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How micronutrients influence the physiology of mosquitoes

Crisalejandra Rivera-Pérez[1], **Mark E. Clifton**[2], and **Fernando G. Noriega**[3,4,*]

[1]CONACyT-Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, B.C.S., México

[2]Collier Mosquito Control District, Naples, FL, 3USA

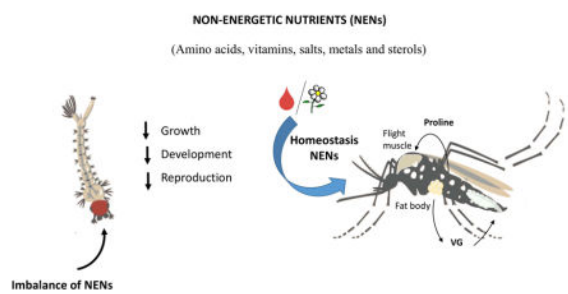
[3]Department of Biological Sciences, Florida International University, Miami, FL, USA

[4]Biomolecular Science Institute, Florida International University, Miami, FL, USA

Abstract

Micronutrients or non-energetic nutrients (NEN) are needed in reduced amounts, but are essential for many mosquito physiological processes that influence biological traits from vector competence to reproductive capacity. The NEN include amino acids (AA), vitamins, salts, metals and sterols. Free AA play critical roles controlling most physiological processes, from digestion to reproduction. Particularly proline connects metabolic pathways in energy production, flight physiology and ammonia detoxification. Metal, in particular iron and calcium, salts, sterol and vitamin homeostasis are critical for cell signaling, respiration, metabolism and reproduction. Micronutrient homeostasis influence the symbiotic relationships with microorganisms, having important implications in mosquitoes' nutrition, physiology and behavior, as well as in mosquito immunity and vector competence.

Graphical abstract



*Corresponding author: fnoriega@fiu.edu.

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Introduction

Adult female mosquitoes of several species are important vectors of human diseases. Due to a primarily hematophagous feeding strategy and the ability to produce offspring, most studies in mosquitoes have naturally focused on the adult female. *Aedes aegypti*, in particular, have received the majority of attention because of its ease of rearing, desiccation resistant eggs, cosmopolitan distribution and vector importance. Those factors have all converged to make *Ae. aegypti* a model organism. Nutrition strongly influences the physiology and behavior of mosquitoes. A sugar meal can sustain the energetic requirements of a female *Ae. aegypti*, and blood meals are required to produce eggs. However, much less is known about particular nutrients required during larval stages or about the importance of specific components of a sugar or blood meal in completing oogenesis.

For the purposes of this review, the nutritional requirements of mosquitoes can be divided into two main classes: energetic nutrients (so-called macronutrients) such as carbohydrates, fatty acids and some amino acids [1,2,3] and micronutrients or non-energetic nutrients (NEN). NEN are needed in reduced amounts but are essential for proper cellular and organismal function [4]. The NEN's include vitamins, salts, metals as well as sterols. Many amino acids (AA) can also be considered micronutrients because they are not used as a main source of energy, yet they are indispensable [4,5]. While energy-providing nutrients have received full attention regarding their impact on the physiology, behavior and ecology of insects, NEN have been largely overlooked. This review summarizes the current knowledge on the influence of NEN homeostasis on physiology of mosquitoes, and stresses some major gaps in our understanding of these processes.

Non-energetic nutrient homeostasis

The physiology of adult female mosquitoes is markedly linked to the gonotrophic cycle. Ovarian development in *Ae. aegypti* can be divided into three distinct phases: previtellogenesis (PVG), oocyte state-of-arrest (ORS) and vitellogenesis (VG) [6,7]. To provide the energy, molecular building blocks and NEN for ovarian development, the female mosquito draws nutrition from three main feeding modalities: larval feeding, adult sugar feeding, and adult blood feeding. Each of these processes provides a unique repertoire of NENs. Mosquito larvae mostly filter feed particulate matter such as phytoplankton, microorganisms, and detritus [8]. The nutrition obtained during larval feeding is considered preimaginal or teneral reserves and is mainly utilized during metamorphosis and PVG. The most commonly recorded sugar sources for mosquitoes are floral nectaries and honeydew [4]. Floral nectar is a complex mixture of chemicals. Sucrose is the major carbohydrate constituent but nectar also includes AA, proteins, lipids, antioxidants, alkaloids, vitamins, organic acids, allantoin and allantoic acid, dextrins, and minerals [9]. Nutrients obtained during nectar feeding contributes critical reserves during the ORS. Finally, a blood meal provides amino acids, cholesterol, lipids and metals. In *Ae. aegypti*, a blood meal triggers VG, and produces up to 120 eggs in a single gonotrophic cycle; therefore, a tightly regulated control of nutrient allocations to the ovaries is critical [10,11].

Amino acids

Vertebrate blood is rich in proteins that are digested into their constituent AA, with hemoglobin making up more than 90% of the dry mass of vertebrate blood [12]. Blood feeding on different host species affects mosquito female egg production [12]. The level of free isoleucine in blood has been shown to account for some of these differences, highlighting the importance of this essential amino acid in female mosquito oogenesis [13]; human blood is severely deficient in isoleucine; thus, female mosquitoes have to make full use of the human plasma-free Ile for their reproduction [14]. A large percentage of the blood-meal derived AA is metabolized and used for energy production, while the rest is used for the synthesis of yolk proteins. Free AA play critical roles controlling key physiological processes (Fig. 1). The presence of free AA in the midgut lumen is an important signal used by the mosquito to regulate the retention of the meal in the midgut [15], as well as for the induction of digestive proteases after a blood meal [16,17,18]. An increase in extracellular AA levels after a blood meal, is critical for 20-OH ecdysone stimulation of vitellogenin synthesis in the fat body [19,20].

The amino acid proline plays a unique role in supporting both the flight and post-blood meal physiology of *Ae. aegypti* [21]. Proline participates in a proline-alanine shuttle system that transports acetyl units, in the form of proline, from the fat body and to the flight muscles [22]. In this way, proline serves as an energetic substrate for flight metabolism. The enzyme alanine aminotransferase (ALAT) is a key component of this shuttle system, which enables transferring of proline and alanine as well as the detoxification of ammonia [22].

One of the major by-products of blood digestion is ammonia. *Ae. aegypti* females have evolved strategies to efficiently detoxify ammonia via multiple metabolic pathways [23,24]. Proline again occupies a central role in nitrogen metabolism and ammonia detoxification by serving as a nitrogen sink during the rapid deamination of proteins and production of ammonia that occurs following a blood meal [25]. Preventing ammonia detoxification by RNAi knockdown of ALAT, causes a massive increase of uric acid in the midgut and a delay in digestion, excretion, and oviposition with a significant reduction in egg production [26]. Treatment with the ALAT inhibitor L-cycloserine resulted in significant increases in mortality and disruptions to motor activity in a dose-dependent manner [27]. Key enzymes components of this “shuttling/detoxification” system are up regulated in mosquitoes reared under nutrient-limited conditions [28]. Interestingly, proline has also been recognized as an osmolyte in osmoconforming mosquito larvae [29]. Could a haemolymph proline store serve yet another purpose in *Ae. aegypti* by helping to buffer osmotic changes after a blood meal? Together these results illustrate a central role for proline connecting metabolic pathways in energy production, flight physiology and ammonia detoxification.

Metals

Iron is an important micronutrient for mosquitoes. A blood meal is the main source of iron for female mosquitoes, with the majority of iron bound to hemoglobin inside erythrocytes (98%), as well as transported by transferrin (2%) [30]. A blood meal provides a surplus of iron, and at the end of the first gonotrophic cycle in *Ae. aegypti*, 87% of the ingested heme iron is excreted, while 7% is incorporated into the eggs and 6% is stored in different tissues [30] (Fig. 2). Iron limited meals reduced egg number and progeny survivorship in *Aedes albopictus* [31].

Hemoglobin digestion also releases large quantities of heme, which has potential pro-oxidant and cytotoxic effects when not bound to proteins [32]. Heme promotes the formation of free radicals; therefore, mosquitoes have evolved different mechanisms to avoid heme toxicity [33]; including the shutdown of the production of reactive oxygen species (ROS) in the gut [32] (Fig. 2). Heme also promotes the generation of xanthurenic acid (XA), a product of the oxidative metabolism of tryptophan, which acts as an antioxidant by binding both heme and iron [34]. Blood meal related increases of heme in *Ae. aegypti* triggers a wide range of pleiotropic changes in the expression of genes associated to antioxidant activities, energy metabolism, cell cycle, cellular signaling, immunity and blood meal digestion [35]. Ferritin is an iron-storage protein found free in hemolymph, as well as intracellularly in most tissues [36,37]. It is transcriptionally induced after a blood meal, and contributes to sequester iron released from heme digestion, preventing oxidative damage [38,39]. Ferritin accumulates into the eggs, providing a source of iron that functions as an antioxidant during the development of embryos [36,40] (Fig. 2).

Copper (Cu^{2+}) is an essential component of key enzymes involved in respiration, oxidative stress protection and pigmentation; it is also a potentially toxic trace element in mosquitoes. Eukaryotic organisms use elaborated systems to regulate copper homeostasis, with the involvement of copper importers, copper chaperones, transcription factors, small metal binding proteins called metallothioneins and copper exporters [41]. Cu^{2+} tolerance physiology also serves as a model for mercury, silver, zinc and cadmium metabolism, because each of these share similar detoxification pathways via metallothionein binding. In *Ae. aegypti*, copper stress exerts multiple effects on larvae and adult physiologies [42,43,44]. Metal stressed larvae emerge with decreased lipid reserves, lowered adult body mass, increased starvation tolerance, reduced fecundity, and show an increase in starvation tolerance of offspring compared with non-metal-stressed individuals [44]. How these physiological changes due to copper exposure may affect transmission dynamics of diseases vectored by *Ae. aegypti* is currently unknown. Metal homeostasis is critical to many additional physiological and behavioral processes. Calcium signaling and regulation in the Malpighian tubules play essential roles in osmoregulation and ion homeostasis, detoxification and immunity [45,46]. Excess of calcium in the diet or defects in calcium homeostasis results in the formation of calcium oxalate crystals in the Malpighian tubules of flies; establishing insects as good models for studying human kidney diseases [47]. The homeostatic regulation of intraneuronal levels of divalent cations plays an important role in the development and functions of the dopaminergic system and associated behaviors [48]. Manganese homeostasis is essential for mitochondrial respiration and cellular metabolism [49].

Salts, vitamins and sterols

Salts are common constituents of larval diet or imbibed by adult mosquitoes as either primary or secondary metabolites from floral nectar and honeydew [1]. In the absence of inorganic salts in the diet, only 30% of *Ae. aegypti* larvae completed development; however, the addition of eight inorganic elements (Ca, Cl, Fe, K, Mg, Na, S, P) in the diet was sufficient for normal growth [4,50, 51]. Studies on *Ae. aegypti* mosquitoes reported better acceptance of sucrose diet containing salt concentrations isotonic to hemolymph [52,53]. Sodium is an essential ion in extracellular fluids that must be kept in a narrow range to maintain osmoregulatory functions in animal cells. Hypoosmotic and isosmotic conditions have shown to affect the expression of aquaporins in the anal papillae of larvae of *Ae. aegypti*, which are important osmo and ion regulatory organs [54].

Vitamins such as thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid and biotin are essential for full growth of mosquito larvae and most metabolic adult functions, while folic acid is required for pupation [4,50,51]. Vitamin A (retinol) or its precursor, β-carotene, are essential for the formation of mosquito visual pigments; deficiency in vitamin A results in abnormal development of photoreceptor cells, altering the compound eye response to light [55]. The B vitamins are chemically heterogeneous, but all function as essential coenzymes that are vital to insects [4]. Studies on *Culex quinquefasciatus* have shown that sucrose diets fortified with vitamin B stimulate ovarian development, and increase viability and longevity [56].

Insects lack the ability to synthesize sterols *de novo*; therefore, they acquire this essential nutrient from their food. Mosquitos use cholesterol, 7-dehydrocholesterol, sitosterol and stigmasterol to satisfy their metabolic demands [8, 57]. Cholesterol in insects is vital to membrane stability and cellular signaling and serves as the precursor to ecdysteroid hormones [58]. Dietary cholesterol in mosquitoes has a positive effect on egg development; small female mosquitoes with low cholesterol reserves show an increased number of eggs after feeding with a blood meal supplemented with cholesterol [59].

Insects that have restricted diets often develop intimate symbioses with bacteria, and it has long been hypothesized that a basis for the symbioses is nutritional [60]. The symbiotic relationships between mosquitoes and several microorganisms have important implications in mosquitoes' nutrition, physiology and behavior [61], as well as in mosquito immunity and vector competence [62,63]. In mosquitoes, antibiotic treatment affects reproductive output, indirectly implying an important role of microbiota in reproductive fitness [64,65]. The endosymbiotic bacteria of the genus *Wolbachia* are one of the most successful symbiont bacteria in the terrestrial ecosystem. *Wolbachia* infects several mosquitoes, including *Aedes* and *Culex* species [65,66]. There is evidence of competition between *Wolbachia* and a mosquito host over AA and dietary cholesterol [67,68]. *Ae. aegypti* infected with *Wolbachia* have reduced cholesterol levels [68], as well as reduced Zika virus transmission [69], suggesting yet another source of competition between mosquito and *Wolbachia* to the detriment of viral propagation.

Conclusions

Larval and adult sugar and blood feedings provide distinct groups of energetic and non-energetic nutrients. Only now are we beginning to learn how non-energetic nutrients contribute to the overall physiology of mosquitoes. Evidence is beginning to accumulate that

NEN influence everything from vector competence to the reproductive capacity of female mosquitoes. However, important questions remain in many areas. For example, little is known about the influence of NEN's in the interplay between virus and mosquito host. Another area worthy of study includes the relationship between the mosquito microbiome and NEN's. Finally, the role of most metals, vitamins and sterols is only superficially understood but potentially important. Early work in each of these areas is beginning to illustrate that there is much more to mosquito nutrition than only energetic molecules such as lipids and carbohydrates.

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Highlights

- Non-energetic nutrients or micronutrients are essential for mosquito physiology.
- Free amino acids play important roles in digestion, flight physiology and ammonia detoxification.
- Metal, sterol and vitamin homeostasis are critical for cell signaling, respiration, metabolism and reproduction.
- Micronutrient homeostasis influence the symbiotic relationships with microorganisms.

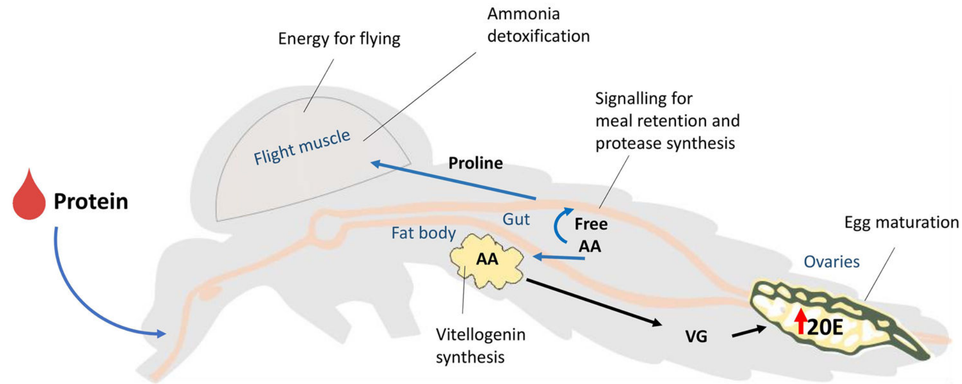

Fig. 1. Amino acid effects on mosquito physiology

Digestion of hemoglobin releases free amino acids in the midgut lumen that act as signaling molecules for meal retention and protease synthesis. Increases in AA titers in hemolymph, together with an increase in 20E synthesis in ovaries, stimulate vitellogenin synthesis by the fat body. An increase in proline connects metabolic pathways in energy production, flight physiology and ammonia detoxification. AA: amino acids; 20E: 20-hydroxyecdysone; VG: vitellogenin.

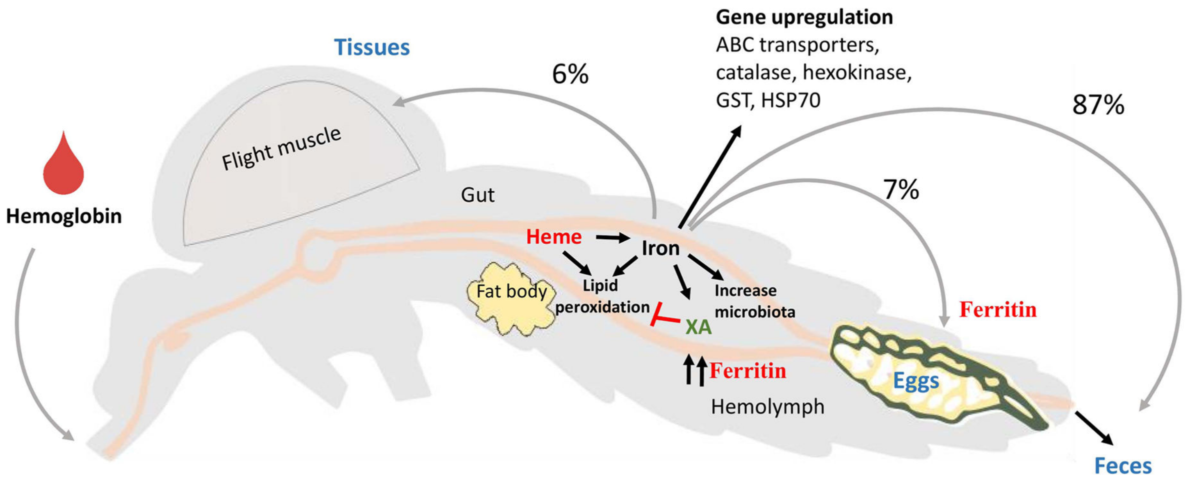

Fig. 2. Iron effects on mosquito physiology

Digestion of hemoglobin releases heme and iron that promote lipid peroxidation and increases in free radical formation. Heme promotes the generation of xanthurenic acid that binds heme and iron. Iron increases upregulate the expression of antioxidant proteins, which block lipid peroxidation. Iron stimulates the proliferation of gut microbiota and increases ferritin titers in hemolymph. Most iron is eliminated in the feces; smaller amounts are incorporated into the eggs or stored in tissues. XA: xanthurenic acid.