
ii. the nutritional effect of inulin addition to the nectar.

2. MATERIALS AND METHODS

In Miramare park (Trieste – Italy), at the Centre for the Safeguard of hummingbirds, in fully air conditioned aviaries in a natural mimic environment with tropical plants and where fruit flies (*Drosophila* sp.) were grown in buckets containing fruits, lived about 90 hummingbirds mainly belonging to *Amazilia amazilia* species. *Amazilia* are medium-sized trochilid (length 8-11 cm, weight 4-7 g) that inhabits arid and semiarid habitats of the tropical Pacific lowlands and adjacent subtropical Andean slopes, eg: edges of deserts scrub and dry forests, of Ecuador and Peru [25]. It is a territorial and nectivorous bird, which by individuals or pairs defends its territories against conspecifics or other nectivorous species [26].

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

The experiment was conducted during the non-reproductive period. Adult *Amazilia amazilia* hummingbirds were randomly assigned to two groups of seven hummingbirds each, 4 males and 3 females, 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 25ºC 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. The nectar powder (Nt) is prepared once a week using the same ingredients. The nectar contains sucrose 47.2%, glucose 19.2%, fructose 19.2%, hydrolyzed soy protein and collagen (Wellesse–Ferndale USA) 1.5%, a mix of essential amino acids 2.2% (Nutricia –Danone), maize and soybean oils 2.5%, flower's Bio-pollen 4.6% (Fior di Loto – Torino, Italy), silimarin (Legalon – Rottapharm-Madaus) 1.1%, Bio-Mos (Alltech) 1.1%, mineral and vitamins supplement 1.3%. As for sugar concentration, Nt and Ni were equimolar, 0.75 M or 0.5 M sucrose equivalent. Inulin was added to the nectar powder (Ni) in the ratio of 7 g/100 g, as fed. We used inulin 90% (Farmalabor-Italy), extracted from *Dahlia variabilis, Helianthus tuberosus* and other *Compositae*. This inulin is classified as "soluble non digestible" fiber (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 [27,28]. To reduce the fractionation, due to precipitation or surfacing, of less soluble or insoluble ingredients and the fermentation, nectar was renewed twice daily, at 7 am and at 3 pm; the volumetric nectar intakes were individually recorded at the 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 -20ºC. Before the chemical analysis, the two pools of feces were weighed and thoroughly mixed in 250 ml of distilled water. DM was measured on 15 ml of sample by drying at 60ºC to constant weight. On the remaining CP (Nx6.25), Ether Extract (EE), ash [29], Neutral Detergent Fiber (NDF) [30], were determined. The gross energy (GE) content (expressed in kJ) was either measured, using an adiabatic calorimeter (IKA C7000), and checked using the average energy values of different nutrients proposed by [31] 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.

The apparent metabolizability of different nutrients was calculated using the following formula: Metabolizability (%)=[1-Intake (g)/*Excreta* (g)] x 100]. Data were statistically analyzed 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). In the tables F is the ratio between variance of the group means / mean of the within group variances; the significance level is obtained from the F distribution with numerator and denominator degrees of freedom (df).

The coefficient of variability (CV), calculated as a percentage of average/standard deviation ratio, was used to describe the range of variation of weights (LW), DM intake and excretion among animals. Body weight was recorded throughout the acclimation and experimental period with an analytical balance (accuracy±0.001·g). Initial LW were 4.08 g and 4.14 g for Nt and Ni groups and at the end of the experimental period 3.98 g and 3.80 g, respectively.

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 and probiotics, and corresponds to about 0.2% on DM.

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 ($P<0.01$).

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 fiber 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). In addition, the contents of CP and EE were significantly increased, while that of ash has been reduced. The amounts of NDF excreted are identical, nearly matching the intakes.

The 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).

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.

Table 1. Dry matter and nutrients content of feeds

Feed (% DM)	**DM**	**CP**	**EE**	**CF**	**Ash**	**NDF**	**NFE**	**GE** (kJ/g)
Nt	93.76	4.28	2.30	0.28	0.95	0.19	92.28	16.63
Ni	93.89	3.99	2.14	0.26	0.88	0.17	92.81	16.66
Inulin	95.64	0.18	-	-	0.02	-	99.80	16.30
Nectar (g/l)								**GE** (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

DM: Dry Matter; CP: Crude Protein; EE: Ether Extract; CF: Crude Fiber; NDF: Neutral Detergent Fiber; NFE: Nitrogen Free Extract; GE: Gross energy; Nt: artificial nectar; Ni: artificial nectar with inulin

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

	Nt	**Ni**	**F**	**df**
ml	11.5^{B}	13.13^{A}	40.89	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
GE (kJ)	34.16^{B}	39.12^{A}	45.37	32
CF	5.72^{B}	6.08^{A}	8.45	32

DM: Dry Matter; CP: Crude Protein; EE: Ether Extract; CF: Crude Fiber; NDF: Neutral Detergent Fiber; NFE: Nitrogen Free Extract; GE: Gross energy; Nt: artificial nectar; Ni: artificial nectar with inulin; F: Anova test; df: degree freedom. In the same row, mean values with different capital letters are significantly different (P< 0.05)

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

	Nt	**Ni**	**F**	**df**
Fresh	1110.53^{B}	2890.08^{A}	84.87	32
DM	110.53^{B}	221.68^{A}	83.86	32
CP	37.8^{B}	46^{A}	8.47	32
EE	26.52^{B}	33.25^{A}	11.11	32
Ash	11.49^{A}	9.62^{B}	7.49	32
NDF	3.70	3.84	0.31	32
NFE	30.39^{B}	128.35^{A}	219.04	32
GE (kJ)	2.07^{B}	3.74^{A}	67.78	32
CF	6	6	0.33	32

DM: Dry Matter; CP: Crude Protein; EE: Ether Extract; CF: Crude Fiber; NDF: Neutral Detergent Fiber; NFE: Nitrogen Free Extract; GE: Gross energy; Nt: artificial nectar; Ni: artificial nectar with inulin; F: Anova test; df: degree freedom

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
GE (kJ)	93.93^{A}	89.99^{B}	51.2376	32
CF	1.95	3.74	0.0822	32

DM: Dry Matter; CP: Crude Protein; EE: Ether Extract; CF: Crude Fiber; NDF: Neutral Detergent Fiber; NFE: Nitrogen Free Extract; GE: Gross energy; Nt: artificial nectar; Ni: artificial nectar with inulin; F: Anova test; df: degree freedom

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, but primarily the ingredients to use to achieve suitable palatability, to satisfy requirements and to compose a stable solution/suspension.

Wild hummingbirds meet their energy requirements essentially from nectar sugars, while protein needs are independently satisfied by eating a changeable amount of small arthropods [32-36], other stuffs and possibly pollen [37]. Hummingbirds prefer flowers providing nectars with sucrose, fructose and glucose [6] and with a higher proportion of sucrose to hexoses. Rarely contains a single sugar [38-43]. According to [44] hummingbirds select nectars containing 64.4±18.5%) of sucrose. Less univocal is the sugars preference in captive hummingbirds, which prefer the more concentrated sugar solution [6,45]. Many factors influence sugars preference and the first adaptation to a definite sugar solution or to a frequent variations sugars solution requires several hours or feeding sessions [6,46-48]. Different results indicate that sucrose is generally preferred alone or when paired with glucose [49,50].

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 [48,51,52].

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

There are some grounds for inclining in the direction of artificial nectar having moderate sugars' concentration, which appears to favor hexoses preferences and absorption [45]; firstly, because the sugars' concentration of nectars does not influence the rate of daily energy intake [53]. 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.

Energy and main nutrients requirements for maintenance, flight and reproduction have been differently studied and indicated [9-11,54,55]. More often energy requirement is associated to time budget since different activities have different energetic cost [34]. 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 [10,56-59]. Typically, the metabolic rate measured in captivity ranges from 7.3 kJ/d to 10 kJ/d per g LW [5,60].

The overall concentration of amino acids 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 [61,62].

For [34], 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 amino acids profile, is variable and usually rather high [63,64]. [1] 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.

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. [12,36] 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 [9], 150 by [12], 283 by [65]. According to [5], a hummingbird of 3 g LW ingests 677 flies/d. In contrast, [35] 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%. [12] observed that *Sephanoides sephaniodes* weighting about 6 g require approximately 150 fruit flies to maintain body mass.

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 dietary fiber.

Hummingbirds do not specifically necessitate protein but are able to live eating a diet containing free amino acids [66]. As the prudential criteria was to guarantee an adequate intake of protein to cover synthesis of semi essential and non-essential amino acids 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 amino acids with the addition of an extra amount of organic N (hydrolyzed protein).

In some experiments, diets were formulated adding from 2% of crude oil [9], to 3% of corn oil [8] and 3.7% of corn oil [67,68]. On fat utilization by hummingbirds, any specific information has been experimentally obtained. 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 [59,69,70]. Body fat content is variable and changes during the day and with season. Highest variations are found in migratory species, which can store more than 40% of body weight as fat [71].

Arthropods should provide hummingbirds also with essential unsaturated fatty acids, being one of the richest animal feed sources [72]. *Drosophila sp.*, are a significant source of fat (8-15%) and of mono (13-51%) and poly unsaturated fatty acids (8-31%) [73,74]. 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.

The hummingbirds do not collect pollen and only groom it off their bills [75]. The hummingbirds' attitude for eating pollen and their capability of extracting nutrients from ungerminated pollen is controversial [37,76]. [7] 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 [77]. [78] comments that pollen digestibility is limited by the fast transit trough gastrointestinal tract, but it also depends upon bird's age and species.

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 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. [53], with 0.75, 0.5, 0.25 M sucrose nectars, measured increasing intakes (8.6, 12.6 and 24.4 ml/d). [49], 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 [45,49]. [79] measured sucrose digestibility of 98-99%; [80] found an efficiency of sucrose extraction of 97.1±0.3% on *Selaphorus rufus* hummingbirds. [81] 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 [10,12,54,58,82]. 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.

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

In trial, with S*ephanoides sephaniodes* weighing about 5.5 g, [12] compared nectars of different sugars and CP content – from zero to 11.2%. They observed an increasing DM intake from 93 to 139 g/d/kgLW$^{0.75}$ and a declining energy metabolizability (from 0.99 to 0.93); when CP was 4.7%, the DM intake was 119.5 g/d/kgLW$^{0.75}$ and the metabolizability 0.97. From an experiment of [68], 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, [54], 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 metabolizability of diets comparable to Nt were similar to those we measured on *Amazilia amazilia*.

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, [12] 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 [67], the DM intake increases by means of protein content of DM. [54] 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 [68,83], 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 [9,12,54,67,83].

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 it is generally recognized that they have low N requirements compared to non-nectivorous birds [12,55,68,83]. [84] 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. [9], 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%.

[68], 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 [12], 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. [83] 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 [85]. [12], 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%. [8] 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, [55] in a notable discussion paper on N requirements of birds, reassessing Robbins' allometric equation [84], propose for nectivorous-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 [55]. Both MNR and TENL varied giving evidence that this requirement is largely influenced by nectar composition, by hummingbirds' species and by 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 [12]. The few information of N metabolizability experimentally measured using different protein supplements are always lowers than that from fruit flies. [83], using casein acid hydrolyzate (SIGMA–St. Louis), measured N metabolizability within 49 and 54%. [12], giving different amounts of hydrolyzed 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 solubilized 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.

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 (Table 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. [86] comments that various hypotheses have been tested to tentatively explain the modifications of the digestion and absorption of macronutrients, especially carbohydrates, triglycerides and minerals, which may occur in animals and humans, either by delaying gastric emptying and/or shortening small intestinal transit time [87]. 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 [88,89]. 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 through 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. [90] 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 favor 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. [57]. In birds, diverse components of the same meal may move at greatly different rates through the different tracts of digestive system [78]. Soluble and insoluble fraction of dietary fiber 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.

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

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

The 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. The average daily intake of DM (2.05 g/d) and the average volumetric consumption of nectar find a good agreement with other experimental evidences obtained with nectar of similar sucrose molarity, digestibility. The intake of sugars and energy, (about 130 g/kg$^{0.75}$LW/d and 2150 kJ kg$^{0.75}$LW/d), seem adequate to cover the maintenance requirements.

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

The addition of dietary fiber 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. Even if the energy requirements of captive hummingbirds can be easily satisfied by sugars, a small amount of vegetal oil is useful to meet essential fatty acids exigency, which is unspecified, but probably not avoidable. 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 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. The addition of soluble fiber modified intakes and decreased the metabolizabilities of nutrients. Positive dietetic effects should compensate this negative consequence.

ETHICAL ISSUES

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

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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