

# Beyond the protein concept: health aspects of using edible insects on animals

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## Abstract

There is an increasing interest in the use of insects in animal feed since they contain high proteins levels, lipids, vitamins and minerals. In particular, insect-derived proteins are seen as one of the potential solution to face the increasing protein shortage and are able to fully substitute soybean meal or fishmeal in aquaculture or livestock feeds. However, beside their interesting nutritional composition, insects are also rich in bioactive compounds such as chitin, antimicrobial peptides or specific fatty acids with immunostimulating, antimicrobial and/or anti-inflammatory properties able to sustain animal health, increase their resistance to diseases. Further studies will also have to investigate whether insects share similarities with bacterial or parasitological pathogens and may act as immunostimulants. These recent findings may launch insects beyond the protein concept into healthy animal feeds. This review presents the effects of insects and their bioactive compounds on fish and crustaceans, poultry, pigs and rabbits immune system, gut health, microbiota and resistance to diseases.

**Keywords:** gut health, microbiota, antioxidant enzymes, immunity, disease resistance

## 1. Introduction

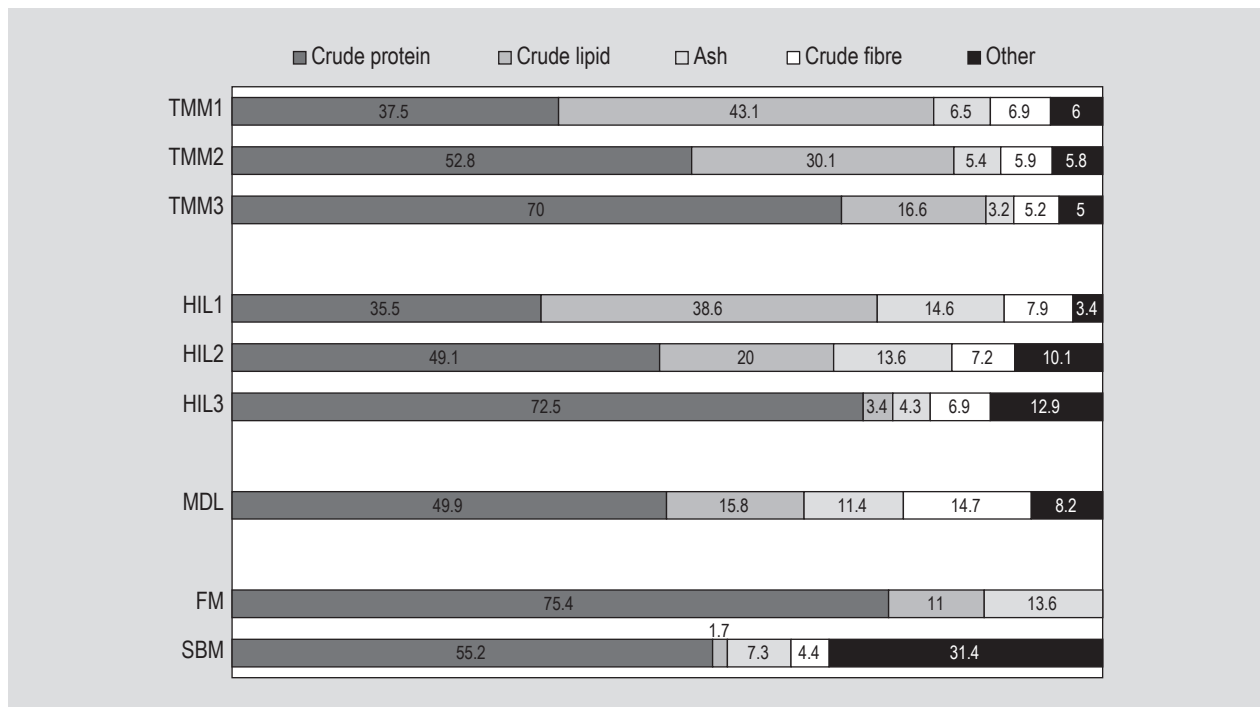
Since the dawn of time, insects have been part of our life, being considered as a pest, a resource, food and, more recently, as a feed for intensive livestock production. Pest insects are known to have detrimental impacts on agricultural and food production as they damage crops and parasitise livestock (Bradshaw *et al.*, 2016; FAO, 2020; Paini *et al.*, 2016), and they can also be a great nuisance and health hazard for human (WHO, 2015).

Nevertheless, apart from being considered a great nuisance, insects are responsible for the production of about one third of our food through the pollination process and, as such, they are a great asset playing a key role in all terrestrial and freshwater ecosystems. If all insects were to disappear, human food supply would run out in about four years (Goulson, 2019; Noriega *et al.*, 2018).

Insects were already a major food source 1.2 million years ago (Hardy *et al.*, 2016; Van Huis, 2017) and they are still

today part of the modern diet of more than two billion people, in particular in non-Western countries (Kouřimská and Adámková, 2016; van Huis, 2020).

The interest in insects has recently turned to their use in animal nutrition. Insect have a high nutritional value and species that undergo a non-feeding phase (pupae), store energy as fat and thus contain large quantities of triacylglycerol which are recalled in periods of high-energy demand. The fatty acid (FA) profile of insects is species-specific and related to the sex, life stage and environmental conditions of the insects (Oonincx *et al.* 2015), and most interestingly, it can be modulated by the rearing substrate to ameliorate the FA profile of the fed animals (Danieli *et al.*, 2019; Liland *et al.*, 2017; St Hilaire *et al.*, 2007). A fraction of this fat is usually extracted during the insect meal production process, and the resulting defatted raw material is a powder which vary in protein content and can exceed 70% (on a dry matter (DM) basis) (Figure 1). (Gasco *et al.*, 2020a; Józefiak *et al.*, 2016). As a result of their high nutritional value, and in particular their high



**Figure 1. Nutrient composition of *Tenebrio molitor*, *Hermetia illucens* and *Musca domestica* larvae meals compared to fishmeal and soybean meal (% of dry matter) (elaborated from Gasco *et al.*, 2018a). FM = fishmeal; HIL1, HIL2, HIL3 = *Hermetia illucens* larvae meals obtained under different production process; MDL = *Musca domestica* larvae meal; SBM = soybean meal; TMM1, TMM2, TMM3 = *Tenebrio molitor* larvae meals obtained under different production process.**

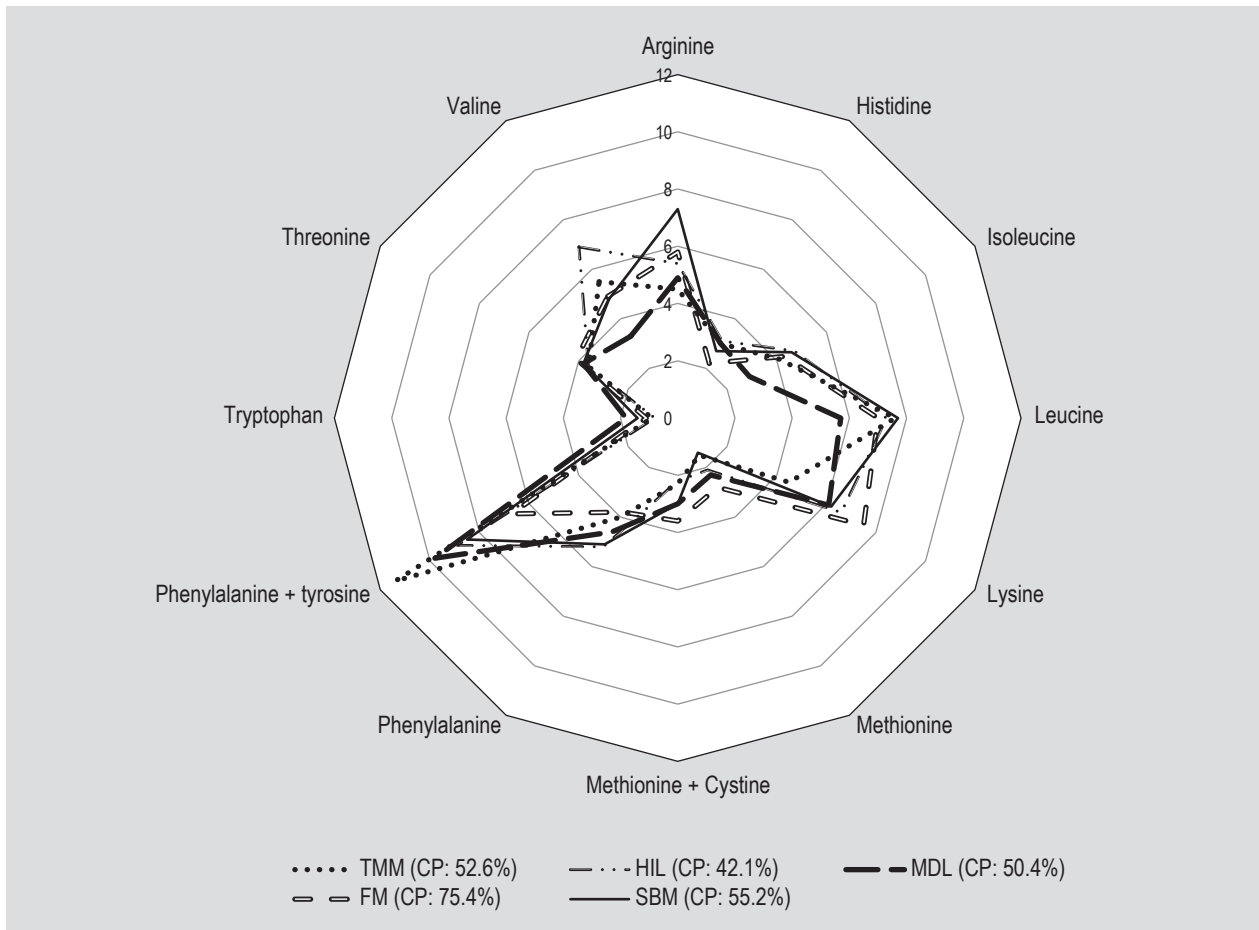
protein content, they represent excellent alternatives to conventional protein sources, such as soybean meal (SBM) or fishmeal (FM) in animal feed (De Souza *et al.*, 2019; Gasco *et al.*, 2018a, 2019a, 2020a,b; Henry *et al.*, 2015; Józefiak and Engberg 2017; Khan, 2018; Koutsos *et al.*, 2019; Lock *et al.*, 2018; Nogales-Mérida *et al.* 2019; Sogari *et al.*, 2019). Moreover, many insect species bio-convert organic substrates into valuable protein- and energy-rich products thus contributing to the Circular Economy principle (Gasco *et al.*, 2020b). In fact, the use of otherwise non-utilised side-streams as substrates for insects enables the production of high-value products with low environmental impacts (Bosch *et al.*, 2019; Smetana *et al.*, 2019).

So far, only a few insect species are being mass reared for feed purposes, the most studied being the black soldier fly (*Hermetia illucens*; HI), the common housefly (*Musca domestica*; MD), and the yellow mealworm (*Tenebrio molitor*; TM). They have a valuable amino acid profile similar to those of SBM and FM partially or totally covering the requirements of fish and livestock species (Figure 2).

Although, at the beginning, insects were mainly appreciated for their protein and energy supply, their newly discovered bioactive compounds may promote animal health and launch insects beyond the 'simple' protein concept (Gasco *et al.*, 2018b; Józefiak and Engberg 2017; Lee *et al.*, 2018; Wu

*et al.*, 2018). This review presents the main effects of insects and their bioactive compounds on fish and crustaceans, poultry, pigs and rabbits.

Preparing this article, an accurate bibliographical search was performed to collect all relevant references on the effects of dietary insects on animal health. The interest on this subject is recent and the search returned increasing numbers of references from 2011 to 2020. In addition to personal databases of articles collected since 2011 by the 3 authors and shared between them, academic articles were sourced from Google, Web of Science, Scopus and Science direct. Different key words were used, alone or in association with each others, such as 'insects', 'edible insects', 'insect feed', 'poultry', 'fish', 'teleost', 'pigs', 'piglets', 'rabbit', 'health effect', 'gut health', 'microbiota', 'immunology', 'antimicrobial peptides'. The search terms also consisted of the insect common names in English (e.g. 'yellow mealworm', 'black soldier fly', 'lesser meal worm' or 'common housefly') or the Latin names (e.g. '*Tenebrio molitor*', '*Hermetia illucens*', '*Zophobas morio*' or '*Musca domestica*'). The full text of each article was downloaded, read and when pertinent, used for the current review. All newly found articles were added to the common database. In all, about 950 articles were found of which 130 on fish, 113 on poultry, 36 on pigs, 7 on crustaceans, 4 on rabbits and more than 93 concerning antimicrobial peptides, chitin or microbiome.



**Figure 2.** Main amino acid composition of *Tenebrio molitor*, *Hermetia illucens* and *Musca domestica* larvae meals compared to fishmeal and soybean meal (% of protein dry matter) (Gasco *et al.*, 2018a and Feedipedia.org). FM = fishmeal; HIL = *Hermetia illucens* larvae meal; MDL = *Musca domestica* larvae meal; SBM = soybean meal; TMM = *Tenebrio molitor* larvae meal.

Considering insect products and their effects on animal health, only peer-reviewed articles written in English language and published during the last decade were considered for further evaluation to ensure contemporary scientific quality and timeliness of the review. At the end, without considering more general articles used in the introduction part, a total of 129 articles were included in this review.

## 2. Microbiota modulation and gut health

The importance of gut health in general health has recently been underlined for both mammals and other animals. Until recently, gut health was mainly assessed through histological observations. The development of 16S RNA based on high throughput sequencing techniques has enabled the precise detection of complex microbial communities in animals gastrointestinal tract (GIT), named microbiota, which has been closely linked not only to digestion but also to immunity and resistance of the animals to diseases (Egerton *et al.*, 2018; Kayama *et al.*, 2020; McCarville *et al.*, 2020). Diet composition is recognised as one of the major

factors affecting this gut microbiota and dietary insects may subsequently modulate the gut microbiota of the animals feeding on these insects (Vogel *et al.*, 2018).

### Fish and crustaceans

Crustaceans and fish gut health and microbiota are influenced by dietary insects (HI, listed in Table 1; TM, listed in Table 2; and other insects listed in Table 3).

### Fish and crustaceans gut morphology and physiology

Insect meal did not generally show any adverse effect on the gut morphology of fish (Caimi *et al.*, 2020; Elia *et al.*, 2018; Lock *et al.*, 2016; Wang *et al.*, 2019) or on their gut digestive enzymes (Belghit *et al.*, 2019; Li *et al.*, 2017a; Wang *et al.*, 2019). Some exceptions concerned dietary HI (Table 1) found to induce some villus irregularities in Jian carp (Li *et al.*, 2017a), villus shortening in clown fish (Vargas-Abundez *et al.*, 2019) and rainbow trout (Cardinaletti *et al.*, 2019), increased gut lipase activity in Japanese sea bass (Wang *et al.*, 2019) but decreased in European sea bass (Magalhaes

**Table 1. Effects of *Hermetia illucens* products on gut health and microbiota in fish and crustacean.<sup>1</sup>**

Animal [IBW-FBW, g] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Type and % of main protein in CTRL diet	% of substitution	Gut health and microbiota	Reference
		CP	CL					
Marron ( <i>Cherax cainii</i> ) [65-89] {60}	HIL	–	–	12, 11	FM: 41% or PBP: 39%	22, 19	Increased richness and diversity of microbiota; Bacteroidetes, Proteobacteria and Firmicutes LAB; decrease of <i>Vibrio</i> and Enterobacter	Foyсал <i>et al.</i> (2019)
Zebrafish ( <i>Danio rerio</i> ) [2-21 dps] {19}	HIP [FF]			10.5, 21	FM: 42%	25, 50	No intestinal inflammation; lipid accumulation in liver of fish fed 21% HIL; higher chitinase expression at 21% HI	Zarantoniello <i>et al.</i> (2018)
Jian carp ( <i>Cyprinus carpio</i> var. Jian) [35-110] {59}	HIL [DF]	–	–	2.6, 5.3, 7.9, 10.6	FM: 10%	25, 50, 75, 100	At HIL>75%, irregularities of gut microvilli shape, no effect on gut digestive enzymes	Li <i>et al.</i> (2017a,b)
Siberian sturgeon ( <i>Acipenser baerii</i> ) [24-159] {118}	HIL [DF]	65.8	4.24	18.5, 37.5	FM: 70%	25, 50	No effect on histology of liver or gut	Caimi <i>et al.</i> (2020)
Siberian sturgeon ( <i>A. baerii</i> ) [640-1,200] {60}	HIL [FF]	40.4	33.5	15	FM: 26%	30	Reduced thickness of mucosa, no difference of villi height; increased richness of microbiota, increased numbers of <i>Clostridium</i> , Enterobacteriaceae, <i>Aeromonas</i> , <i>Bacillus</i> , <i>Carnobacterium</i> , <i>Enterococcus</i> , <i>Lactobacillus</i>	Józefiak <i>et al.</i> (2019a)
Rainbow trout, ( <i>Oncorhynchus mykiss</i> ) [53-166] {71}	HIP [FF]	56.3	23.5	20	FM: 34.8%	30	Slight decrease of villi height in the proximal intestine; no change of mucosal thickness; increased richness of microbiota; increased <i>Clostridium</i> and LAB	Józefiak <i>et al.</i> (2019b)
Rainbow trout ( <i>O. mykiss</i> ) [66-223] {84}	HIL [DF]	48.6	20.6	10, 20, 30	FM: 60%	10, 20, 30	Increased richness and diversity of microbiota in faecal samples, increased lactic acid and butyrate-producing bacteria; Actinomycetaceae, Bacillaceae, Lactobacillaceae, Staphylococcaceae, reduced Vibrionaceae, Pseudomonaceae and Enterobacteriaceae; Firmicutes rich in faeces of HIL-fed trout, not in gut mucosa. Gut mucosa (adhered intestinal microbiota) of HIL-fed fish showed increased diversity but not richness; reduction of Proteobacteria, increased abundance of <i>Mycoplasma</i> in gut mucosa.	Terova <i>et al.</i> (2019) (gut content); Rimoldi <i>et al.</i> (2019) (gut mucosa)
Rainbow trout ( <i>O. mykiss</i> ) [137-300] {98}	HIP [FF]	39.0	41.9	10.5, 21	FM: 42%	25, 50	Increased goblet cells (hyperplasia) producing neutral mucins in hind intestine; shortening of mid intestine villi; no intestinal inflammation; increased lipid accumulation in liver at 21% HIP	Cardinaletti <i>et al.</i> (2019)
Rainbow trout ( <i>O. mykiss</i> ) [179-540] {78}	HIL [FF]	55.3	18	20, 40	FM: 60%	25, 50	No change of gut morphology; no effect on histology of liver, spleen or gut, acidic and neutral mucin; increased diversity of microbiota especially at 20%, Proteobacter and Tenericutes most abundant in HIL-fed trout	Elia <i>et al.</i> (2018); Bruni <i>et al.</i> (2018)

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Table 1. Continued.

Animal [IBW-FBW, g] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Type and % of main protein in CTRL diet	% of substitution	Gut health and microbiota	Reference
		CP	CL					
Rainbow trout ( <i>O. mykiss</i> ) [202-276] {35}	HIP [FF]	–	–	30	FM: 50%	30	Best diversity and richness of microbiota; rich in Actinobacteria	Huyben <i>et al.</i> (2019)
	HIL [FF]	–	–				Intermediate diversity and lowest richness of microbiota between 3 HIL but still richer than FM-fed fish; high firmicutes; high LAB	
	HIL [DF]	–	–				Lowest diversity and intermediate richness of microbiota still better than FM; very high Firmicutes (Bacillaceae)	
Atlantic salmon ( <i>Salmo salar</i> ) [49-143] {56}	HIL [DF]		–	60	FM: 35% + SPC: 29.6%	85	Decreased hyper-vacuolisation of proximal intestinal enterocytes; normal histological structure of mid- and distal intestine but of increased weight compared to ctrl fish, maybe related to increased butyrate	Li <i>et al.</i> (2019); Belghit <i>et al.</i> (2018)
Atlantic salmon ( <i>S. salar</i> ) [247-from 359 to 575] {105}	HIL [FF]	54.1	26.4	5, 10, 25	FM: 20%	25, 50, 100	No effect on gut histology	Lock <i>et al.</i> (2016)
		61.0	17.8	5, 25		25, 100		
Atlantic salmon ( <i>S. salar</i> ) [1,400-3,702] {112}	HIL [DF]	52	18	5, 10, 15	FM: 10%	33, 66, 100	At 15% HIL, lower enterocytes steatosis in proximal intestine, increased distal intestine weight. At 5, 10 and 15% HIL, no effect on digestive brush border enzymes or total bile acids in the digesta	Belghit <i>et al.</i> (2019); Li <i>et al.</i> (2020)
European sea bass ( <i>Dicentrarchus labrax</i> ) [50-129] {62}	HIP [DF]	55.8	5.5	6.5, 13, 19.5	FM: 32.4%	15, 30, 45	No effect on gut digestive enzymes except for lipase lower at 6.5 compared to FM ctrl and 19.5% HIP in both anterior and posterior intestine	Magalhaes <i>et al.</i> (2017)
Clownfish ( <i>Amphiprion ocellaris</i> ) [juveniles]{106}	HIL [DF]	–	–	20, 40, 60	FM: 60%	25, 50, 75	No intestinal inflammation, reduced villi at HIL>40%	Vargas-Abundez <i>et al.</i> (2019)

<sup>1</sup> CL = crude lipid; CP = crude protein; CTRL = control diet; dps = days post-spawning; DF = defatted; DM = dry matter; FBW = final body weight; FF = full fat; FM = fishmeal; HIL = *Hermetia illucens* larvae meal; HIP = *Hermetia illucens* prepupae meal; IBW = initial body weight; LAB = lactic-acid producing bacteria; PBP = poultry by-product meal; SBM = soybean meal; SPC = soy protein concentrate.

*et al.*, 2017), increased weight of the distal intestine of Atlantic salmon (Li *et al.*, 2019, 2020) and reduced steatosis (i.e. abnormal fat retention) of the proximal intestine in Atlantic salmon (Li *et al.*, 2020). The dietary inclusion of HI also increased submucosa cellularity and production of neutral mucin by the goblet cells in the proximal intestine of rainbow trout (Cardinaletti *et al.*, 2019; Elia *et al.*, 2018). Mucosal and muscular layer thickening of the GIT was also shown in sturgeon fed TM (Józefiak *et al.*, 2019a; Table 1 and 2). This may be related to the production of short-chain

fatty acids (SCFA; such as butyrate), which are metabolites of the microbiota known to stimulate the proliferation of mucosal cells in the colon of humans (Mortensen *et al.*, 1999). Microbiota studies on crustaceans and fish have been performed on rainbow trout and few other freshwater and marine fish species fed mainly on HI (Table 1) and TM (Table 2), but recent studies have also investigated the effects of MD, *Gryllus sigillatus*, *Blatta lateralis* and *Zophoba morio* (ZM) (Table 3).



**Table 2. Effects of *Tenebrio molitor* products on gut health and microbiota in fish and crustacean.<sup>1</sup>**

Animal [IBW-FBW, g] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Type and % of main protein in CTRL diet	% of substitution	Gut health and microbiota	Reference
		CP	CL					
Siberian sturgeon ( <i>Acipenser baerii</i> ) [640-1,200] {60}	TMM [FF]	56.3	25.3	15	FM: 26%	40	Increased thickness of muscular layer, no difference of villi height; reduced richness of microbiota; no effect on intestinal <i>Clostridium</i> , Enterobacteriaceae, <i>Lactobacillus</i> or <i>Enterococcus</i> . Increased <i>Bacillus</i> , <i>Carnobacterium</i> and <i>Enterococcus</i>	Józefiak <i>et al.</i> (2019a)
Rainbow trout ( <i>Oncorhynchus mykiss</i> ) [53-166] {71}	TMM [FF]	56.3	25.3	20	FM: 34.8%	40	Decrease of villi height in the proximal intestine; no change of mucosal thickness; strongly increased richness of microbiota; strongly increased <i>Clostridium</i> ; Enterobacteriaceae and LAB	Józefiak <i>et al.</i> (2019b)
Rainbow trout ( <i>O. mykiss</i> ) [115-no data] {90}	TMM [FF]	51.9	23.6	60	FM: 70%	86	Decreased diversity of microbiota	Antonopoulou <i>et al.</i> (2019)
Sea trout ( <i>Salmo trutta m. trutta</i> ) [5-21] {56}	TMM [hydrolysed]	47	29.6	10	FM: 25%	58	Slight increase of villi height; no change in muscular layer thickness of the anterior intestine; no change in microbiota richness; reduction of <i>Carnobacterium</i> and <i>Lactobacillus</i>	Mikołajczak <i>et al.</i> (2020)
European sea bass ( <i>Dicentrarchus labrax</i> ) [5-from 17 to 22] {70}	TMM [FF]	51.9	23.6	50	FM: 70%	71	Increased diversity of microbiota, increased abundance of Tenericutes	Antonopoulou <i>et al.</i> (2019)
Gilthead seabream ( <i>Sparus aurata</i> ) [105-240] {163}	TMM [FF]	51.9	23.6	50	FM: 50%	74	increased diversity of microbiota, increased abundance of Tenericutes	Antonopoulou <i>et al.</i> (2019)

<sup>1</sup> CL = crude lipid; CP = crude protein; CTRL = control diet; dps = days post-spawning; DM = dry matter; FBW = final body weight; FF = full fat; FM = fishmeal; IBW = initial body weight; LAB = lactic-acid producing bacteria; TMM = *Tenebrio molitor* larvae meal.

### Fish and crustaceans gut microbiota

The microbiota of fish is closely correlated to the host diet and dietary insects have been suggested to act as prebiotics through both an improvement of the gut absorptive area and the stimulation of the host immunity to fight pathogenic bacteria, thus favouring beneficial commensal bacteria (Guerrero *et al.*, 2018; Józefiak *et al.*, 2019a). In crustaceans, a dietary supplementation of HI increased the richness and diversity of the gut microbiota of marron (*Cherax cainii*) (Foysal *et al.*, 2019). The opposite was true in swamp eel where the replacement of a control diet with whole MD larva every 3 or 7 days reduced the gut microbiota richness and diversity. However, the fish did not grow well in this unique study showing adverse effect of dietary insects on fish microbiota (Xiang *et al.*, 2019). Indeed, although dietary TM or ZM did not affect the richness of sea trout's microbiota, they significantly reduced the occurrence of potential pathogens (Mikołajczak *et al.*, 2020). Moreover, several studies have shown that both HI and TM

increased the gut microbiota diversity of rainbow trout (Antonopoulou *et al.*, 2019; Huyben *et al.*, 2019; Józefiak *et al.*, 2019b). Dietary crustacean chitin and chitosan have been shown to enrich and diversify fish microbiota (Askarian *et al.*, 2012; Ringø *et al.*, 2012; Udayangani *et al.*, 2017), acting as prebiotics and contributing to a healthy gut, decreasing the abundance of fish pathogenic bacteria and thus increasing disease resistance (Qin *et al.*, 2014; Terova *et al.*, 2019; Zhou *et al.*, 2013). Insect chitin may have a similar prebiotic effect (Huyben *et al.*, 2019), although this has not been demonstrated as yet. Nevertheless, dietary insects have been shown to cause a shift of microbiota of crustaceans and fish towards an increased importance of Tenericutes, Actinomycetes, Clostridiales and/or Firmicutes suggested to be chitinolytic bacteria facilitating the digestion of chitin-rich nutrients (Elia *et al.*, 2018; Bruni *et al.*, 2018; Antonopoulou *et al.*, 2019; Foysal *et al.*, 2019; Huyben *et al.*, 2019; Józefiak *et al.*, 2019b; Terova *et al.*, 2019). In particular, Firmicutes lactic acid producing bacteria (LAB), often used as probiotics

**Table 3. Effects of *Musca domestica*, Chironomidae, *Gryllus sigillatus*, *Blatta lateralis* and *Zophoba morio* on gut health and microbiota in fish and crustacean.<sup>1</sup>**

Animal [IBW-FBW, g] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Type and % of main protein in CTRL diet	% of substitution	Gut health and microbiota	Reference
		CP	CL					
Swamp eel ( <i>Monopterus albus</i> ) [27-31] {56}	MDL [whole]	64	24.3	1 day every 3 (1d/3) or 7 days (1d/7)	FM: 45%		At 1d/7, poorer microbiota and less diverse, decreased <i>Pseudomonas</i> . At 1d/3, more Bacteroidaceae, decreased <i>Pseudomonas</i>	Xiang <i>et al.</i> (2019)
Rainbow trout ( <i>Oncorhynchus mykiss</i> ) [0.13-0.65] {28}	ChL [whole frozen]	66	9	100	FM: 12.4%	48	Distal intestine more folded, with higher brush border, less goblet cells	Ostaszewska <i>et al.</i> (2011)
Rainbow trout trout ( <i>O. mykiss</i> ) [53-166] {71}	GSA [FF]	61.3	19.5	20	FM: 34.8%	48	Decrease of villi height in the proximal intestine; decrease of mucosal thickness; increased richness of microbiota; increased <i>Clostridium</i> and LAB	Józefiak <i>et al.</i> (2019b)
Rainbow trout trout ( <i>O. mykiss</i> ) [53-166] {71}	BLA [FF]	54.6	26.1	20	FM: 34.8%	42	Decreased growth; Increase of villi height in the proximal intestine; increase of mucosal thickness; increased richness of microbiota; increased <i>Clostridium</i> and LAB	Józefiak <i>et al.</i> (2019b)
Sea trout ( <i>Salmo trutta m. trutta</i> ) [5-21] {56}	ZMM [hydrolysed]	49.3	33.6	10	FM: 25%	56	No change in villi height or muscular layer thickness of the anterior intestine; no change in richness of microbiota; reduction of <i>Aeromonas</i> , <i>Carnobacterium</i> and <i>Enterococcus</i>	Mikołajczak <i>et al.</i> (2020)

<sup>1</sup> BLA = *Blatta lateralis* adults; ChL = Chironomidae larvae; CL = crude lipid; CP = crude protein; CTRL = control diet; dps = days post-spawning; DM = dry matter; FBW = final body weight; FF = full fat; FM = fishmeal; GSA = *Gryllus sigillatus* adults; IBW = initial body weight; LAB = lactic-acid producing bacteria; MDL = *Musca domestica* larvae meal; ZMM = *Zophoba morio* larval meal.

and known to improve mucosal activity and to produce antimicrobial bacteriocins, were more abundant in the intestines of rainbow trout and Siberian sturgeon fed with HI (Bruni *et al.*, 2018; Huyben *et al.*, 2019; Józefiak *et al.*, 2019a,b, Terova *et al.*, 2019). However, this was not the case of sea trout fed TM, where *Lactobacillus* counts reduced compared to FM-fed fish (Mikołajczak *et al.*, 2020). Other commensal bacteria with antibacterial or antiviral activity (e.g. *Pseudomonas stutzeri*, *Carnobacterium divergens*, *Acinetobacter* spp.) and with the potential to help the host fight or compete with pathogens were observed to be particularly abundant in HI-fed rainbow trout (Bruni *et al.*, 2018). Moreover, bacterial genera associated with common fish or human pathogens (*Pseudomonas*, *Vibrio*, *Aeromonas* and *Enterococcus*) decreased in HI-fed rainbow trout (Rimoldi *et al.*, 2019; Terova *et al.*, 2019), TM- and ZM-fed sea trout (Mikołajczak *et al.*, 2020) as well as in MD-fed swamp eel (Xiang *et al.*, 2019). Insect meals are usually rich in saturated SCFA such as lauric acid, propionate and butyrate, which are antibacterial and antiviral, and may thus be responsible for some of the modulating activity of dietary insects on the gut microbiota of fish described above

(Terova *et al.*, 2019). In turn, dietary insects were shown to favour commensal bacteria of the Clostridiales order, which also produce SCFA reinforcing their contribution to the gut microbiomic homeostasis of fish (Józefiak *et al.*, 2019 a,b; Terova *et al.*, 2019). Moreover, SCFA are also known to be anti-inflammatory (Vargas-Abundez *et al.*, 2019) and may be involved in the absence of gut inflammation observed in insect meal-fed fish (Cardinaletti *et al.*, 2019; Li *et al.*, 2019; Zaratoniello *et al.*, 2018).

## Poultry

In nature, most birds are primarily insectivorous. For example, wild red jungle fowl (*Gallus gallus*) is known to eat ants and termites and juvenile wild turkeys eat approximately 60% of insects (Klasing, 2005). When farmed poultry have access to outdoor areas, they dedicate part of their time to searching for and ingesting live preys including insects (Józefiak *et al.*, 2016; Schiavone *et al.*, 2019). Table 4 and 5 report the main literature concerning the effects of insect-derived products inclusion in poultry diets.

**Table 4. Effects of *Hermetia illucens* products in gut health and microbiota modulation in poultry.<sup>1</sup>**

Poultry species [IBW-FBW, g] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Gut health and microbiota	Reference
		CP	CL			
Laying hens (Lohmann Brown Classic) [1,410-no data] {168}	HIL [DF]	60.0	9.0	7.3, 14.6	In the duodenum, villi height and villi/crypt ratio were lower in both HIL groups than in control. In ileum, only the villi height was reduced in birds fed the highest level of HIL.	Moniello <i>et al.</i> (2019)
Laying hens (Lohmann Brown Classic) [1,780-2,009] {147}	HIL [DF]	62.7	4.7	17	Increased relative abundance of Elusimicrobia, Lentisphaerae and Cyanobacteria and decreased Fusobacteria. The HIL group showed a higher villi height in the duodenum, while the opposite happened in the jejunum and the ileum. Only in the ileum, higher crypt depth in the HIL group than in the SBM.	Borrelli <i>et al.</i> (2017); Cutrignelli <i>et al.</i> (2018)
Broiler chickens (Ross 308) [40.2-2,278] {35}	HIL [DF]	55.3	18.0	5, 10, 15	The HIL 15% showed a lower villus height, a higher crypt depth and a lower villus height-to-crypt depth ratio than the other groups. Caecal digesta analysis of HIL 15% birds displayed significant increase of the relative abundance of Proteobacteria phylum than HIL 10%. Characteristic microbiota for 5% HIL: <i>L-Ruminococcus</i> (Lachnospiraceae family), <i>Faecalibacterium</i> , <i>Blautia</i> and <i>Clostridium</i> genera were found to be characteristic in caecal microbiota. Characteristic microbiota for 10% HIL: <i>Lactobacillus</i> and <i>Ruminococcus</i> and <i>Bacteroides</i> , <i>Roseburia</i> . Characteristic microbiota for 15% HIL: <i>Helicobacter</i> genera. Lower mucin in small and large intestine of chicken fed 10 and 15% addition of HIL compared to CTRL.	Biasato <i>et al.</i> (2020a); Dabbou <i>et al.</i> (2018)
Broiler chickens (Ross 308) [42-2,101] {35}	HIL [FF]	40.4	33.5	0.2	Crop: increase in <i>Clostridium coccoides</i> – <i>Eubacterium rectale</i> cluster and <i>Lactobacillus</i> spp./ <i>Enterococcus</i> spp. Ileum: increase in <i>C. coccoides</i> – <i>E. rectale</i> cluster and decrease in <i>Lactobacillus</i> spp./ <i>Enterococcus</i> spp. Caeca: increase in <i>Bacteroides-Prevotella</i> cluster, <i>Streptococcus</i> spp./ <i>Lactococcus</i> spp., <i>C. coccoides</i> – <i>E. rectale</i> cluster and <i>Lactobacillus</i> spp./ <i>Enterococcus</i> spp.	Józefiak <i>et al.</i> (2018)
Broiler chicken (Ross 308) [817.8-3,751.3] {27}	HIF	–	–	3.43, 6.87	No influence on gut morphometric indexes	Schiavone <i>et al.</i> (2018)
Muscovy ducks ( <i>Cairina moschata</i> <i>domestica</i> ) [70.4-2,554] {47}	HIL [DF]	56.7	10.7	3, 6, 9	No effect on gut morphology	Gariglio <i>et al.</i> (2019)
Turkey (B.U.T. 6, AVIAGEN) [179-1,396] {35}	HIF	–	–	2.5, 5	Decrease of Enterobacteriaceae counts	Sypniewski <i>et al.</i> (2020)

<sup>1</sup> CL = crude lipid; CP = crude protein; CTRL = control diet; DF = defatted; DM = dry matter; FBW = final body weight; FF = full fat; HIL = *Hermetia illucens* larvae meal; HIF = *Hermetia illucens* fat; IBW = initial body weight; SBM = soybean meal.



**Table 5. Effects of *Tenebrio molitor*, *Shelfordella lateralis* and *Zophobas morio* meals in gut health and microbiota modulation in poultry.<sup>1</sup>**

Poultry species [IBW-FBW, g] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Gut health and microbiota	Reference
		CP	CL			
Broiler chickens (Ross 308) [42-2,122] {35}	TMM [FF]	47.0	29.6	0.2, 0.3	Caeca: TML 0.2% tended to show highest relative abundance of the family Ruminococcaceae and <i>Lactobacillus reuteri</i> . Significant increase in level of Clostridia.	Józefiak <i>et al.</i> (2020)
Broiler chickens (Ross 308) [42-2,127] {35}	TMM [FF]	56.3	25.3	0.2	Crop: decrease in <i>Bacteroides-Prevotella</i> cluster. Ileum: increase in <i>Clostridium coccoides</i> – <i>Eubacterium rectale</i> and <i>Lactobacillus spp./ Enterococcus spp.</i> Caeca: increase in <i>C. coccoides</i> – <i>E. rectale</i> .	Józefiak <i>et al.</i> (2018)
Broiler chickens (Ross 708) [no data-2,309] {40}	TMM [FF]	55.3	25.1	5, 10, 15	No effect on gut morphology.	Biasato <i>et al.</i> (2017)
Free-range broiler chickens [716-no data] {53}	TMM [FF]	55.3	25.1	7.5	Significant increase of the relative abundances of <i>Firmicutes</i> and decrease of <i>Bacteroidetes</i> phyla. No effect on gut morphology. Higher relative abundance of <i>Clostridium</i> , <i>Oscillospira</i> , <i>Ruminococcus</i> , <i>Coprococcus</i> and <i>Sutterella</i> genera compared to CTRL.	Biasato <i>et al.</i> (2018a)
Broiler chickens (Ross 708) [no data-3,641] {53}	TMM [FF]	57.5	30.7	5, 10, 15	TMM 15% showed lower villus height, higher crypt depth, and lower villus height to crypt depth ratio compared to CLRL and TMM 5%.	Biasato <i>et al.</i> (2018b)
Broiler chickens (Ross 308) [42-2,076] {35}	SLI [FF]	54.6	26.1	0.05, 0.1, 0.2	Crop: increase in <i>Clostridium leptum</i> subgroup. Ileum: increase in <i>C. coccoides</i> – <i>E. rectale</i> cluster and <i>Lactobacillus spp./ Enterococcus spp.</i> Ceca: increase in <i>Bacteroides-Prevotella</i> cluster.	Józefiak <i>et al.</i> (2018)
Broiler chickens (Ross 308) [42-2,122] {35}	ZMM [FF]	49.3	33.6	0.2, 0.3	Caeca: ZML 0.2% resulted in an increase in relative abundance of the Actinobacteria, including the family Bifidobacteriaceae, with the highest relative abundance of genus <i>Bifidobacterium pseudolongum</i> . ZML 0.2% resulted in an increase in the number of <i>Lactobacillus agilis</i> .	Józefiak <i>et al.</i> (2020)

<sup>1</sup> CL = crude lipid; CP = crude protein; CTRL = control diet; DM = dry matter; FBW = final body weight; FF = full fat; IBW = initial body weight; SLI = *Shelfordella lateralis* imago meal; TMM = *Tenebrio molitor* larvae meal; ZMM = *Zophobas morio* larvae meal.

On the one hand, the effects of dietary insect meals inclusion on GIT morphometry in poultry, the results are often inconsistent. For instance, a decrease in the villi height, crypt depth and villi/crypt ratio was reported in the GIT segments (duodenal, jejunal and ileum) of laying hens (Cutrignelli *et al.*, 2018; Moniello *et al.*, 2019) and broilers (Dabbou *et al.*, 2018) fed with HI especially when the inclusion levels surpassed 10%. On the contrary, no histopathological changes were reported for Muscovy ducks (Gariglio *et al.*, 2019) or broiler chickens (Biasato *et al.*, 2018a) fed HI larva meal or for chickens fed TM meal (Biasato *et al.*, 2016, 2017, 2018b). The use of insect-derived oils/fats did not seem to affect the GIT morphology (Schivavone *et al.*, 2018; Sypniewski *et al.*, 2020).

On the other hand, dietary insects consistently modulate the microbiota populations in the GIT of poultry, but it may vary depending on the considered gut tract, the insect species or the level of insect inclusion (Benzeriha *et al.*, 2019; Józefiak *et al.*, 2018, 2020). Low levels of insect meals in poultry diets diversified the GIT microflora and increased the bacteria with positive effects on gut health (Józefiak *et al.*, 2019a). However, levels higher than 10% (and in particular 15%) of insect meal inclusion seemed to reduce microbial complexity and richness in beneficial bacteria including those with mucolytic activity (Biasato *et al.*, 2019a, 2020a). Following the ingestion of 0.2% of defatted TM, potentially pathogenic bacteria, such as the *Bacteroides-Prevotella* cluster, reduced in crops and caeca, while the ileum showed an increase in *Lactobacillus spp./*

*Enterococcus* spp. (Benzertiha *et al.*, 2019; Józefiak *et al.*, 2018). An increase in butyrate producing bacteria, such as the *Clostridium coccoides* – *Eubacterium rectale* cluster and the *Clostridium leptum* subgroup, was observed in broiler fed 0.2% of full fat HI in all the GIT and ileum, respectively (Józefiak *et al.*, 2018).

Similar results were found in broilers fed 0.2 and 0.3% of TM or ZM full fat meals where *Bacteroides*-*Prevotella* cluster decreased in the caeca and a decrease in *Clostridium perfringens* counts decreased in groups treated with TM (0.2 and 0.3%) and ZM (0.2%). In contrast, *C. perfringens* counts were increased in the birds fed 0.3% ZM resulting in a negative effect as this very pathogenic bacteria causes necrotic enteritis in poultry (Benzertiha *et al.*, 2019).

Similarly, low inclusion levels (0.2, 0.3%) of TM and ZM in broiler chicken also improved caecal commensal microbiota with an increase in Clostridiales and particularly Lactobacillales and Bifidobacteriaceae with *Bifidobacterium pseudolongum* showing the highest abundance in the caecum digesta of birds fed 0.2% ZM (Józefiak *et al.*, 2020).

The dietary inclusion of 7.5% of full fat TM larva meal positively modulated the gut microbiota of free-range broiler chickens, inducing an increase in Firmicutes and a decrease in Bacteroidetes, but did not influence the mucin composition (Biasato *et al.*, 2018a).

Insect meal was also shown to positively modulate the gut microbiota composition of laying hens. Borrelli *et al.* (2017), for instance, indicated that the total substitution of SBM by defatted HI larvae increased the relative abundance of Elusimicrobia, Lentisphaerae and Cyanobacteria and decreased Fusobacteria, compared with SBM-fed laying hens. These changes were correlated with the production of butyric acid in the caeca, potentially linked to chitin degradation.

Dietary oil replacement by insect fat also influenced the microbiota as shown in turkey where total substitution of soybean oil (SBO) by HI fat reduced the proliferation of potentially pathogenic bacteria (i.e. Enterobacteriaceae spp.) (Sypniewski *et al.*, 2020).

The modulation of the animal microbiota may be due to antimicrobial peptides (AMPs) produced by the insects introduced in the animal diet (Józefiak and Engberg, 2017). The addition of 2 AMPs (A3 and P5) to the chicken diet showed beneficial effects not only on growth performance and nutrient retention, (Choi *et al.*, 2013a,b) but also on the histomorphology (dose-dependent increase in villi height and depth) and intestinal microflora through a decrease in the population of anaerobic bacteria in the ileal and caecal digesta. The same was true of chicken fed with a cecropin

hybrid which showed increased villus height and villus height: crypt depth ratio, but showed a negative effect on the crypt depth of the duodenum and ileum and decreased total aerobic bacteria in the jejunal and caecal digesta (Wen and He, 2012).

## Pigs and rabbits

Literature on the influence of insect products on the microbiota and GIT health of pigs and rabbits is scarce as evident from Table 6. The somehow controversial results may be due to differences in composition of the used insect products.

Sprangers *et al.* (2018) did not observe any difference in the histo-morphological traits or in the bacterial count in the intestinal segments of weaned pigs fed full fat or defatted HI meal. Nevertheless, as an increase in lauric acid was found in all the gut segments of piglets fed HI meal, with significant differences between pigs fed 8% full fat HI meal compared to all the other treatments in the digestive tract compartments, the authors suggested a possible dose-dependent antibacterial effect (Sprangers *et al.*, 2018).

Yu *et al.* (2019, 2020a) highlighted that the inclusion of a full fat HI larvae meal in finishing diets for pigs induced changes in the bacterial composition of the gut that were correlated to an enhanced colonic mucosal immune homeostasis. They suggested a prebiotic effect of low levels (2%) of HI meal inclusion, likely due to chitin. Their results showed an increase in the abundance of health-promoting bacteria, such as *Lactobacillus* and *Bifidobacterium*, which are known to preserve the integrity of the intestine lining. At the same time, they also reported an increase in the abundance of butyrate-producing bacteria (*Roseburia*, *Pseudobutyrvibrio* and *Faecalibacterium*) and in the number of *Clostridium* XIVa clusters in the colon, with a consequent significant increase in the production of butyrate and SCFAs. As far as bacteria or bacterial metabolites with potential negative effects are concerned, their results showed a decrease in *Streptococcus* sp., *Escherichia coli* and in the metabolites involved in amino acids metabolism. The same authors (Yu *et al.*, 2020b) also reported an increase in the relative weight of the small intestine of piglets fed 2 and 4% of HI meal and in the villus height in the jejunum of piglets fed 2% of HI meal, thus suggesting a promoting effect on gut development and metabolism. In contrast, Biasato *et al.* (2019b) reported no effect on the intestinal morphology of pigs fed HI defatted meal.

Feeding weaning piglets with an inclusion of up to 10% of defatted HI larvae meal had a positive effect on the caecal microbiota and the small intestine mucin composition, showing a modulation of the potentially beneficial bacteria and a preservation of the mature mucin secretory

Table 6. Effects of insect products in gut health and microbiota modulation in pigs and rabbits.<sup>1</sup>

Animal [IBW-FBW, kg] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Type and % of main protein in CTRL	% substitution	Gut health and microbiota	Reference
		CP	CL					
Weaning pigs [6.1-no data] {15}	HIL [FF]	42.01	42.13	4, 8	Toasted soybeans: 12%	50 and 100%	No effects on gut morphology. Increase in lauric acid in gut segment of pig fed 8% FF HIL	Sprangers <i>et al.</i> (2018)
	HIL [DF]	62.84	8.06	5.42		72%		
Weaning piglets [7.9-17.6] {28}	HIL [FF]	37.92	38.48	1, 2, 4	FM: 4%	25, 50 and 100%	Increase of villus height in the jejunum of piglets fed 2% HIL	Yu <i>et al.</i> (2020a,b)
Weaning pigs [6.1-32.4] {61}	HIL [DF]	59.0	8.97	5, 10	SBM: 20% (phase I); 18.5% (phase II)	Phase I: 30 and 60%; Phase II: 32 and 65%	No effects on gut morphology. Increase of $\beta$ diversity in HIL fed groups. Increase in SCFAs producing bacteria. Decrease in jejunal inflammation. Increase in neutral mucins	Biasato <i>et al.</i> (2019b, 2020b)
Finishing pigs [76.0-116] {46}	HIL [FF]	37.92	38.48	4, 8	SBM: 16.98%	18.4 and 36.7%	Increase in <i>Lactobacillus</i> , <i>Pseudobutyrvibrio</i> , <i>Roseburia</i> , and <i>Faecalibacterium</i> . Decrease in <i>Streptococcus</i>	Yu <i>et al.</i> (2019)
Rabbits [1.05-2.9] {41}	HIF and TMO			0.75, 1.5	SBO: 1.5%	50 and 100%	No effects on gut morphology	Gasco <i>et al.</i> (2019b)

<sup>1</sup> CL = crude lipid; CP = crude protein; CTRL = control diet; DF = defatted; DM = dry matter; FBW = final body weight; FF = full fat; HIL = *Hermetia illucens* larvae meal; HIF = *Hermetia illucens* fat; IBW = initial body weight; SBM = soybean meal; SBO = soybean oil; SCFAs = short chain fatty acids; TMO = *Tenebrio molitor* oil.

architecture together with an attenuation of jejunal inflammation associated with an increased proportion of SCFA-producing bacteria in the caecal microbiota (Biasato *et al.*, 2020b).

The dietary ingestion of HI and TM fats in substitution of 50 and 100% of soybean oil in the diets of growing rabbits did not influence the gut morphometry or alter the liver functions (Gasco *et al.*, 2019b), although a positive effect on microbiota has been suggested (Dabbou *et al.*, 2020). Indeed, an increase in the relative abundance of *Bacteroides*, *Clostridium*, *Akkermansia* and *Ruminococcus* has been observed. *Akkermansia* has been indicated as being able to degrade the mucin in the intestine, with a consequent production of SCFAs and consequent positive effects on gut health. *Ruminococcus* are also butyrate producing bacteria that contribute to the positive effect on the caecal microbiota of rabbits (Dabbou *et al.*, 2020).

### 3. Immunological status

Beside their nutritional interest, insects contain biological active components such as chitin, which is a structural polysaccharide forming the outer shell of crustaceans and the exoskeleton of insects. It has been shown that crustacean chitin and chitin derivatives activate and boost the innate immune response of animals (Ringo *et al.*, 2012) and it seems that insect chitin may play a similar role (Józefiak and Engberg, 2017; Lee *et al.*, 2008).

#### Fish and crustaceans

In fish, dietary crustacean chitin and chitosan were potent immunostimulants and improved bacterial and parasitological disease resistance (Esteban *et al.*, 2001; Lee *et al.*, 2008; Shanthi Mari *et al.*, 2014). However, few studies have investigated the effects of insects on the crustaceans and fish immune system (Table 7, 8 and 9). In crustaceans, dietary TM significantly increased both the total hemocyte counts and phenoloxidase activity of the Pacific white

Table 7. Effects of *Hermetia illucens* meals on antioxidant enzymes, immune parameters in crustacean and fish.<sup>1</sup>

Animal [IBW-FBW, g] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Type and % of main protein in CTRL diet	% of substitution	Antioxidant enzymes	Immune parameters	Reference
		CP	CL						
Marron ( <i>Cherax cainii</i> ) [65-89] {60}	HIL	–	–	12, 11	FM: 41% or PBP: 39%	22, 19		Increased lysozyme, total haemocyte counts, increased IL1 $\beta$ , IL8, IL10, IL17F and TNF $\alpha$ expression in intestine	Foyсал <i>et al.</i> (2019)
Zebrafish ( <i>Danio rerio</i> ) [2-21 dps] {19}	HIP [FF]	–	–	10.5, 21	FM: 42%	25, 50		Transient increase in HSP70 at 14 dps then significantly lower in HIP 10.5 and 21% than in ctrl fish, higher TNF $\alpha$ and IL6 at 21% HIP	Zarantoniello <i>et al.</i> (2018)
Jian carp ( <i>Cyprinus carpio</i> , var Jian) [35-110] {59}	HIL [DF]	–	–	2.6, 5.3, 7.9, 10.6%	FM: 10%	25, 50, 75, 100	Reduced cholesterol; no effect on serum SOD or MDA, increased serum CAT; more apoptotic hepatocytes in fish fed HIL100%	At HI>75%, enhanced hepatic HSP70 gene expression suggesting induced stress	Li <i>et al.</i> (2017a,b)
Siberian sturgeon, ( <i>Acipenser baerii</i> ), [24-159] {118}	HIL [DF]	65.8	4.24	18.5, 37.5	FM: 70%	25, 50	Alteration of hepatic oxidative stress biomarkers (increased SOD, GR, decreased GPx at 37.5%). SOD, GR, EROD and GST increased in kidney of fish fed 37.5% HIL		Caimi <i>et al.</i> (2020)
Yellow catfish ( <i>Pelteobagrus fulvidraco</i> ) [1.5-48.5] {65}	HIL [FF]	47	17.7	5.5, 10.8, 16.5, 22.3, 34.3, 46.2, 58.5	FM: 40%	13, 25, 37, 48, 68, 85, 100	Increased serum SOD at 10.8% inclusion. Hepatopancreas SOD not affected	Tendency for increased lysozyme and phagocytosis activities at 5.5 and 10.8% inclusion levels	Xiao <i>et al.</i> (2018)
Rainbow trout ( <i>Oncorhynchus mykiss</i> ) [137-300] {98}	HIP [FF]	39	41.9	10.5, 21	FM: 42%	25, 50	No difference in cholesterol or hepatic GR	HSP70 increase at 21% HIP; increased IL10 and TNF $\alpha$ in mid-intestine	Cardinaletti <i>et al.</i> (2019)
Rainbow trout ( <i>O. mykiss</i> ) [179-540] {78}	HIL [FF]	55.3	18	20, 40	FM: 60%	25, 50	Unchanged liver and kidney MDA, SOD, CAT, GPx, GR, reduced SeGPx in liver and kidney, increased GSH and EROD in liver, increased GST in kidney		Elia <i>et al.</i> (2018)
Atlantic salmon ( <i>Salmo salar</i> ) [49-143] {56}	HIL [DF]	–	–	60	FM: 35% + SPC: 29.6%	83	Elevated xenobiotic detoxification response in intestine (cyp1a1 expression)	Stimulation of regulatory T-cell activity; no difference in anti-IPVN antibodies	Li <i>et al.</i> (2019); Belghit <i>et al.</i> (2018)
Atlantic salmon ( <i>S. salar</i> ) [1,400-2,550] {56}	HIL [DF]	52	18	10, 15	FM: 10%	66;100	down-regulated SOD and GPx expression in LPS-triggered cells	No effect on inflammation response to bacteria and virus; antioxidant and stress response to bacteria downregulated (SOD, GPx, HSP70, C/EBP $\beta$ , p38MAPK and TLR22)	Stenberg <i>et al.</i> (2019)
Atlantic salmon ( <i>S. salar</i> ) [1,400-3,702] {112}	HIL [DF]	52	18	5, 10, 15	FM: 10%	33;66; 100		No effect on haemoglobin	Belghit <i>et al.</i> (2019)
Japanese sea bass ( <i>Lateolabrax japonicus</i> ), [14-58] {56}	HIL [DF]	55.4	1.6	5, 10, 15, 20	FM: 25%	16, 32, 48, 64	no difference in serum CAT, GPx, SOD; lower MDA	No effect on complement, alkaline phosphatase or lysozyme activity or on concentration of intestinal inflammatory cytokines	Wang <i>et al.</i> (2019)

<sup>1</sup> CAT = catalase; C/EBP $\beta$  = CCAAT-enhancer-binding proteins; CL = crude lipid; CP = crude protein; CTRL = control diet; DF = defatted; EROD = ethoxyresorufin O-deethylase; FBW = final body weight; FF = full fat; FM = fishmeal; GPx = glutathione peroxidase; GR = glutathione reductase; GST = glutathione S-transferase; GSH = glutathione; HIL = *Hermetia illucens* larvae meal; HIP = *Hermetia illucens* prepupae meal; HSP70 = heat shock proteins 70; IBW = initial body weight; IL10 = interleukin-10; IL17F = interleukin-17F; IL1 $\beta$  = interleukin 1 beta; IL8 = interleukin-1; LAB = lactic-acid producing bacteria; LPS = lipopolysaccharide; MDA = malondialdehyde; p38MAPK = phosphokinase p38; PBP = poultry by-products; SBM = soybean meal; SOD = superoxide dismutase; SPC = soy protein concentrate; TNF- $\alpha$  = tumour necrosis factor-alpha; TLR22 = toll-like receptor 22.

**Table 8. Effects of *Tenebrio molitor* larvae meal on antioxidant enzymes, immune parameters and resistance to bacterial diseases in crustacean and fish.<sup>1</sup>**

Animal [IBW-FBW, g] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Type and % of main protein in CTRL diet	% of substitution	Antioxidant enzymes	Immune parameters	Resistance to diseases	Reference
		CP	CL							
Pacific white shrimp ( <i>Litopenaeus vannamei</i> ) [1.5-6] {56}	TMM [DF]	74.8	12.6	5, 10, 15, 20	FM: 25%	25, 50, 75, 100		Increased phenoloxidase activity and total haemocyte counts; decreased bacterial number	Increased resistance to <i>Vibrio parahaemolyticus</i> (55; 75; 84; 87; 86)	Motte <i>et al.</i> (2019)
Mandarin fish ( <i>Siniperca scherzeri</i> ) [21-36] {56}	TMM [FF]	52.5	34.1	10, 20, 30	FM: 65%	11, 22, 32	GPx increased at 30%; SOD not affected	Reduced cholesterol et 30%; lysozyme increased, significantly at 30%; myeloperoxidase not affected		Sankian <i>et al.</i> (2018)
Yellow catfish ( <i>Pelteobagrus fulvidraco</i> ) [10-no data] {35}	TMM [-]			9, 18, 27	FM: 24%	25, 50, 75	Lower MDA; increased plasma SOD	Enhanced immune (lysozyme, IgM); up- regulation of hepcidin, MHC II and IL2	Enhanced resistance to <i>Edwardsiella ictaluri</i> (67; 70; 74; 87%)	Su <i>et al.</i> (2017)
Rainbow trout ( <i>Oncorhynchus mykiss</i> ) [116-314] {90}	TMM [FF]	51.9	23.6	25, 50	FM: 75%	35, 66	increase of intestinal SOD, CAT, G6DP; reduced MDA	More rapid antibacterial activity of complement and increased myeloperoxidase and anti-protease activities; no effect on inflammation or lysozyme activity		Henry <i>et al.</i> (2018b); Belforti <i>et al.</i> (2015)
Sea trout ( <i>Salmo trutta</i> ) [5-21] {56}	TMM [hydrolysed]	47	29.6	10	FM: 25%	58		No change in IgM or lysozyme activity		Mikolajczak <i>et al.</i> (2020)
European sea bass ( <i>Dicentrarchus labrax</i> ) [65] {42}	TMM [FF]	51.9	23.6	25	FM: 70%	35		Decreased inflammatory and stress- related response (myeloperoxidase and nitric oxide); increased anti-protease activity. No effect on lysozyme or complement		Henry <i>et al.</i> (2018a,b); Gasco <i>et al.</i> (2016)
Pearl gentian groupers ( <i>Epinephelus lanceolatus</i> × <i>E. fuscoguttatus</i> ) [7-67] {50}	TMM [DF]	65	3	2.5, 5, 7.5, 10, 12.5	FM: 40%	6, 12, 18, 25, 31	decreased SOD and MDA at 7.5%, increased GR at 2.5%		Better survival to <i>Vibrio harveyi</i> with 7.5% TMM (35; 33; 30; 50; 10;5%)	Song <i>et al.</i> (2018)
Red seabream ( <i>Pagrus major</i> ) [24-34] {56}	TMM [DF]	76.5	5.3	5, 10	FM: 50%	10, 20	SOD not affected		Increased survival to <i>Edwardsiella tarda</i> (21; 55; 67%)	Ido <i>et al.</i> (2019)

<sup>1</sup> CAT = catalase; CL = crude lipid; CP = crude protein; CTRL = control diet; DF = defatted; FBW = final body weight; FF = full fat; FM = fishmeal; GPx = glutathione peroxidase; GR = glutathione reductase; G6DP = anti oxidative enzyme; IBW = initial body weight; IgM = immunoglobulin M; IL2 = interleukin-2; MDA = malondialdehyde; MHC = major histocompatibility complex gene; SOD = superoxide dismutase; TMM = *Tenebrio molitor* larvae meal.



**Table 9. Effects of *Bombyx mori*, *Musca domestica*, Chironomid meals and *Zophoba morio* on antioxidant enzymes, immune parameters and resistance to bacterial diseases in crustacean and fish.<sup>1</sup>**

Animal [IBW-FBW, g] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Type and % of main protein in CTRL diet	% of substitution	Antioxidant enzymes	Immune parameters	Resistance to diseases	Reference
		CP	CL							
Prawns ( <i>Litopenaeus vannamei</i> ) [0.6-2.4] {30}	BM			0.0125, 0.25, 5 µg/g	FM: 24.5%				Increased survival to <i>Vibrio penaeicida</i> (0; 90; 89.5; 100)	Ali et al. (2018)
Prawns ( <i>Marsupenaeus japonicus</i> ) [0.9-1.4] {15}	BM [DF]			0.001, 0.01, 0.1	FM: 20%				Increased survival to <i>V. penaeicida</i> (0; 73; 77; 76)	
Asian swamp eel ( <i>Monopterus albus</i> ) [27-31] {56}	MDL [whole]	64	24.3	1 day every 3 (1d/3) or 7 days (1d/7)	FM: 45%			HSP70 and IL1β downregulated, no difference in IgM	Better resistance to <i>Aeromonas hydrophila</i> (73; 93 for 1d/3; 83% for 1d/7)	Xiang et al. (2019)
Asian swamp eel ( <i>M. albus</i> ) [30-58] {40}	MDL [DF]	60	2.6	10, 20, 30	70% earthworm	not isonitrogenous and isolipidic	Increased anti-oxidant enzymes (SOD, GPx, CAT)	Increased lysozyme, acid and alkaline phosphatase; lower serum triglycerides	Increased survival to <i>A. hydrophila</i> (30; 37.5; 45; 37.5)	Pei et al. (2019)
Black carp ( <i>Mylopharyngodon piceus</i> ) [72-no data] {60}	MDL [-]			2.5	together with 150 mg/kg carnitine		Increased serum GPx and liver SOD,GPx and CAT; reduced MDA	Increased lysozyme and complement	Improved resistance to <i>A. hydrophila</i>	Ming et al. (2013)
Red seabream ( <i>Pagrus major</i> ) [26-no data] {60}	MD pupae [FF]	50.6	21.9	5	FM: 50%	10			Increased resistance to <i>Edwardsiella tarda</i> (0; 100%)	Ido et al. (2015)
Red seabream ( <i>P. major</i> ) [48-no data]{10}				0.75, 7.5		1.5, 15		Increased phagocytic activity		
Rainbow trout ( <i>Oncorhynchus mykiss</i> ) [0.13-0.65] {28}	ChL [whole]	66	9	100	FM: 12.4%			Higher proliferation and apoptotic indexes in the proximal intestine, no difference in distal intestine		Ostaszewska et al. (2011)
Sea trout ( <i>Salmo trutta</i> ) [5-21] {56}	ZMM [hydrolysed]	49.3	33.6	10	FM:25%	56		No change in IgM or lysozyme activity		Mikolajczak et al. (2020)

<sup>1</sup> BM = *Bombyx mori*; CAT = catalase; ChL = Chironomidae frozen larvae; CL = crude lipid; CP = crude protein; CTRL = control diet; DF = defatted; FBW = final body weight; FF = full fat; FM = fishmeal; GPx = glutathione peroxidase; HSP70 = heat shock proteins 70; IBW = initial body weight; IgM = immunoglobulin M; IL1β = interleukin 1 beta; MDA = malondialdehyde; MDL = *Musca domestica* larvae meal; SOD = superoxide dismutase; ZMM = *Zophoba morio* larvae meal.

shrimp, *Litopenaeus vannamei* (Motte *et al.*, 2019), and dietary HI increased lysozyme, total haemocyte counts and cytokine expression in the intestine of crayfish marron (*C. cainii*) (Foysal *et al.*, 2019). In fish, only two studies have shown no effect of dietary insects on the immune system of fish (Mikołajczak *et al.*, 2020; Wang *et al.*, 2019). All other studies, concerning dietary inclusion of whole chironomid larvae or meals from HI, TM or MD, showed modulation of the fish immune system through a more rapid or increased antibacterial complement activity (Henry *et al.*, 2018b; Ming *et al.*, 2013), increased lysozyme (Ming *et al.*, 2013; Pei *et al.*, 2019; Sankian *et al.*, 2018; Su *et al.*, 2017), increased phagocytic activity (Ido *et al.*, 2015) and increased myeloperoxidase (MPO) activity in rainbow trout (Henry *et al.*, 2018b), but decreased MPO in European sea bass (Henry *et al.*, 2018a), increased trypsin-inhibition (Henry *et al.*, 2018a,b), increased alkaline phosphatase (Pei *et al.*, 2019), increased IgM titres (Su *et al.*, 2017), increased proliferation and apoptotic indexes of the proximal intestine (Ostazewska *et al.*, 2011) and stimulation of T-cells (Li *et al.*, 2019). As for the expression of the genes involved in immunity, and cytokines in particular, dietary insects induced an up-regulation of hepcidin, major histocompatibility complex II (Su *et al.*, 2017), HSP70 (Cardinaletti *et al.*, 2019; Li *et al.*, 2017a; Zarantoniello *et al.*, 2018) pro-inflammatory cytokines, such as interleukin (IL)1 $\beta$ , IL17F, tumour necrosis factor (TNF) $\alpha$  and IL6 expression (Foysal *et al.*, 2019; Zarantoniello *et al.*, 2018) anti-inflammatory cytokines such as IL10 (Foysal *et al.*, 2019), IL2, a cytokine which contributes to the differentiation of T-cells (Su *et al.*, 2017), while HSP-70 and the pro-inflammatory cytokine IL1 $\beta$  were down-regulated in swamp eel (Xiang *et al.*, 2019). Some studies have shown no effect of dietary insects on inflammation or on the expression of inflammation-related cytokines (Henry *et al.*, 2018b; Li *et al.*, 2020; Stenberg *et al.*, 2019), but inflammation has been observed to decrease in European sea bass, as suggested by the ceruloplasmin activity (Henry *et al.*, 2018a). Dietary insects certainly affect the cytokines related to the inflammatory response, but this effect was not found to be consistent between fish species, and it is somewhat difficult to define their precise role. It has been suggested that chitin acts as a pathogen-associated molecular patterns by binding to pathogens recognition receptors and stimulating the production of several cytokines and immune mediators (Stenberg *et al.*, 2019). Regarding the anti-oxidant and stress-response, an *in vitro* study showed that HI down-regulated the bacteria triggered in head-kidney cells of Atlantic salmon (Stenberg *et al.*, 2019). Dietary insects have also been shown to modulate the hepatic or intestinal antioxidant response of fish (Caimi *et al.*, 2020; Elia *et al.*, 2018; Henry *et al.*, 2018b; Ming *et al.*, 2013; Pei *et al.*, 2019; Sankian *et al.*, 2018; Song *et al.*, 2018; Su *et al.*, 2017) and to increase the detoxification capacity in the distal intestine of fish, as suggested by the elevated expression of the Cyp1a1 gene in

the intestine of Atlantic salmon (Li *et al.*, 2019). However, this may be linked to potentially higher concentrations of heavy metals in IM, and the absence of effects on toxicity indicators (cell apoptosis or tissue regeneration) or on gut histology tended to infirm this hypothesis (Li *et al.*, 2020).

## Poultry

Diet is one of the factor affecting the immunological mechanism of poultry (Kaiser and Balic, 2015). Immunological effects of poultry fed insects products are reported in Table 10.

A recent study in broiler chickens showed that small amounts (0.2 and 0.3%) of TM and ZM significantly decreased IgM levels but up-regulated IL-2. Both dietary insects also decreased the weight of Bursa of Fabricius associated with the differentiation of B-lymphocytes. (Benzertiha *et al.*, 2020). Moreover, insect oil extracted from both TM and ZM affected the expression of selected genes involved in immunological homeostasis, such as GIMAP5, a key regulator of hematopoietic integrity and lymphocyte homeostasis, Kierończyk *et al.* (2018).

The ingestion of low levels of HI (1, 2 and 3%) also immunostimulated broilers by increasing the CD4+ T lymphocyte counts, serum lysozyme activity, spleen lymphocyte proliferation and phagocytic activity in a dose-dependent manner (Lee *et al.*, 2018).

Blood analyses of birds fed insect meals have shown that levels corresponding to about 1 g/d of ingested chitin increased the globulin levels and decreased the albumin to globulin ratio indicating a better immune response (Bovera *et al.*, 2015, 2018; Marono *et al.*, 2017). Feeding laying hens with defatted HI also reduced triglycerides and cholesterol levels (Bovera *et al.*, 2018). On the other hand, the inclusion of HI meal in jumbo quail diets increased the albumin/globulin ratio, which may have compromised the birds' immunity and caused plasma disorders (Mbhele *et al.*, 2019). However, other researchers did not show any differences in blood traits following the ingestion of insect meals, thus suggesting the need to further study the mechanism of action (Biasato *et al.*, 2016, 2017, 2018b; Dabbou *et al.*, 2018; Elahi *et al.*, 2020).

The partial substitution of SBO by HIL fat down-regulated the pro-inflammatory TNF- $\alpha$ , while the total substitution reduced the proliferation of potentially pathogenic bacteria (i.e. Enterobacteriaceae spp.) and down-regulated the pro-inflammatory IL-6, thereby supporting the immune response of turkeys (Sypniewski *et al.*, 2020). Dietary HIF however showed no effect on broiler chicken haematological traits (Schiavone *et al.*, 2018).

Table 10. Immunological status of poultry fed insect products.<sup>1</sup>

Poultry species [IBW-FBW, kg] {no. days}	Insect species and form	Insect composition (% DM)		% insect inclusion	Immunological status	References
		CP	CL			
Jumbo quails [no data-223] {42}	HIL [DF]	55.5	11.1	2.5, 5, 7.5, 10	Increase of albumin/globulin ratio.	Mbhele <i>et al.</i> (2019)
Laying hens (Hy-line Brown) [1,410-1,857] {140}	HIL [DF]	60.0	9.0	7.3, 14.6	Decrease in albumin/globulin ratio. Decrease in cholesterol and triglycerides levels.	Bovera <i>et al.</i> (2018)
Laying hens Lohmann Brown Classic [1,790-1,890] {140}	HIL [DF]	62.7	4.7	17	Increase level of globulin. Decrease albumin/globulin ratio.	Marono <i>et al.</i> (2017)
Broiler chickens (Ross) [no data-1,300] {30}	HIL [FF]	no data	no data	1, 2, 3	Increase in frequency of CD4+ T lymphocyte, serum lysozyme activity, and spleen lymphocyte proliferation.	Lee <i>et al.</i> (2018)
Broiler chickens (Ross 308) [70,7-2,554] {35} [40,2-2,278] {35}	HIL [DF]	55.3	18.0	5, 10, 15	No differences in haematological traits. No differences in histopathological examination.	Dabbou <i>et al.</i> (2018); Biasato <i>et al.</i> (2020a)
Muscovy ducks ( <i>Cairina moschata domestica</i> ) [70,4-2,554] {47}	HIL [DF]	56.7	10.7	3, 6, 9	No influence on haematological traits.	Gariglio <i>et al.</i> (2019)
Broiler chickens (Ross 308) [42-2,104] {35}	TMM [FF]	47.0	29.6	0.2, 0.3	Significantly decreased IgM levels. Significant increase of IL-2 and TNF- $\alpha$ at 0.3% TMM. No significant changes in level of IgY, IgA, and IL-6. Decreased of Bursa of Fabricius	Benzertih <i>et al.</i> (2020)
Broiler chickens (Shaver brown) [1,760-3,470] {32}	TMM [FF]	55.3	23.0	30	Decreased albumin/globulin ratio- Increased aspartate aminotransferase and alanine aminotransferase.	Bovera <i>et al.</i> (2015)
Broiler chickens (Ross 708) [no data-2,309] {40}	TMM [FF]	55.3	25.1	5, 10, 15	Correlation between increasing TMM and changes in the blood and serum parameters: increased erythrocytes level and albumin, while the gamma glutamyl transferase decreased.	Biasato <i>et al.</i> (2017)
Broiler chickens (Ross 308) [42-2,253/2,273] {42}	TMM [FF] fresh	20.15	11.49	10.48	No differences in haematological traits.	Elahi <i>et al.</i> (2020)
	TMM [FF] dried	52.89	30.05	2, 4, 8		
Broiler chickens (Ross 708) [no data-3,641] {53}	TMM [FF]	57.5	30.7	5, 10, 15	No differences in histopathological examination. Histopathological alterations varied from absent/minimal to severe in spleen, thymus, bursa of Fabricius and liver for each dietary treatment.	Biasato <i>et al.</i> (2018b)
Broiler chickens (Ross 308) [42-2,122] {35}	ZMM [FF]	49.3	33.6	0.2, 0.3	Significant decrease of IgY at 0.2% ZMM, IgM at 0.2 and 0.3%ZMM. Significant increase of IL2. No significant changes in level of IgA and TNF- $\alpha$ . Decreased of Bursa of Fabricius.	Benzertih <i>et al.</i> (2020)
Broiler chickens (Ross 308) [42-1,555/1,566] {28}	TMO, ZMO	–	–	5	Liver: ZMO diet: the <i>APOA1</i> gene was upregulated; TMO diet: the <i>HNF4A</i> gene was downregulated; ZMO and TMO diets: the <i>GIMPA5</i> gene was significantly downregulated.	Kieronczyk <i>et al.</i> (2018)
Broiler chickens (Ross 308) [817.8-3,751.3] {27}	HIF	–	–	3.43, 6.87	No influence in haematological traits.	Schiavone <i>et al.</i> (2018)
Turkey (B.U.T. 6, AVIAGEN) [179-1,398] {35}	HIF	–	–	2.5, 5	Partial substitution of SBO: reduction of TNF- $\alpha$ concentration. Total substitution of SBO: reduction of IL-6.	Sypniewski <i>et al.</i> (2020)

<sup>1</sup> APOA1 = apolipoprotein A1; CF = crude fat; CP = crude protein; DF = defatted; DM = dry matter; FBW = final body weight; FF = full fat; GIMPA5 = guanosine triphosphatases of the immune-associated protein 5; HIL = *Hermetia illucens* larvae meal; HIF = *Hermetia illucens* fat; HNF4A = hepatocyte nuclear factor 4 alpha; IBW = initial body weight; IgA = immunoglobulin A; IgM = immunoglobulin M; IgY = immunoglobulin Y; IL-2 = interleukin-2; IL-6 = interleukin-6; SBO = soybean oil; TMM = *Tenebrio molitor* larvae meal; TMO = *Tenebrio molitor* oil; TNF = tumour necrosis factor-alpha; ZMM = *Zophobas morio* larvae meal; ZMO = *Zophobas morio* oil.

## Pigs

Immunological effects of pigs and rabbits fed insect products are reported in Table 11. Dietary HI larvae meal at levels varying between 3.5 and 10% had no effect on the haematological parameters of piglets (Biasato *et al.*, 2019; Driemeyer, 2016). Indeed, these authors reported no major effects on the blood profile parameters, all of which fell within the physiological range for pigs, although a linear increase in monocytes and a quadratic response of neutrophils were observed (Biasato *et al.*, 2019b). Dietary TM had no effect on the blood profile or immune system of weaned piglets (Ao *et al.*, 2020). At dietary levels up to 6%, TM meal did not affect immunoglobulin G (IgG) and immunoglobulin A (IgA) titres, but increased the serum insulin-like growth factor (Jin *et al.*, 2016).

An increase in neutrophils was found in growing pigs fed diets with increasing levels of HI full fat larvae meal in

partial and total substitution of FM (Chia *et al.* 2019), thus suggesting an immunostimulating activity of dietary HI. The authors ascribed the antimicrobial response to the high level of lauric acid in HI larva meal and speculated an increased protection effect against pathogens such as bacteria, viruses and fungi.

The beneficial effect of full fat HI meal (2 and 4%) dietary inclusion on the immune homeostasis of piglets is linked to the increase in serum anti-inflammatory cytokines IL-10 and IgA and simultaneous decrease in the pro-inflammatory cytokine IFN- $\gamma$  (Yu *et al.* 2020b).

*In vitro* MD extracts were shown to have a strong anti-inflammatory effect (induced by lipopolysaccharide) on mice macrophage cell line probably through low-molecular extracted components of the insect (Chu *et al.*, 2013).

**Table 11. Immunological status of pigs and rabbits fed insect products.<sup>1</sup>**

Animal [IBW-FBW, kg] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Type and % of main protein in CTRL	% substitution	Immunological status	Reference
		CP	CL					
Piglets [1.4-6.7] {28}	HIL [FF]	35.9	48.1	3.5	SBM FF: 12%; FM: 11.32%	SBM FF: 35%; FM: 35%	No effects on haematological parameters	Driemeyer (2016)
Weaning pigs [6.1-no data] {15}	HIL [FF]	42.01	42.13	4; 8	Toasted soybean: 12	50 and 100%	No effects on bacterial counts (Lactobacilli and D-Streptococci) in stomach, proximal small intestine and distal small intestine.	Spranghers <i>et al.</i> (2018)
	HIL [DF]	62.84	8.06	5.42		72%		
Weaning pigs [6.1-32.4] {61}	HIL [DF]	59.0	8.97	5; 10	SBM: 20% (phase I); 18.5% (phase II)	Phase I: 30 and 60%; Phase II: 32 and 65%	Increase in monocytes (linear response) and neutrophils (quadratic response)	Biasato <i>et al.</i> (2019b)
Weaning piglets [7.9-17.6] {28}	HIL [FF]	37.92	38.48	1, 2, 4	FM: 4%	25, 50 and 100%	Decrease in serum cytokines (IFN- $\gamma$ ) (minimum in pigs fed 4% HIL). Increase in IgA (maximum in pigs fed 2% HIL). Increase in anti-inflammatory cytokine IL-10 in pig fed 2% of HIL	Yu <i>et al.</i> (2020b)
Growing pigs [18.3-53.2] {63}	HIL [FF]	46.6	–	9, 12, 14.5, 18.5	FM: 10%	FM: 25, 50, 75 and 100%	Increase in neutrophils	Chia <i>et al.</i> (2019)
Weaning piglets [6.9-21.4] {35}	TMM [FF]	58.0	31.65	1, 2	FM: 2%	50 and 100%	No effect on red blood cells, white blood cells, lymphocyte, total protein, blood urea nitrogen, insulin-like growth factor and IgG.	Ao <i>et al.</i> (2020)
Weaning pigs [8.0-from 17.8 to 20.2] {35}	TMM [FF]	43.27	32.93	1.5, 3.0, 4.5, 6.0	SMB: 35.1% (phase I); 27.3 (phase II)	Phase I: 5, 10, 15 and 20%; Phase II: 6, 12, 18, 24%	No effect on IgG and IgA. Increase in serum insulin-like growth factor.	Jin <i>et al.</i> (2016)

<sup>1</sup> CL = crude lipid; CP = crude protein; CTRL = control diet; DF = defatted; DM = dry matter; FBW = final body weight; FF = full fat; FM = fishmeal; HIL = *Hermetia illucens* larvae meal; IBW = initial body weight; IgA = immunoglobulin A; IgG = immunoglobulin G; SBM = soybean meal; TMM = *Tenebrio molitor* larvae meal.



#### 4. Antimicrobial effects and resistance to diseases

The natural biotope of insects is often infested with hostile microorganisms (Józefiak and Engberg, 2017). To protect themselves, insects produce a wide range of bioactive substances with anti-microbial activity. Thus, HI larvae grown on chicken manure have been shown to reduce the load of Gram negative potential pathogens in the substrate (Józefiak and Engberg, 2017). For example, insects produce many AMPs exhibiting activity against bacteria, fungi, parasites and viruses (Alvarez *et al.*, 2019; Dang *et al.*, 2010; Elhag *et al.*, 2017; Faruk *et al.*, 2016; Imamura *et al.* 1999; Józefiak and Engberg, 2017; Li *et al.*, 2017b; Moon *et al.*, 1994; Mylonakis *et al.*, 2016; Ohta *et al.*, 2014, 2016; Park *et al.*, 2014, 2015; Ravi *et al.*, 2011; Schuhmann *et al.* 2003; Vogel *et al.*, 2018; Wu *et al.* 2018). AMPs' mode of action is usually based on their cationic nature enabling them to form pores in the microbe cell membranes making them natural alternatives to medicinal treatments reducing the risk of developing microbial resistance (Zhang and Gallo, 2016). Moreover, peptides extracted from the larvae of MD and HI were shown to have an anti-tumoral activity (Sun *et al.*, 2014; Tian *et al.*, 2018).

Beside AMPs, FAs may have antimicrobial activities (Suresh *et al.* 2014). Insects are rich in SCFAs and medium chain FA, such as valeric (5:0), enanthic (7:0), caprylic (8:0), pelargonic (9:0), capric (10:0), myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1) and linoleic (18:2) which are known to have antifungal and antibacterial activity against both Gram-negative and Gram-positive bacteria (Urbanek *et al.*, 2012). Lauric acid (12:0) in particular has a strong impact on Gram-positive bacteria (Ankaku *et al.*, 2017; Dayrit, 2015).

Chitin and its deacetylated form, chitosan, have also been recognised as having an antimicrobial effect against bacteria, fungi and yeast (Benhabiles *et al.*, 2012; Shin *et al.* 2019). Their antibacterial activity relies on the interaction between the positively charged chitin/chitosan and the negatively charged microbial cell membranes (Goy *et al.*, 2009). Chitin also has a wound-healing action which could complement its immunostimulating and disease resistance promoting effect (Goy *et al.*, 2009).

The GIT of some carnivorous and omnivorous fish species show enzymatic activity. The chitinases produced by gastric glands and the pancreas are able to hydrolyse the glycosidic bonds of polysaccharides in the chitin of insects and crustaceans (Rangaswamy, 2006; Nogales-Mérida *et al.*, 2019). In general, fish and poultry produce chitinases and present GIT genetically adapted to consume insects, which have been part of their natural diet since ancient times and chitinase activity may increase in animals fed a chitin-rich diet (Nogales-Mérida *et al.*, 2019). Apart

from the enzymatic hydrolysis of chitin, chitinase may also be antioxidant and immunostimulant with a potential protective role against bacterial and parasitical infections (Di Rosa *et al.*, 2016; Ngo and Kim, 2014) and such an activity could also partly explain the improved resistance of animals to the bacterial diseases listed below.

#### Fish and crustaceans

The rich intestine microbial diversity shown in the first section of this review increases the competition with pathogens for nutrient and colonisation sites in the intestine, and thus may improve resistance to diseases (Cerezuela *et al.*, 2013). Moreover, the modulation of the immune system of fish and crustaceans, as a result of the inclusion of insects in their diets described in the previous section, has been translated in a remarkably improved resistance to bacterial diseases as shown in the few existing studies on dietary TM, MD or SWP listed in Table 8 and 9. To our knowledge, no study has been done so far on the effects of dietary HI on crustaceans or fish resistance to diseases. In Pacific white shrimps, *L. vannamei*, 10-20% dietary TM replacing 50-100% of FM drastically reduced *Vibrio parahaemolyticus*-induced mortality, from 45 to 13-16% (Motte *et al.*, 2019). Very low dietary doses of SWP, or silkrose purified from SWP, greatly increased the survival of prawns subjected to an immersion challenge with *Vibrio penaeicida*, from 0% for control diet-fed prawns to 73-77 and 90-100% for SWP- and silkrose-fed prawns, respectively (Ali *et al.*, 2018). In fish, 5 and 10% of dietary TM increased the survival of red seabream (*Pagrus major*) infected with *Edwardsiella tarda*, from 21% in the control fish to 55-67% in the insect-fed fish (Ido *et al.*, 2019). Moreover, 7.5% of dietary TM improved the resistance of pearl gentian grouper to *Vibrio harveyi*, but other levels of dietary inclusions either had no effect or even reduced the fish survival rate (Song *et al.*, 2018). The resistance of yellow catfish (*Pelteobagrus fulvidraco*) to *Edwardsiella ictaluri* was significantly improved by the addition of 27% of dietary TM (Su *et al.*, 2017). Dietary MD also improved the survival of swamp eel and black carp (*Mylopharyngodon piceus*) to *Aeromonas hydrophila* (Ming *et al.*, 2013; Pei *et al.*, 2019; Xiang *et al.*, 2019) and of red seabream (*P. major*) to *E. tarda* (Ido *et al.*, 2015). Because of the similarities between the exoskeletons of insects and parasites, it can be hypothesised that they share some pathogen-associated molecular patterns which may not only improve fish resistance to bacterial diseases as listed before but also resistance to parasitic or even fungal diseases. Although no such study has been undertaken with insect chitin, the closely related crustacean chitin and/or chitosan, have recently been shown to reduce the prevalence and intensity of monogenean in rohu, *Labeo rohita* (Kumar *et al.*, 2019) and to greatly increased survival of Indian major carp, *Cirrhina mrigala*, to 75-80%, compared to 10% in control-fed fish when infected with the aquatic fungus *Aphanomyces invadans* (Shanthi *et al.*, 2014). These results



taken together are very positive and suggest that dietary inclusion of insects may represent a healthy supplement for aquafeed of the future.

## Poultry

The immunostimulation of broiler chickens fed 3% HI discussed in the previous section was translated into an improved survival of chicken when experimentally infected with *Salmonella* Gallinarum (Lee *et al.*, 2018) (Table 12).

Islam and Yang (2017) showed that 0.4% of dietary full-fat TM or ZM decreased mortality and increased IgG and IgA levels in broiler chickens challenged with *Salmonella* and *E. coli*. The authors speculated that the chitin in TM and ZM larva meal had a probiotic effect that was able to act as a natural antibiotic (Table 12).

## Pigs and rabbits

The antimicrobial effects of HI fat on the microbiota of pigs has been investigated *in vitro* (Spranghers *et al.* 2018). The results showed a strong antibacterial effect against Lactobacilli and in particular against D-Streptococci, a Gram-positive bacteria genus that can cause severe damage in the pig industry. The authors did not report any effect against Gram-negative bacteria. The *in vitro* antibacterial activity of insect fats extracted from both HI and TM larvae were also investigated in rabbit against common Gram-positive and Gram-negative bacterial pathogenic strains (*Salmonella tiphymurium*, *Salmonella enteritidis*, *Yersinia enterocolitica*, *Pasteurella multocida* and *Listeria monocytogenes*) (Dabbou *et al.*, 2020). These bacteria are foodborne pathogens posing public health concern (De Cesare *et al.* 2017; Kylie *et al.* 2017; Massacci *et al.* 2018; Rodriguez-Calleja *et al.* 2006). The growth of

*Y. enterocolitica*, *L. monocytogenes* and *P. multocida* was impaired by HI fat, while TM fat only inhibited the growth of *P. multocida* (Table 13).

*In vivo*, Ji *et al.* (2016) suggested a positive effect of AMPs from insects and reported a decrease in the incidence of diarrhoea in weaning piglets fed 5% of TM, MD or ZM in total substitution of plasma protein meal. However, this effect was not found by Yu *et al.* (2020b) who fed piglets with up to 4% of full fat HI meal (Table 13).

## 5. Conclusions

Dietary insects can successfully be used to partially replace conventional protein sources (FM, SBM) without affecting the growth of the animals. However, the lack of large-scale production units (with the exception of housefly) and legislation uncertainties have resulted in high prices and limited amounts of available quantities, both of which currently impair their massive use in animal nutrition. However, the exoskeleton of insects resembles that of parasites, and insects may share pathogen-associated molecular patterns with bacterial or parasitological fish pathogens. They are also rich in short- and medium-chain fatty acids and in chitin, and produce polysaccharides and peptides, all of which may have antimicrobial and/or immunostimulating activity. The immunostimulation and drastically improved disease resistance highlighted in the present publication (particularly in crustacean and fish studies) suggest that low dietary levels of insects may represent a potent supplement providing 'healthy animal feeds' or 'natural non-specific oral vaccines' to boost the animal immune system. They could be used as a 'preventive cure' before stressful events, such as transport or seasonal temperature changes, which are usually linked to a rise of infection risk.

**Table 12. Antimicrobial effects and resistance to diseases induced by insect products in poultry.<sup>1</sup>**

Poultry species [IBW-FBW, kg] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Antimicrobial and diseases resistance	References
		CP	CL			
Broiler chickens (Ross) [no data-1,300] {30}	HIL [FF]	–	–	1, 2, 3	Reinforced bacterial clearance and increased survival against <i>Salmonella</i> Gallinarum. Prophylactic properties: Reduced bacterial burden against <i>S. Gallinarum</i> . Increased survival rate of chicken experimentally infected with <i>S. Gallinarum</i> in 3% HIL group.	Lee <i>et al.</i> (2018)
Broiler chickens (Ross 308) [39.3-74.8] {7}	TMM [FF]	27.26	11.50	0.4%	Insect meals were fermented with <i>Lactobacillus plantarum</i> and <i>Saccharomyces cerevisiae</i> to form probiotics. Increase in survival rate in birds challenged with <i>Escherichia coli</i> and <i>Salmonella</i> spp.	Islam and Yang (2017)
	ZMM [FF]	27.15	8.70			

<sup>1</sup> CL = crude lipid; CP = crude protein; DM = dry matter; FBW = final body weight; FF = full fat; HIL = *Hermetia illucens* larvae meal; IBW = initial body weight; TMM = *Tenebrio molitor* meal; ZMM = *Zophoba morio* larvae meal.

**Table 13. Antimicrobial effects and resistance to diseases induced by insect products in pigs and rabbits.<sup>1</sup>**

Animal [IBW-FBW, kg] {no. days}	Insect [form]	Insect composition (% DM)		% insect inclusion	Type and % of main protein in CTRL	% substitution	Antimicrobial and diseases resistance	Reference
		CP	CL					
<b><i>in vivo trial</i></b>								
Weaning pigs [4.7-no data] {56}	TMM [FF]	50.2	29	5	Plasma protein powder: 5%	100%	Decrease in diarrhoea rate between 15 and 28 days of trial	Ji <i>et al.</i> (2016)
	MDL [FF]	45.6	30.1					
	ZMM [FF]	45.1	41.7					
<b><i>in vitro trials</i></b>								
Weaning pigs [6.1-no data] {15}	HIF	Addition of 0.20, 0.50, 1.00, or 1.50 g/100 mL of HIF (corresponding to 0.1, 0.29, 0.58 and 0.87% C12:0) to an incubation medium containing synthetic diet + phosphate buffer (pH 5) + a microbial inoculum from one donor piglet					0.58% had a strong <i>in vitro</i> effect against D-streptococci	Sprangers <i>et al.</i> (2018)
Rabbits [1.05-2.9] {41}	HIF TMO			0.75, 1.5	SBO: 1.5%	50 and 100%	<i>In vitro</i> inhibitory effect of HIO against <i>Yersinia enterocolitica</i> , <i>Pasteurella multocida</i> and <i>Listeria monocytogenes</i> and of TMO against <i>P. multocida</i>	Dabbou <i>et al.</i> (2020)

<sup>1</sup> CL = crude lipid; CP = crude protein; CTRL = control diet; DM = dry matter; FBW = final body weight; FF = full fat; HIF = *Hermetia illucens* fat; IBW = initial body weight; MDL = *Musca domestica* larvae meal; TMM = *Tenebrio molitor* larvae meal; SBO = soybean oil; TMO = *Tenebrio molitor* oil; ZMM = *Zophoba morio* larvae meal.

Further studies will have to determine for each insect species with a potential for mass production, doses and duration of administration and the range of diseases protection offered to aquatic and terrestrial farmed animals.

## Conflict of interest

The authors declare no conflict of interest.

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