Nutritional requirements of natural enemies

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Abstract
Because parasitoids are living animals, nutrition inevitably plays an important role in augmenting these natural enemies of pests. For nutrition is about nourishment; that is, it is the action or processes of transforming substances found in foodstuff into body materials and energy to do all the things attributed to life. Nutritional requirements depend on the synthetic abilities of the organism and the basis is genetical. Therefore, through nutrition we have a direct and essential connexion between an environmental factor, foodstuff, and the vital processes of the insect organism. A comprehensive knowledge of the nutritional ecology of insects is a requirement to understand their biology, behavior, and impact in natural ecosystems and agro-ecosystems. Although, major insect nutritional requirements for growth and reproduction are known for years, only a few dozen insects have been studied to learn their nutritional requirements. Extraordinary nutritional requirements were found in a few insects from even these few studies. It is important to understand comparative nutritional requirements in order to study insects. In this review, emphasis will be placed on insect nutritional requirements for natural enemies.

Keywords: Natural enemies, diet, nutritional requirement, amino acid, carbohydrate and lipid

1. Introduction
Nutrition is the action or processes of transforming substances found in foods into body materials and energy to do all the things attributed to life. Nutritional requirements are dependent on the synthetic abilities of the organism, which is controlled genetically. It is generally admitted that the basic qualitative nutritional requirements for parasitoids and predators are not different from those of free-living insects. Essential resources for natural enemies differ in species but may broadly classify as food other than host, water, special sites for breeding and protective refuges. Resources for some enemies may be found in sufficiency within a given crop, other enemies may have more complex requirements for some requisite. Significant advances have been made in our knowledge of the nutritional requirements of entomophagous insects since the topic has been discussed by Doutt (1964) [15] and Hagen (1964) [26]. Many nutritional factors important for growth, development and reproduction have been identified and success has been achieved at feeding and rearing of natural enemies in absence of natural foods.

Nutrition of natural enemies represents a complex and tritrophic interaction of physiological, behavioural and ecological factors involving the entomophage, its host and host food source. Contrary to the species developing at the expense of growing stages (koinobionts), idiobiont parasitoids and especially egg parasitoids develop in closed systems, for a short time, without external nutritional supply (Mellini, 1986) [41]. Consequently they need very rich and concentrated food. The nutritional plasticity of some predator species is a positive characteristic regarding their possibility to be reared on artificial food (Specty et al., 2003) [56]. Sometimes, a diet designed for a species can be appropriate for another species, possibly with small adaptations, as observed with Harmonia axyridis diet used for Chrysoperla carnea (El Arnaouty et al., 2006) [17]. Sufficient flower abundance and appropriate vegetation structure are required to support diverse populations of insects (Zurbrug and Frank, 2006) [33] and therefore manipulation of structurally resource-poor habitats through the addition of flowering plants and grasses can increase beneficial insect populations in agricultural landscapes.

Many beneficial insects, including natural enemies, require access to alternate hosts, overwintering habitats, a constant food supply, and appropriate microclimates in order to survive (Johnson and Triplehorn, 2005) [33]. The majority of predators and parasitoids are omnivores and require non-prey food, such as pollen and nectar, as their diet. Natural enemies
from a broad range of orders including Hymenoptera, Diptera, Coleoptera, Heteroptera, Neuroptera, Araneae, and Acarina have been observed to require and/or benefit from access to flowering resources (Wackers et al., 2008) [68]. Access to pollen and nectar sources can significantly increase the activity, longevity and fecundity of these predators and parasitoids (Wackers et al., 2008; Hogg et al., 2011) [68, 20]. The availability of flowering resources can be essential to natural enemy efficacy in biological control of pest insects (van Rijn and Sabelis, 2005) [65]. These non-prey requirements can be fulfilled with a diverse assemblage of flowering plants, which will provide necessary resources that support populations of predators and parasitoids throughout the season. Simple addition of flowering plants to farms may not be sufficient to gain the expected increase in biological control, however, and in some cases it may be counter-productive due to supplying resources for pest insects.

House (1977) [32] stated that “through nutrition we have a direct and essential connection between an environmental factor, food stuff and the vital processes of the insect organism.” Most nutrition research with insects has been aimed at improving rearing and not developing a basic understanding of their nutrition. In contrast to parasitoids, few studies have been done on the effects of various natural foods on the biological character of predators. Smith (1965) [53] reported that 10 coccinellid species fed dried, powdered aphids, grew larger and faster when feeding on *Acerthosiphon pisum* (Harris) and *Rhopalosipum maidis* (Fitch) than on *Aphis fabae* Scopoli. *Coccinella septempunctata* L. gained more weight when feeding on *Lipaphis erysimi* (Kaltenbach) than on two other aphid species, and it was demonstrated that *L. erysimi* had higher protein levels (Atwal & Sethi, 1963) [4].

### 2. History of Parasitoid Nutrition

Probably was the first to emphasize the complexity of parasitoid nutrition in studies that demonstrated that the host influences growth and survival of the developing parasitoid as well as sex ratio, fecundity, longevity and vigor of the adult wasp. It has long been known that there is a relationship between host biomass and size of solitary parasitoids, larger parasitoids developing from larger hosts. This relationship exists for parasitoids which attack every host developmental stage, but applies more generally to parasitoids of host eggs and pupae where host size is fixed (Sandlan, 1982) [48]. The size of adult *Trichogramma pretiosum* Riley reared on the eggs of five hosts showed a direct correlation between parasitoid size and the volume of the host egg from which it emerged (Bai et al., 1989) [5]. The success of parasitoids in parasitization activity is directly related to nutritional factors.

Smith (1957) [55] found differences in larval mortality and adult size, sex ratio and reproductive rate of several species when reared on *Aonidiella aurantii* (Maskell) and *Comperiera bifasciata* (Howard) maintained on different food plants. *Habropeles rouxi* Compare displayed limited mortality on *A. aurantii* when feeding on citrus, but 100% cent mortality when feeding on sago palm. Pimentel (1966) [45] and Altahtawy et al. (1976) [2] showed differences in parasitoid fecundity and longevity depending on host food source. *Aphelinus asychis* required a longer larval developmental time and showed a decreased adult longevity when reared on *Myzus persicae* fed on defined diets deficient in sucrose or iron (Zohdy, 1976) [72]. The effects seemed related to decreased host size rather than a difference in nutritional quality of the host, however. Differences in larval development and adult size, fecundity and sex ratio were observed in *Tetrastichus israeli* (Mani and Kurian) when reared on several host species, which was correlated to the total level of essential amino acids in host tissues (Nadarajan and Jayaraj, 1975) [42]. Even though parasitoids reared from some host species with high levels of essential amino acids were larger and longer-lived, the results were variable, as were the specific amino acid compositions of the different hosts. In general it may be assumed that parasitoid fecundity, reproductive size, sex ratio and longevity are correlated with host size and nutritional factors (Strand, 1986) [57]. The importance of rearing *Chelonus sp.* and *curvimaculatus* on the natural host for vigor retention was demonstrated by Legner and Thompson (1977) [57]. In contrast to parasitoids, few studies have been done on the effects of various natural foods on the biological character of predators. Smith (1965) [53] reported that 10 coccinellid species fed dried, powdered aphids, grew larger and faster when feeding on *Acerthosiphon pisum* and *Rhopalosipum maidis* than on *Aphis fabae* Scopoli. *Coccinella septempunctata* L. gained more weight when feeding on *Lipaphis erysimi* than on two other aphid species, and it was demonstrated that *L. erysimi* had higher protein levels (Atwal and Sethi, 1963) [4].

### 3. The Nutritional Requirements

#### 3.1 Nitrogen sources

The sources of nitrogen are a very important parameter in the nutrition of entomophagous insects, because of the very fast growth of many species (Grenier et al., 1974) [122]. For example, the weights of newly hatched and mature larvae of the tachinid, *Lixophaga diatraeae* are respectively, 12 μg and 33 mg. The larval growth is completed in 8 days with a weight doubling time of about 17 hours. The supply in amino acids (AA) has to fit the needs, to avoid a lot of time and energy in conversion between amino acids, and the production of toxic metabolites. Parasitoids and predators are carnivorous species needing a protein-rich diet, with some specific requirements in aromatic amino acids especially in parasitoid diptera at the end of their larval development for cuticle tanning (Bonnot et al., 1976) [8]. Nevertheless some free amino acid like phenylalanine, may be toxic at high concentration, or have a very low solubility like tyrosine, enforcing to deliver the aromatic AA as tyrosine-rich peptides or proteins. The 10 “essential” AA are required, but some other ones are highly beneficial for a normal growth (Grenier et al., 1994) [20, 24]. Many species need a complementary supply of several non-essentials AA because they fail to develop normally in diets containing only these 10 essential AA. The modification of the balance between the different AA of a medium that only permits the larval survival of the tachinid fly *Phryxe cauda*, could induce the start of its growth (Grenier et al., 1975) [23]. For the first time for a tachinid, the complete development from egg to adult was obtained with *L. diatraeae*, in a medium containing 19 AA in well-balanced proportions. To maintain the osmotic pressure (OP) within acceptable values, part of the AA could be provided as proteins, protein hydrolysates or peptides, but free AA could be required for some species (Nettles, 1987) [43]. Casein, lactalbumine, ovalbumine, serum albumine, soybean extract and yeast are the most common used proteins (Grenier, 1994) [20, 24].
3.2 Lipids
The similarity of the composition in total fatty acids of many parasitoids with that of their host, suggests they may copy to a certain extent the host composition (Delobel and Pageaux, 1981) [44]. It could be the same for predators (Sighinolfi, 2008) [46]. Itoplectis conquistor can develop in a diet without any fatty acids, but their addition improves the yield and the fecundity of the adults obtained, but conversely polyunsaturated fatty acids may be required for normal growth of several entomophagous insects (Sighinolfi, 2008) [46]. Dietary sterols are required by a great number of parasitoids and predators, such as Exeristes roborator (Thompson, 1990) [63], P. caudata (Grenier et al., 1975) [23] and Geocoris punctipes. Fatty acids may be supplied as free fatty acids or triglycerides, and need the use of emulsifying agents to obtain their homogeneous dispersion in the aqueous phase. The most employed emulsifying agents are Tween 80 (polysorbate sorbitan monoooleate), lecithin (phosphatidyl-choline) or lauryl sulphate. Egg yolk frequently incorporated into artificial media for egg parasitoids provides highly well emulsified concentrations in fatty acids, cholesterol and lecithin. Free fatty acids are toxic for the tachinid P. caudata (Grenier et al., 1974) [22].

3.3 Carbohydrates
Carbohydrates are often considered as energy sources as well as some lipids. It is usually admitted that there are no specific needs for carbohydrates, but glucose promotes growth and lipogenesis, increasing the level of unsaturated fatty acids in E. roborator (Thompson, 1990) [63]. Trehalose, the most common non-reducing disaccharide in insects, plays an important role in metabolism and stress resistance (Qin et al., 2011) [46]. It could be used instead of sucrose or glucose, and also partly replaced hemolymph in media for Trichogramma (Lu et al., 2011) [28]. To reduce the OP in medium/diet it is recommended to replace oligosaccharides by polysaccharides, like glycogen, but OP being not so critical for many predators, sucrose has been used in place of glycogen to reduce the cost of the diet. Moreover, sucrose may act as a feeding stimulant on parasitoid, as well as on predator insects.

4. Other needs
4.1 Inorganic salts
They are generally required for the normal development of insects, but their level and the balance between the different captions, especially K+/Na+ is of prime importance and varies according to the species. The predator G. punctipes prefers to feed on diets containing K/Na ratio exceeding 2 than on diets with a ratio lower than 1.

4.2 Vitamins
The accurate determination of the needs in vitamin very delicate experiments, requiring vitamin free components, and taking into account the egg stocks. Very few specific investigations were conducted for entomophagous insects. It was usually admitted that their needs would be no different from those of other insects. Habitually about 12 vitamins were added in the diets, mainly hydrosoluble ones including vitamins B as well as C, and 2 liposoluble ones (retinol-A, andtocopherol-E). Commercial preparations are available and often used by many authors, like Vanderzant vitamin mixture for insects (Vanderzant, 1969) [66].

4.3 Miscellaneous
Ribonucleic acids (RNA) are sometimes incorporated in medium/diet, but their dispensability is questionable. RNA could increase survival or promote growth. Mineral cations also are needed for many (House, 1972) [30]. Lioplectis conquistor develops well on a diet containing 0.63 per cent of inorganic salts, whereas this level had a harmful effect on a closely related species, Pimpla turionellae, for which the best yield of adults was obtained from a diet with 0.39 per cent of salt mixture (Yazgan, 1981) [70]. The proper ratio of K+ to Mg++ also was found to stimulate oviposition by Trichogramma females (Nettles et al., 1982) [44].

4.4 Other physiological requirements
For endoparasitoids, the medium is not only the food source, but also the environment in which they are bathed for all their larval life. Thus, besides the nutritional needs, the medium must have acceptable physicochemical parameters, and provide for other requirements concerning essential physiological functions like respiration, excretion and general protection.

5. Nutritional Requirements in Development
All insects have nearly similar requirements that include protein and/or 10 essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine), the B-vitamin complex (biotin, folic acid, nicotinic acid, panthothenic acid, pyridoxine, riboflavin and thiamin), as well as other water soluble growth factors, including choline and inositol, some fat soluble vitamins, cholesterol or a structurally similar phytosterol, a polyunsaturated fatty acid, minerals and an energy source usually provided by simple or complex carbohydrates and/or lipids. The rule has been confirmed by recent studies with parasitic and predaceous insects. In assessing the need for nutrients, it is important to consider that rearing a single generation on a synthetic or semi-synthetic diet did most studies. Some investigations overlooked the potential contribution of nutrients stored within the egg. Stored nutrients may support limited development and, in the case of trace nutrients, supply a sufficient quantity to ensure development of at least one generation. Thompson (1981) demonstrated partial larval development on diets lacking various essential amino acids and B-complex vitamins with Exeristes roborator. Other studies have demonstrated that entomophagous insects have no unusual qualitative nutritional requirements. A requirement for asparagine by Euclatoria bryani Sabrosky and the absence of a requirement for a polyunsaturated fatty acid by A. housei (House and Barlow, 1960) [31] were consistent with findings for non parasitic Diptera. The predominant foods of both parasitic and predaceous insects are of animal origin and, thus, are generally high in protein content and low in carbohydrate and fat (House, 1977) [32]. Thompson (1983) [61, 62] described the effect of nutritional balance on larval growth of Brachymeria lasus. Media containing 0-10 per cent glucose with 2 per cent amino acids, and 1-8 per cent amino acids with or without 2 per cent glucose were tested. All media contained 15 per cent albumin and 2.5 per cent lipids. Weight gain increased on diets containing 2 per cent glucose when the amino acid level was increased from 1-4 per cent, but was reduced at the higher amino acid levels. Similar effects of varying the amino acid level were obtained with diets lacking glucose, but the overall
weight gain was less than observed with the diets containing glucose. On diets containing 2 per cent amino acids, weight gain increased dramatically when glucose was increased from 0.5-4 per cent, but decreased at higher glucose levels. Growth rates on the above diets were generally in the range of 15-200 mg/g/day. The maximal rate, 260 mg/g/day, was obtained on a medium containing 2 per cent glucose and 2 per cent amino acids. The effects of nutrient balance were closely related to the osmolality of the artificial medium (Thompson, 1983) [61, 62].

House (1966) [29] demonstrated similar quantitative requirements to those of hymenopterous parasitoids in the dipteran Agria houi. Shewell. Maximal growth and survival were achieved when all nutrients were increased proportionately over the levels in a basal diet that contained 2.25 per cent amino acids, 0.05 per cent salts, 1.16 per cent lipids and 2.25 per cent other ingredients, including glucose, ribonucleic acid, vitamins and agar. When amino acid level alone was increased, survival was reduced. On a diet containing nutrient levels equivalent to pork liver (20 per cent amino acids, 4 per cent glucose, 3.5 per cent lipids, 2 per cent salts and 0.75 per cent ribonucleic acid), survival was >80 per cent. Non-nutritional factors are intimately and intrinsically involved in food acceptance and ingestion. These include physical properties such as form, texture, etc., but also non-nutritive chemicals that elicit specific behavioral and/or physiological responses essential for finding and accepting foodstuff and in some cases for initiating behaviors associated with the feeding process itself (Bernays and Simpson 1982, Bernays 1985) [6]. Although such factors have been best shown in phytophagous insects, they also play a role in the biology of entomophaga and will likely be of importance in the development of continuous in vitro culture.

6. Presentation of the Food in Artificial Rearing
The medium consistency could be limiting factor for the larval and pupal parasitoids, mainly in relation with respiration, but the presentation of the food is more crucial for egg parasitoids and predators. To simplify the production of biocontrol agents, research has been conducted in several laboratories to develop techniques enabling mass culture in artificial media. For mass production, media are suitable if they are inexpensive relative to the use of conventional hosts, and if they support continuous growth of entomophages (Grenier et al., 1989) [25]. Chemically defined media as well as those containing raw materials derived from insects have been used. Furthermore, artificial media may facilitate experimental studies of the biology, physiology and behavior of parasitoids and predators. They will contribute to improve our knowledge of the very complex relationships between parasitoids and hosts. In cases where the research goal is to enrich our understanding of nutritional requirements, it is advisable to use a well defined diet. Artificial media also open possibilities for studying the pre imagine conditioning of parasitoids. For endoparasitoids, the diet is not only the food source but also the environment in which they are bathed for all their larval life, so that besides the nutritional needs, the medium must provide for all other requirements concerning respiration, excretion and protection from desiccation (Grenier et al., 1989) [25].

6.1 Egg parasitoids
Especially for non-gregarious species, larvae need a limited quantity of food because they cannot regulate their food intake; otherwise the development will terminate mainly at pupation, or will produce adults showing abnormalities (Grenier and Bonnot, 1988) [21]. Thus, for egg parasitoids the medium is presented in various ways to create an artificial host egg. The hanging drop technique consists in the deposition of droplets of medium on a flat surface in which the Trichogramma eggs are deposited for development. In wax eggs, paraxylylene eggs or plastic egg cards, the artificial egg shell is made wax/paraffin mixture, polymerized paraxylylene or polyethylene/ polypropylene film, respectively (Grenier and Bonnot 1988) [21]. Egg laying occurs directly in these artificial host eggs, but the stimulation of the oviposition by the Trichogramma females is usually enhanced by smearing the surface of the artificial eggs by some chemicals like moth scales extracts, or polyvinyl alcohol solutions (Consoli and Grenie, 2010) [13]. In wax and paraxylylene eggs the development usually stops at pupation, probably because of gas exchange limitations. The artificial egg shell has to be permeable to oxygen and carbon dioxide, but not to water vapour, in order to avoid desiccation.

6.2 Predators
For predators, the presentation of the diet is a key parameter although the respiration is not mainly involved. Liquid diets that were fully or semi defined were presented within wax capsules for the neuropteran C. carnea and hemipteran predator G. punctipes. Diets for different lacewing species (chrysopids) were encapsulated, presented on cellulose sponge or in the form of a free hygroscopic powder. Diets for predaceous coccinellids could be presented in gelled cubes or as powder, or dry pellets. Stretched Parafilm was used to package diets with a paste-like consistency for several hemipterous predators, as well as for some species of coccinellids (Bonte and De Clercq, 2010) [9]. Parafilm enclosing synthetic foam cubes soaked with diet devoid of insect components was successfully used to rear Macrophosca lignosus (Grenier et al., 1989) [25]. For Orius laevigatus, the diets were encapsulated in Para film using an encapsulation device (ARS, Gainesville, USA) forming small hemispherical domes (35ul) sealed with transparent tape. The Para film was stretched before encapsulation to facilitate stylet penetration by early instars of the predator (Bonte and De Clercq, 2010) [9]. The same presentation with 2 artificial diets was successfully used for the development and reproduction of M. caliginosus.

6.3 Predator Culture in vitro
The artificial rearing of predators has stressed maintenance of the adult stage for maximizing egg production rather than complete in vitro culture. Predator larvae are the preferred biological control agent, and eggs and larvae produced by adults are placed directly in the field. However, some effort has been aimed at complete artificial culture of predators (Grenier et al., 1989) [25]. Among the first reared artificially from egg to adult was the coccinell Coleomegilla maculata maculate by Szumkowski (1952) [59]. Adults fed on raw liver or meat being kept for months on this food in the absence of prey. However, survival of larvae was poor on meat products alone and only 38per cent reached the adult stage. Supplementing vitamins resulted 86 per cent of the larvae reaching adults. Oviposition and egg viability were increased by addition of vitamin E to the adult diet. Smith (1966) [54] reared several coccinellid species including C. maculata lenti on dried aphids supplemented with pollen. Success also was
achieved on a diet of 40 per cent brewer's yeast, 55 per cent sucrose, inorganic salts, cholesterol, RNA, wheat germ oil and vitamins. Adults were fed the same diet supplemented with powdered liver. Attallah and Newsom (1966) [29] reared 8 generations of this coccinellid on a defined diet of casein, sucrose, wheat germ, soybean hydrolysate, glycogen, butter fat, corn oil, a liver factor, dextrose, cotton leaf extract (with carotenoids and steroids), brewer's yeast, ascorbate, inorganic salts, vitamins and agar. Adults reared in vitro were fecund and mating was stimulated by addition of vitamin E to the diet. In vitro culture attempts with Chrysopa species did not succeed until Hagen and Tassan (1965) [27] got a complete culture of C. carnea (Stephens) on an encapsulated liquid medium (in paraffin droplets). The diet consisted of enzymatic yeast, protein hydrolysate, ascorbate, fructose, choline and casein hydrolysate. Vanderzant (1969) [66] then successful cultured C. carnea for 7 generations on pieces of cellulose sponge soaked in enzymatic casein and soy hydrolysates, fructose, inorganic salts, lecithin, cholesterol, choline, ascorbate, vitamins and inositol. Development on this diet was slow, but 50-65 per cent of larvae reached the adult stage compared with 85 per cent when reared on natural insect eggs. Cai et al. (1983) [10] reared this species on an encapsulated medium of soybean and beef hydrolysates, egg yolk, sucrose, honey, brewer's yeast, ascorbate and linoleic acid, with similar success reported by Zhou and Zhang (1983) [71].

The hemipteran predator, Geocoris punctipes may be reared on several diets. Media were nevertheless supplemented with insects. Reported in vitro culture of G. punctipes from 1st stage nymph to adult on encapsulated semi defined diets. Six media containing casein hydrolysates, yeast, sucrose, cholesterol, corn oil, lecithin, agar, inorganic salts, phenylalanine and a vitamin mixture were formulated and encapsulated in different forms. Best results were with vitamin-enriched medium encapsulated in a mixture of 5 per cent polybutene 32 and 95 per cent dental impression wax. Development of G. punctipes in vitro was better than when reared on Spodoptera exigua. The percent of nymphs that reached adults and survival of the in vitro reared predators were significantly greater on the artificial diet (Tschopp, 2013) [64].

6.4 Parasitoid Cultures in vitro

In vitro culture offers a simple alternative for mass culture (Mellini, 1978; Greany et al., 1984) [40, 19], and also enables dietary and nutritional manipulations for fundamental studies of nutrition and biochemistry. Some benefits of in vitro culture were given by Greany et al. (1984) [19]. However, the physiological and metabolic adaptations exhibited by insect parasitoids in relation to their parasitic way of life are of critical importance for successful in vitro culture. Parasitoid/host relationships are often incorrectly thought to lack the complex physiological interactions typical of the host associations of other Metazoa (Tschopp, 2013) [64]. The extent that parasitoid/host physiological interactions need to be considered in the successful development of in vitro culture must still be determined but will undoubtedly vary with the parasitoid species.

7. Conclusion

Because parasitoids are living animals, nutrition inevitably plays an important role in augmenting these natural enemies of pests. For nutrition is about nourishment; that is, it is the action or processes of transforming substances found in foodstuff into body materials and energy to do all the things attributed to life. Nutritional requirements depend on the synthetic abilities of the organism and the basis is genetical. Therefore, through nutrition we have a direct and essential connexion between an environmental factor, foodstuff, and the vital processes of the insect organism.

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