Nutritional ecology, infection and immune defence - exploring the mechanisms

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Highlights

- Diet can impact host-parasite outcomes in insects but there are no clear patterns.
- Protein is beneficial in some insects, fats or carbohydrates are better in others.
- Diet can act directly, or can change physiology, immunity or the microbiome.
- An understanding of the mechanisms of diet-related impacts on parasites is lacking for most systems.
- More studies are needed to find commonalities across host and parasite taxa.

Declaration of interest: none

Abstract

Diet can impact the outcome of parasitic infection in three, non-mutually exclusive ways: 1) by changing the physiological environment of the host, such as the availability of key nutritional resources, the presence of toxic dietary chemicals, the pH or osmolality of the blood or gut, 2) by enhancing the immune response and 3) by altering the presence of host microbiota, which help to digest nutrients and are a potential source of antibiotics. We show that there are no clear patterns in the effects of diet across taxa and that good evidence for the mechanisms by which diet exerts its effects are often lacking. More studies are required to understand the mechanisms of action if we are to discern patterns that can be generalised across host and parasite taxa.

Introduction

Food plays a vital role in organismal health with under- and over-nutrition contributing to poor health outcomes across taxa [1]. Nutrition is also important in the response to parasitic infection with potential *direct* and *indirect* effects of a host's diet and nutrient stores on a parasite's ability to successfully propagate in/on a host [1,2]. With diverse diets and a range of micro- and macro-parasites, insects are interesting models with which to address key questions around how dietary components can impact parasitism. In this review we use the word 'parasite' in the ecological sense, as an organism that lives in or on another organism (the host), to the detriment of host fitness. Using this definition, the term micro-parasite covers unicellular bacteria and fungi, protozoa and viruses (often referred to as pathogens), whilst macro-parasite covers the larger organisms such as nematode worms, that are typically considered parasites.

The host as a hostile environment

The insect host encompasses the parasite's living environment in which it must grow and reproduce, and this environment can be both beneficial and costly to the parasite. Insect hemolymph provides an excellent example of an environment that has costs and benefits. It is now well established that hemolymph is the main mediator of nutritional and immunological homeostasis in insects. A prime location for nutrient storage [3], hemolymph contains sugars such as trehalose and glucose, and sugar alcohols like mannitol and sorbitol [4], free amino acids, as well as various peptides and proteins [3]. For a microorganism, therefore, insect hemolymph is the best of places because it is a nutrient-rich medium of balanced ionic composition and near-neutral pH [5]. In contrast, the hemolymph is also the worst of places due to the presence of sequestered dietary chemicals, and immune components that can variously kill, engulf and encapsulate invading microbes [6]. Hemolymph is therefore a hostile environment for microorganisms, and microbiologically sterile in healthy insects [7].

The suitability of an insect as a host depends on the parasite's ability to access nutrients in the insect's body whilst overcoming detrimental aspects of the environment to be successful [8], and the balance of these effects is likely to vary across taxa [9]*. These detrimental aspects include: the basic physiological environment, such as the balance and abundance of nutrients [9*,10], the presence of toxic sequestered dietary chemicals and the pH and osmolality of the blood or gut matrix [11,12]; the presence of host microbiota as competitors for nutrients and a potential source of antibiotics, but which also play a key role in digestion, thus helping to deliver nutrients to the host (see [13,14] for recent reviews of this topic); and the strength and rapidity of the deployment of immune effectors, all of which are dependent upon or moderated by host diet (Figure 1). In this review we will first consider the evidence that an insect's diet can impact the outcome of host-parasite interactions, examine the potential mechanisms underpinning these effects and finally address areas for future research.

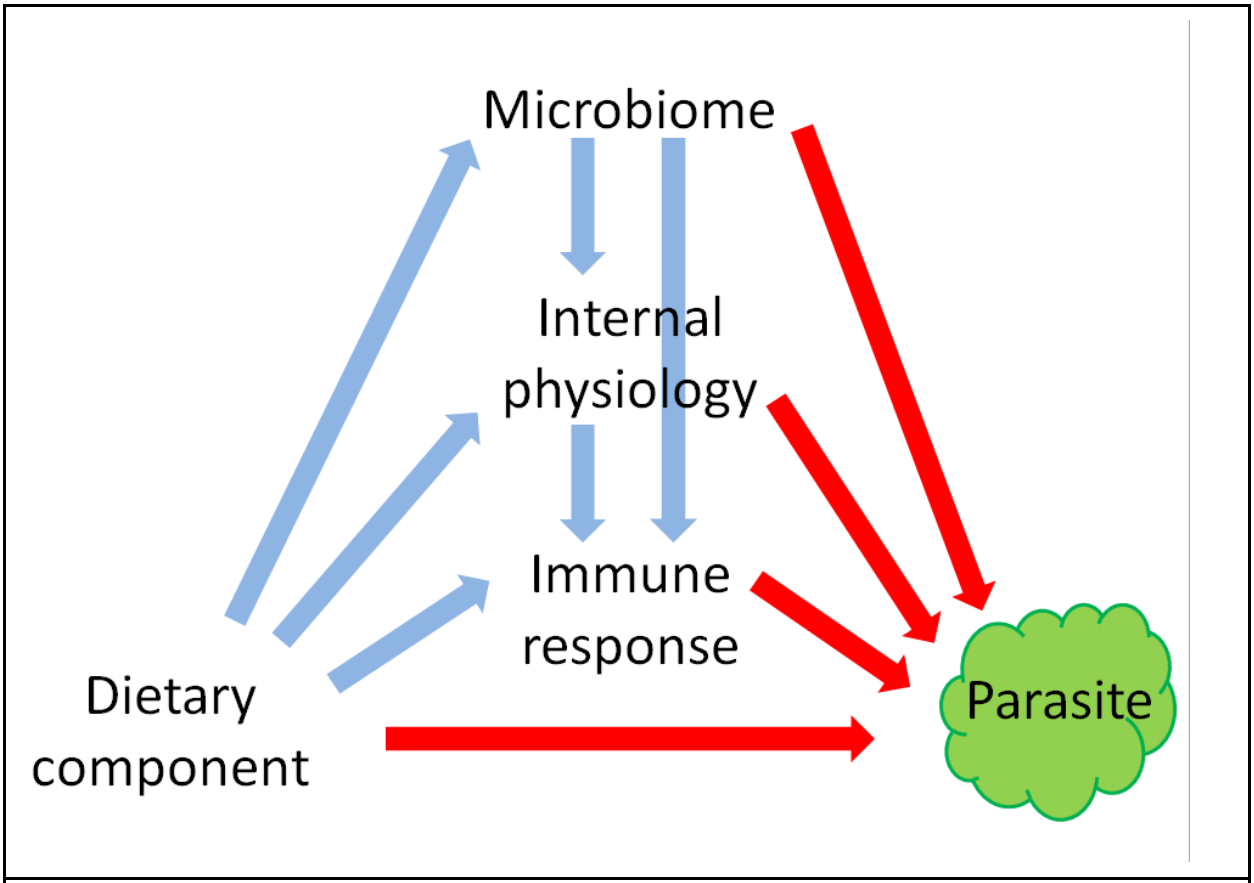

Figure 1. Potential routes of action for dietary components to negatively* impact parasite fitness. Red lines indicate negative action and blue lines positive action. For example, a dietary component could have a direct toxic effect on a parasite (e.g. Octanoic acid in noni fruit appears to kill parasitoid wasps [15]). Alternately, a dietary component could change the internal physiology such that it was detrimental to parasite growth (e.g. [16]**). None of these mechanisms are mutually exclusive, and indeed, diet components may have negative effects on some components of the response (e.g. downregulate immunity [17]), but still negatively impact parasites via one of the other routes.
*It is also possible for parasite fitness to be augmented by some of these routes, e.g. direct metabolic benefits of dietary components on parasite growth, but those interactions are not considered here.

Evidence for diet mediating host parasite interactions

The quality and quantity of the diet has long been shown to impact host-parasite interactions across host and parasite taxa. Many studies have used starvation or energy restriction, or the restriction of specific dietary components in the host's diet, typically resulting poorer infection outcomes for the host in e.g. bumblebees, *Bombus terrestris* [18], *Galleria mellonella* waxworms [19] *Culex pipiens* mosquitoes [20] and *Drosophila melanogaster,* though these effects can be parasite specific e.g. [21,22]. For example, diet-restricted *Drosophila* flies had higher bacterial counts when infected with *L. monocytogenes*, but counts did not change for

either *E. faecalis* or *S. typhimurum* [21]. Other studies note that effects of dietary composition rather than energy content, i.e. quality rather than quantity of diet, could impact host-parasite interactions. High protein relative to carbohydrate diets have been shown to be beneficial to many insects during infection e.g. *Spodoptera littoralis* [23], *Spodoptera exempta* [24,25], *Drosophila melanogaster* larvae [26], Mormon crickets, *Anabrus simplex* [27], the true fruit fly, *Bactrocerca dorsalis* larvae [28]* and bumblebees, *Bombus terrestris* [29]. In contrast, diets high in carbohydrates relative to protein have been shown to improve infection outcomes for other hosts e.g. the Australian plague locust, *Chortoicetes terminifera [30], Bactrocerca dorsalis* adults [31]** and Drosophila melanogaster* flies [32]. A diet high in fat relative to protein was beneficial for carnivorous burying beetles, *Nicrophorus vespilloides [33]*, whilst high fat relative to carbohydrate diets have been shown to be detrimental in the field cricket, *Gryllus texensis* [34].

Although most studies have focussed on the energy or macronutrient content of diets, the availability of micronutrients and trace elements can also impact host-parasite outcomes. For example, the availability of iron in the haemolymph is an important determinant of micro-parasite fitness, with low iron levels reducing parasite growth rates [35**]. Similarly, low levels of Phosphorus in the diet result in lower replication rates of the bacteria *Pasteuria ramosa* in the waterflea, *Daphnia magna* [36]. Plant-derived secondary chemicals can also be protective against infection. For example, woolly bear caterpillars, *Grammia incorrupta,* are more successful at fighting off parasitism by tachinid flies when pyrrolizidine alkaloids are incorporated into their diets [11]. Monarch butterfly larvae sequester cardenolides from milkweeds [12], providing protection against the protozoan parasite, *Ophryocystis elektroscirrha* [37].

What is not clear from many of the studies above is the mechanisms behind these diet-driven changes in host-parasite interactions. The immune response is energetically costly [38] and many effectors require key amino acids [39,40], so are the dietary manipulations described above increasing the efficacy of the host immune response?

Potential mechanisms - 1. Diet can impact immune efficacy

Immune activation is energetically costly [41,42] and protein consumption supplies important amino acids that are necessary for the structure of immune pathway peptides [39,43] and effectors such as antimicrobial peptides (AMPs) [40]. Protein production from dietary amino acids is costly, consuming ~50% of the ATP in growing yeast cells [44]. Dietary carbohydrates supply the energy needed for metabolic actions in both humoral and cellular immune responses and can equip secondary plant metabolites that have antibacterial activity [45]. Recently, investigators have identified the role of nutrient-sensing pathways, particularly the insulin signalling (ILS) pathway in regulating components of the immune response [46,47]. Therefore, both food quality and quantity can have direct effects on the ability of the host to mount an effective immune response.

In this context, deprivation of *Galleria mellonella* larvae of food leads to a reduction in cellular and humoral immune responses [19]. The hemocytes from starved larvae were as effective at killing *Candida albicans* cells as those from control larvae but they occurred at a lower density. In addition, hemolymph from starved larvae displayed reduced expression of AMPs [19]. Mormon crickets reared on high protein diets had higher levels of the immune enzyme

phenoloxidase (PO), its precursor prophenoloxidase (proPO) and a stronger encapsulation response than those on low protein diets [27]. In Australian locusts (*Chortoicetes terminifera*), circulating haemocyte densities were greater in locusts fed on balanced or protein-biased diets compared to those fed carbohydrate-biased diets, whilst proPO did not differ among diet treatments [30]. Despite this boost to the immune response, high protein diets reduced survival from fungal infection. Graham *et al.* [30] argue that high protein and low carbohydrate substrates boost mycelial growth and toxin production by *M. acridum*, and so the fungus might use dietary protein circulating in the hemolymph against the locust. In adult *Drosophila* infected with *M. luteus*, AMP expression levels were higher on low protein diets throughout the course of infection [32]. However, *Drosophila* larvae maintained on high protein diets show an improved immune response as adults [48] suggesting that the impact of nutritional components on immunity may differ by life stage as nutritional requirements change. In black soldierfly larvae, the addition of proteins or plant oils, on top of the standard diet, increased AMP expression and increased antibacterial activity from whole body extracts [49].

It isn't as simple as more protein and/or more energy results in a stronger immune response. Cotter et al [50] systematically manipulated both the energy content and the protein to carbohydrate ratio (P:C) of the diet for *Spodoptera littoralis* caterpillars, then measured constitutive PO activity, lysozyme-like antibacterial activity and cuticular melanism. Whilst lysozyme peaked at high energy intakes, PO peaked at low energy intake on a balanced P:C diet and melanism increased weakly with protein [50]. A similar trend was found in the same species using natural plant diets; PO activity was higher on maize but antibacterial activity was lower, potentially mediated by its nutritional quality [51]. A recent study using *Manduca sexta* found remarkably similar results, with PO and haemocyte density both peaking at intermediate intakes, but at a balanced P:C for PO, and at a high P:C for haemocyte density [52]. These results show that different immune effectors might respond differently to the deprivation of both energy and specific nutrients. This was examined in more detail recently using *Spodoptera littoralis* challenged with dead or live *Xenorhabdus nematophila* bacteria [53]*. The expression of 4 immune genes in control larvae correlated weakly with protein or carbohydrate intake, though expression after challenge with live bacteria tended to peak in similar regions of nutrient space. However, the expression of lysozyme and PPO, correlated poorly with the activity of lysozyme and PO in the haemolymph, with both traits correlating more strongly with the amount of protein in the diet. This suggests that immune gene expression is not necessarily a good indicator of immune efficacy, as translation might be dependent on amino acid availability [53]*. When amino acids are limiting, gene expression can still occur but the gene may not be translated until their availability increases [54].

Plant secondary compounds have also been shown to modify immune efficacy, and not always positively [55]. In monarchs, although cardenolides increase resistance to *O. elektroscirrha* parasites, they also downregulate a handful of immune genes in the gut and body, suggesting that the effect of cardenolides on the parasite is via direct toxicity [17]. A separate study on the same system looked at the functional antibacterial response in haemolymph from naive and immune stimulated caterpillars, with host plant cardenolide content having no significant effect on either [56]. Nicotine, in contrast, has been shown to enhance PO activity and encapsulation in *Manduca sexta* caterpillars [57]. The Anicia checkerspot butterfly, *Euphydryas*

anicia, sequesters greater amounts of the iridoid glycoside (IG), catalpol, from *Penstemon glaber*, than it does from *Penstemon virgatus* host plants. It also has higher PO activity on *Penstemon glaber*, but whether this is caused by the higher levels of catalpol is unclear [58]. However, the buckeye caterpillar, *Junonia coenia,* also shows an improved PO response when reared on high IG plants and higher survival after viral infection [59], suggesting that IGs may stimulate the PO response. It is clear therefore, that dietary components can both up or downregulate specific immune responses, but that not all immune effectors react in the same way to a dietary manipulation.

Potential mechanisms - 2. Diet modifies the internal physiological environment

Rather than modifying the immune response, some dietary manipulations could impact parasitism directly, by starving the parasite of key nutrients, or via toxicity to the parasite, or by changing the physiological environment, such as pH or osmolality, to harm the parasite. First, the parasite relies on the host for food, and so by changing the food available to the host, it may change the quality of the host as a nutritional resource for the parasite. This could result in the host having non-preferred nutrient ratios in terms of macronutrients (proteins, carbohydrates and fats) [60] or in terms of its elemental composition [10]. For example, microsporidian parasites grow less well in *Daphnia magna* and *Daphnia galeata*, kept on reduced food rations, suggesting that the parasite is unable to compete with the host for limiting nutrients [61]. Some parasites have lost the ability to synthesise key nutrients, for example glycogen in many bacteria [62], purines/ pyramidines in protozoa [63], cholesterols in trypanosomes and *Plasmodium* (reviewed in [64]) and so a manipulated host diet could potentially starve a parasite of specific nutrients, potentially enhancing some of the immune-related effects on infection outcomes cited in the previous section. Finally, transition metals are vital for many key biological processes and micro-parasites rely on the availability of Iron, Zinc, Manganese and Copper in particular, for replication [65]. Insects can restrict the availability of these metals by shuttling them out of the blood and into the fat body via proteins called transferrins [66] or by chelating them such that they are not bio-available [65]. This has been known to occur in mammals but the immune role of transferrins in insects has only recently been confirmed *in vivo* [35]**. *Drosophila* use the transferrin, Tsf1, to shuttle iron out of the blood and into the fat body in response to bacterial infection. Tsf1 mutant flies are more susceptible to infection and *Pseudomonas aeruginosa* bacteria that lack the iron-scavenging siderophore, pyoverdine, are successful in the Tsf1 *Drosophila* mutants but cannot infect wild type flies [35]**.

Other dietary components show direct toxicity to parasites. For example, *Orgyia antiqua* larvae are protected from infection by *Metarhizium anisopliae* fungal infection when consuming high levels of phenolic glycosides, even though there was no marked improvement in the encapsulation response that acts against fungi in the haemocoel [67]. Several phytochemicals ingested in pollen and nectar can directly impact *Crithida bombi* gut parasites of bees, both *in vivo* [68] and *in vitro* [69], indicating direct toxicity. *Drosophila sechellia* preferentially breed on ripe noni fruit [15], which is rich in toxic octanoic acid [70]. Ripe noni extract, reduces the success of parasitoids attacking *Drosophila* flies, with adult wasps appearing to succumb to the toxin, reducing the parasitism rate [15]. Thus, the ability of *D. sechellia* to cope with the plant toxin appears to have reduced the need for an immune response targeted against parasitoids [15].

In contrast, Sun et al [71]** tested the role of glucosinolate detoxification in diamondback moth, *Plutella xylostella,* on the interaction with its endoparasitoid wasp. To do this, they used plant-mediated RNAi to silence the glucosinolate sulphatases that *P. xylostella* use to detoxify plant defensive glucosinolate compounds. When feeding on RNAi- modified *Arabadopsis*, the caterpillars accumulated toxic isothiocyanates, resulting in greater mortality of the endoparasitoid and delayed emergence of those that survived [71]**. This effect was independent of the immune response, PO activity was not affected by the treatment. Therefore, the plant defensive compounds are likely to be directly toxic to the wasp, but detoxification by the caterpillar provides them some protection.

Fermentation compounds, such as alcohols, produced by bacteria and fungi are often toxic [72], but *Drosophila* fruit flies can tolerate alcohol [73]. Not only does alcohol consumption by *Drosophila* larvae deter generalist parasitoid wasps, but it increases alcohol levels in the haemolymph, increasing larval wasp mortality [73]. Alcohol did not increase the likelihood of encapsulation and resulted in a reduction in the density of the haemocyte type responsible for encapsulation in the haemolymph [73]. It appears, therefore, that ingested plant secondary compounds and alcohol can have direct negative effects on parasites attempting to grow inside a host. This is not surprising as these compounds have long been considered toxic, but what about nutritional dietary components such as proteins, fats and carbohydrates?

A high fat/low protein diet in *Nicrophorus vespilloides* burying beetles increased survival from infection substantially, without altering the bacterial load in the haemolymph [33]. The high fat diet also marginally decreased PO activity, suggesting that the improved survival was not mediated by the immune system [33]. Instead, the high fat diet may have increased host tolerance of bacterial toxins which play a key role in host killing [74]. A recent study by Wilson et al [16]** found that a high protein diet in armyworm, *Spodoptera littoralis,* caterpillars increased resistance to *Xenorhabdus nematophila* bacteria. They also showed that high protein diets increased the level of solutes in the blood resulting in high osmolality, which is detrimental to bacterial growth *in vivo* and *in vitro*, irrespective of whether it is induced with proteins or salts. This shows a *direct* impact of the food eaten on pathogen growth in the insect's body, without invoking the immune response [16]**.

Potential mechanisms - 3. Diet affects host microbiota

The insect gut microflora is tightly linked to diet [75] and gut microflora can impact the host's ability to resist or tolerate parasites e.g. [76]. The interaction between diet, host microbiota and immune function has been reviewed recently (see [13,14]) and so here we will note recent studies that have addressed this issue. *Bactrocera dorsalis* larvae, whose gut microbiota had been cleared, died more rapidly from infection, had reduced PO activity and antibacterial activity of the haemolymph and this was compounded when the protein content of the diet was reduced [28]*. Removing gut flora also reduced the levels of circulating haemolymph nutrients, suggesting that the effects on immunity may be mediated via nutrient deprivation due to the role of microflora in digestion [28]*. The honeybee gut microbiome is sensitive to changes in diet [77], and a recent study has shown that infection with the gut microsporidian, *Nosema ceranae,* was associated with gut dysbiosis [78]. Furthermore, an intact gut microbiota halved the daily risk of death from Deformed Wing Virus in honeybees, though it did not reduce viral

load, suggesting that the microbiome mediates tolerance to infection and its activity extends beyond the gut [79].

Interestingly, multiple mechanisms can come together to determine the outcome of infection. Several studies have shown that gut microflora of mosquitoes can influence their susceptibility to *Plasmodium* (see [80] and references therein) but mechanisms were lacking. Mosquitoes fed supplementary glucose or trehalose showed increased susceptibility to infection, but only when the gut microflora was intact [81]**. Wang et al [81]** showed that these additional sugars increased the abundance of *Asaia bogorensis* in the gut, which increased the gut pH, increasing the rate of sexual maturation of the *Plasmodium*. Simultaneously, the additional sugars downregulated immune genes in the Imd pathway. To test whether the change in pH had a causative role in susceptibility to infection, mosquitoes were fed NaHCO3 to increase gut pH independent of diet, and the alkalisation of the gut alone was sufficient to increase oocyst numbers and downregulate the Imd pathway genes. Therefore, rather than diet modifying the immune response or changing the internal physiological conditions of the mosquito directly, the changes occur as a result of diet affecting the balance of gut microorganisms.

Summary and future directions

In summary, dietary components can impact parasitism in many, non-mutually exclusive ways, from direct toxicity, to upregulating the immune response, to changing the physiology of the gut or haemolymph to make it refractory to parasite growth. At present there do not appear to be many universal truths in this field [9*], with different effects of nutrient ratios or the deprivation/addition of specific dietary components, and outcomes that differ between parasite and host taxa, and even within host taxa across life stages. Many studies report the effect of nutrition on one aspect of the host or parasite, e.g. survival or the immune response, but very few studies systematically address the potential mechanisms underlying the response (but see [16**], [81**]). The task for future studies is for researchers to address how nutrition modulates parasite infection outcomes in their study species via detailed mechanistic studies. Identifying commonalities across host and parasite taxa will further our understanding of how diet can impact parasitism.

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