

Gut microbiota changes in insect-fed monogastric species: state-of-the-art and future perspectives

Ilaria Biasato,[†] Laura Gasco,[†] Achille Schiavone,[‡] Maria Teresa Capucchio,[‡] and Ilario Ferrocino[†]

[†]Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Paolo Braccini 2, Grugliasco (TO), 10095, Italy

[‡]Department of Veterinary Sciences, University of Turin, Largo Paolo Braccini 2, Grugliasco (TO), 10095, Italy

Corresponding author: ilaria.biasato@unito.it

Implications

- Insects exert their influence on gut microbiota of monogastric animals by increasing microbiota alpha diversity, selecting bacteria able to produce short-chain fatty acids, reducing potential pathogens, and decreasing the nutrient digestibility.
- Gut microbiota of fish and pigs seems to respond better to the administration of insect-based diets, while that of poultry generally displays a more favorable outcome when fed low inclusion levels of insect meals.
- As most of research has only dealt with the characterization of gut microbiota of insect-fed animals, the “-omics” technologies appear to be fundamental to investigate the functional relevance of microbiota changes.

Key words: Insects, gut microbiota, microbiome, monogastric animals

Introduction

The intestinal microbiome can be defined as “the genes and genomes of the gut microbiota, as well as their products and the host environment” (Berg et al., 2020). Therefore, three major microbiome components can easily be unearthed, as the term “microbiome” does not only comprise the “assemblage of living microorganisms present in the intestinal environment” (the

so-called “microbiota”), but also its “theatre of activity”, which is the “collection of their genomes and genes” (the so-called “metagenome”) and the “whole spectrum of molecules produced by them, including their structural elements, metabolites, and molecules produced by coexisting hosts and structured by the surrounding gut conditions” (the so-called “metabolome”) (Berg et al., 2020). One of the main factors affecting the intestinal microbiome in either humans or animals is the diet, as feed nature and characteristics exert a significant influence on nutrient specificity and availability for microbiome members, thus, in turn, selecting/excluding taxa that are adept at/deficient in processing the available biomolecules. In particular, the relationship between diet and gut microbiome seems to have a key role in the animal production systems, as they represent two of the main components involved in the establishment and maintenance of a proper health status of the intestine, which is of vital importance to animal health and growth performance (Biasato et al., 2018). Nowadays, when choosing a diet for monogastric species, insects (especially *Hermetia illucens* [HI] and *Tenebrio molitor* [TM]) cannot be taken out of the picture, not only for their excellent nutritional profile, but also for their interesting nutraceutical components (i.e., chitin, antimicrobial peptides [AMPs], and lauric acid), which have recently been suggested to exert a primary influence on animal gut microbiota (Biasato et al., 2022). So far, most of the research dealing with the binomial “insects-animal gut” focused on the intestinal microbiota (on the bacterial composition only), with very few studies had started exploring the metagenome potential and metabolome as well. Microbiome study (applying various -omics technologies, such as metataxonomics, metagenomics, metaproteomics, metabolomics, and metatranscriptomics) can be used for an in-depth characterization of the complexity of the microbial ecosystems to highlight any shift related to dietary modifications. Indeed, the gut microbiome has an enormous functional potential for the host, as changes in dietary nutrients play a fundamental role in shaping the structure of the gut microbiome and, in turn, determining the inter-relationship between the latter and host. The present review

© Biasato, Gasco, Schiavone, Capucchio, Ferrocino

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
<https://doi.org/10.1093/af/vfad025>

aims to critically summarize—for the first time—the current knowledge about the intestinal microbiota (and microbiome, when available) of monogastric species intended for production purposes (poultry, fish, pigs, and rabbits) fed diets including or supplemented with insect-based products (meals, fats, and live larvae). In particular, a focus on the mode of action of insect-based products and the different species-specific response is herein provided, with final remarks about the future challenges and perspectives as well.

Mode of Action of Insects in Animal Gut

The insect-based products (mainly insect meals) seem to exert their influence on animal gut microbiota in four different ways (Figure 1):

- 1) Increase in microbiota alpha diversity, which can be attributable to the chitin fermentation (as recently suggested in humans [Refael et al., 2022]);
- 2) Selection of short-chain fatty acids (SCFAs)-producing bac-

teria, as a consequence of their ability to degrade the chitin (Borrelli et al., 2017; Rangel et al., 2022);

- 3) Reduction in pathogens, which can be related to the antimicrobial properties (i.e., chitin, AMPs, and lauric acid) of insects (Dabbou et al., 2021; Biasato et al., 2022);
- 4) Decrease in nutrient digestibility (especially crude protein [CP]), as a consequence of the chitin presence (Biasato et al., 2020a) or the use of full-fat meals (Basto et al., 2021).

Furthermore, a different species-specific response can be highlighted, as fish and pigs overall respond better to the administration of insect-based diets, while poultry generally displays a more favorable outcome when fed low inclusion levels of insect meals (i.e., 5–10%). Differently, data about gut microbiota changes in insect-fed rabbits are still too limited to include them in such scenario. The following sections and subsections will provide a rationale for all these aspects. Tables 1–4 will also summarize the main gut microbiota (and microbiome, when available) findings in the different monogastric species.

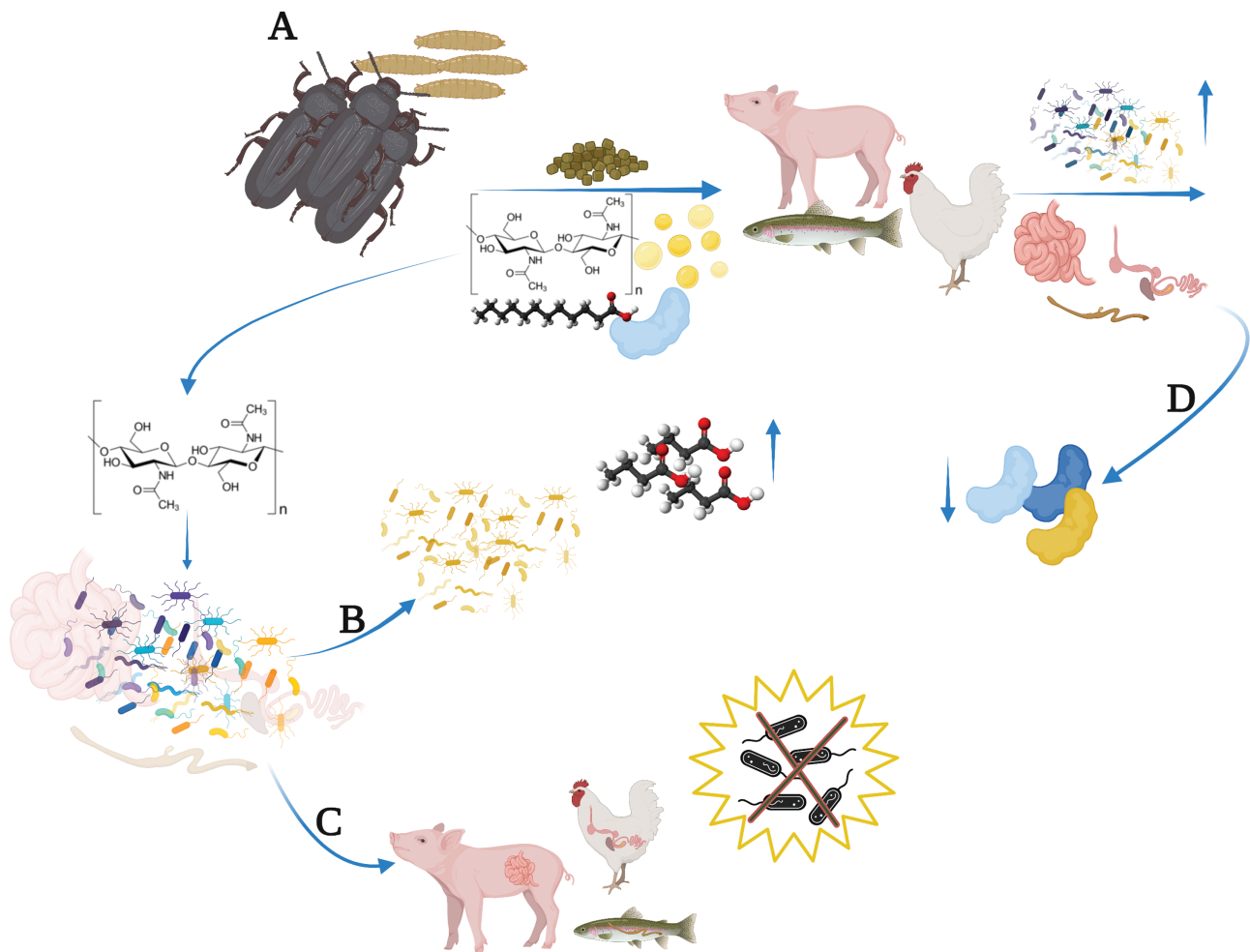


Figure 1. Graphical summary of the different modes of action of insects on the gastrointestinal tract of monogastric animals. **A)** Insects and their derivatives (chitin, lauric acid, proteins, and oils) increase microbiota richness and diversity. **B)** Chitin selects beneficial bacteria, and it is used as the main fermentation component to increase short chain fatty acids (SCFAs) in the gut. **C)** Antimicrobial compounds present in insects reduce the incidence of pathogenic bacteria in the gut. **D)** Insects decrease nutrient digestibility.

Table 1. Main intestinal microbiota and microbiome findings in insect-fed poultry

Poultry strain	Insect species, stage, and form	Inclusion levels	Intestinal segment	Main findings	Reference
Lohmann Brown Classic laying hens	Highly defatted HI larva meal	17%	Cecum (digesta)	<ul style="list-style-type: none"> • ↑alpha-diversity (Shannon index and number of observed species) • ↑<i>Bacteroides plebeius</i>, <i>Elusimicrobium minutum</i>, <i>Alkaliphilus transvaalensis</i>, <i>Christensenella minuta</i>, <i>Vallitalea guaymasensis</i>, and <i>Flavonifractor plautii</i> • ↑β-N-acetylhexosaminidases (K01207) and N-acetylglucosamine 6-phosphate deacetylase (K01443) • ↑SCFAs 	Borrelli et al. (2017)
Label Hubbard hybrid chickens	Full-fat TM larva meal	7.5%	Cecum (digesta)	<ul style="list-style-type: none"> • ↑alpha-diversity (Shannon index) • ↑Firmicutes and Firmicutes:Bacteroidetes ratio • ↓Bacteroidetes • ↑<i>Clostridium</i>, <i>Oscillospira</i>, <i>Ruminococcus</i>, <i>Coprococcus</i>, and <i>Sutterella</i> • ↓<i>Bacteroides</i> 	Biasato et al. (2018)
Ross 308 broiler chickens	Full-fat TM larva meal	5%, 10%, and 15%	Cecum (digesta)	<ul style="list-style-type: none"> • =alpha-diversity • ↓Firmicutes and Firmicutes:Bacteroidetes ratio (10 and 15% vs. 5%) • ↑<i>Clostridium</i>, <i>Alistipes</i>, and <i>Sutterella</i> • ↓<i>Ruminococcus</i> 	Biasato et al. (2019)
Julia laying hens	Full-fat HI larva and pupa meal	10%	Cecum (digesta)	<ul style="list-style-type: none"> • ↑alpha-diversity (Chao1 index) • ↓<i>Lactobacillus</i> and <i>Bifidobacterium</i> • =SCFAs 	Kawasaki et al. (2019)
Ross 308 broiler chickens	Partially defatted HI larva meal	5%, 10%, and 15%	Cecum (digesta)	<ul style="list-style-type: none"> • ↓alpha-diversity (Shannon index; 15% vs. control diet) • ↑Proteobacteria (15% vs. 10%) • ↑<i>L-Ruminococcus</i>, <i>Faecalibacterium</i>, <i>Blautia</i>, and <i>Clostridium</i> (5%) • ↑<i>Lactobacillus</i> and <i>Ruminococcus</i> (10%) • ↑<i>Bacteroides</i>, <i>Roseburia</i>, and <i>Helicobacter</i> (15%) 	Biasato et al. (2020b)
Ross 308 broiler chickens	Full-fat TM larva meal	5%, 10%, and 15%	Cecum (digesta)	<ul style="list-style-type: none"> • =alpha-diversity • ↓Firmicutes and Firmicutes:Bacteroidetes ratio (15% vs. control diet) • ↓<i>Clostridium</i>, <i>Coprococcus</i>, <i>L-Ruminococcus</i>, and <i>Ruminococcus</i> 	Biasato et al. (2020c)
Ross 308 broiler chickens	Live HI and TM larvae	5% of the expected daily feed intake (supplementation)	Cecum (digesta)	<ul style="list-style-type: none"> • =alpha-diversity • ↓Firmicutes and Firmicutes:Bacteroidetes ratio (15% vs. control diet) • ↑<i>Clostridium</i>, <i>Saccharibacteria</i>, and <i>Victivallaceae</i> (HI), and <i>Collinsella</i> (TM) 	Colombino et al. (2021)
Ross 308 broiler chickens	HI larva fat	0.16% and 0.29%	Cecum (digesta)	<ul style="list-style-type: none"> • =alpha-diversity • ↓<i>Clostridium</i> and <i>Corynebacterium</i> 	Dabbou et al. (2021)

Increase in microbiota alpha diversity

When comparing intestinal microbiotas, the quantification of the existing differences among groups can be performed at two levels: the alpha (within-sample) and beta (between-sample) diversity. Alpha diversity indices (such as phylogenetic diversity [PD], observed number of amplicon sequence variants, Chao1, Simpson, and Shannon) summarize the structure of a microbial community with respect to its richness (number of taxonomic groups) and/or evenness (distribution of abundances of the groups) (Kers and Saccenti, 2022). Including different levels of TM (5–15% [poultry], 18–25% [fish], and 5–10% [pigs]) and HI (5–17% [poultry], 8–60% [fish], and 5–30% [pigs]) meals—as well as oils (0.16–0.29% [poultry, HI], 1.5% [rabbits, TM and HI], and 6.24% [fish, HI])—in diets for monogastric species led to unaffected (Biasato et al., 2019, 2020a, 2020c;

Yu et al., 2019; Meyer et al., 2020; Colombino et al., 2021; Fabrikov et al., 2021; Håkenåsen et al., 2021; Jin et al., 2021; Terova et al., 2021; Rangel et al., 2022; Dabbou et al., 2020, 2021) or increased alpha diversity (in terms of higher Shannon [Borrelli et al., 2017; Biasato et al., 2018; Terova et al., 2019; Kar et al., 2021], Chao1 [Kawasaki et al., 2019; Terova et al., 2019; Biasato et al., 2022], and Simpson [Rimoldi et al., 2019; Terova et al., 2019] indices, and PD [Terova et al., 2019; Weththasinghe et al., 2022] and number of observed species [Borrelli et al., 2017; Terova et al., 2019; Rimoldi et al., 2021]) than that of animals fed the control diet. Even if limited microbial diversity may be desirable as not all microbes are beneficial, high α-diversity of gut microbiota is generally favorable for the overall health and productivity of production animals, as it helps in maintaining the stability of intestinal microbiota and determining the colonization resistance against invading

Table 2. Main intestinal microbiota and microbiome findings in insect-fed pigs

Pig strain	Insect species, stage, and form	Inclusion levels	Intestinal segment	Main findings	Reference
Finishing crossbred pigs	Full-fat HI larva meal	4% and 8%	Colon (digesta)	<ul style="list-style-type: none"> • =alpha diversity • ↑<i>Lactobacillus</i>, <i>Pseudobutyrvibrio</i>, <i>Roseburia</i>, and <i>Faecalibacterium</i>, and ↓<i>Streptococcus</i> (4% vs. control diet) • ↑butyrate and isobutyrate, and ↓total amines, cadaverine, tryptamine, phenol, p-cresol, and skatole (4% vs. control diet) 	Yu et al. (2019)
Weaned crossbred piglets	Partially defatted HI larva meal	5% and 10%	Cecum (digesta)	<ul style="list-style-type: none"> • =alpha diversity • ↑<i>Blautia</i>, <i>Chlamydia</i>, <i>Coprococcus</i>, <i>Eubacterium</i>, <i>Prevotella</i>, <i>Roseburia</i>, unclassified members of Ruminococcaceae, <i>Ruminococcus</i>, and <i>Staphylococcus</i> 	Biasato et al. (2020a)
Weaned crossbred piglets	Highly defatted TM larva meal	5% and 10%	Cecum (digesta)	<ul style="list-style-type: none"> • =alpha diversity • ↓Bacteroidetes (10% vs. control diet) • ↑<i>Bifidobacterium</i> and ↓<i>Coprococcus</i> and <i>Treponema</i> (10% vs. control diet) • ↑isobutyric, isovaleric, and valeric acids 	Meyer et al. (2020)
Weaned crossbred piglets	Full-fat HI larva meal	4.76%, 9.52%, and 19.06%	Jejunum and colon (digesta)	<ul style="list-style-type: none"> • =alpha diversity • ↓<i>Lactobacillus</i> (19.06% vs. control diet) in colon • =SCFAs 	Håkenåsen et al. (2021)
Weaned crossbred piglets	Full-fat HI larva meal	4% and 8%	Feces	<ul style="list-style-type: none"> • =alpha diversity • ↑<i>Lactobacillus</i> and ↓<i>Streptococcus</i> 	Jin et al. (2021)
Post-weaned crossbred piglets	Partially defatted HI larva meal	30.5%	Jejunum and ileum (digesta)	<ul style="list-style-type: none"> • ↑alpha-diversity (Shannon index; jejunum) • ↓Firmicutes (jejunum) • ↑Actinobacteria • ↓<i>Corynebacterium</i> 1, <i>Streptococcus</i> and <i>Sarcina</i> (jejunum) • ↑<i>Bifidobacterium</i> and ↓<i>Corynebacterium</i> 1 (ileum) 	Kar et al. (2021)

pathogens (Biasato et al., 2018). Refael et al. (2022) recently performed an in vitro oral, gastric and intestinal digestion of powders from crickets (*Acheta domesticus*), silkworm pupae (*Bombyx mori*) or isolated chitin and their subsequent fermentation in anaerobic bioreactors inoculated with human feces. Interestingly, they demonstrated that chitin alone supported an increase in Shannon index more than the whole insect powders, thus potentially suggesting its active role in the modulation of gut alpha diversity. However, considering that the abovementioned studies mainly characterized the gut microbiota rather than microbiome, further research is recommended to confirm this hypothesis in monogastric species as well.

Selection of short-chain fatty acids-producing bacteria

High Throughput 16S amplicon target sequencing (or metataxonomics) allows for identifying a potential microbial signature associated with the use of a specific diet or ingredient. Beta diversity metrics (such as Bray-Curtis dissimilarity, Jaccard, unweighted UniFrac, and weighted UniFrac) summarize the differences among intestinal microbiotas by considering sequence abundances or considering only the presence-absence of sequences (Kers and Saccenti, 2022). Significant changes in β -diversity and relative abundances of phyla have commonly been highlighted in monogastric animals after administering insect-based diets. At the highest taxonomic level, insect-fed birds may display higher Firmicutes and/

or lower Bacteroidetes and higher Firmicutes to Bacteroidetes ratios when compared to the control groups (Biasato et al., 2018). The identification of increased Firmicutes (Panteli et al., 2021; Rimoldi et al., 2021; Rangel et al., 2022; Weththasinghe et al., 2022) and decreased Proteobacteria (Rimoldi et al., 2019, 2021; Terova et al., 2019; 2021; Weththasinghe et al., 2022)—sometimes resulting in higher Firmicutes to Bacteroidetes and lower Proteobacteria to Bacteroidetes ratios (Panteli et al., 2021)—seem to be characteristics of fish species fed diets containing insect-based products, while Actinobacteria and Bacteroidetes phyla are, instead, less constant in their changes (Terova et al., 2019; Panteli et al., 2021; Biasato et al., 2022). Lastly, dietary insect meal inclusion in pigs usually leads to higher Actinobacteria (Meyer et al., 2020; Kar et al., 2021) and lower Firmicutes (Kar et al., 2021), as well as heterogeneous Bacteroidetes variations (Meyer et al., 2020; Håkenåsen et al., 2021). As an interesting aspect to consider, independently of the monogastric species, these phyla profiles are mainly driven by the selection of specific genera that are able to SCFAs, such as *Clostridium*, *Ruminococcus*, *Lactobacillus*, *Oscillospira*, *Coprococcus*, *Alistipes*, *Faecalibacterium*, *Blautia*, *Roseburia*, *Eubacterium*, and *Bifidobacterium* in poultry (Biasato et al., 2018, 2019, 2020b; Colombino et al., 2021), *Actinomyces*, *Bacillus*, *Enterococcus*, *Lactobacillus*, *Staphylococcus*, *Mycoplasma*, *Pediococcus*, and *Carnobacterium* in fish (Rimoldi et al., 2019, 2021; Terova et al., 2019; Biasato et al., 2022; Li et al., 2022; Rangel et al., 2022; Weththasinghe et al., 2022), *Bifidobacterium*, *Roseburia*, *Lactobacillus*, *Pseudobutyrvibrio*,

Table 3. Main intestinal microbiota and microbiome findings in insect-fed fish

Fish species	Insect species, stage, and form	Inclusion levels	Intestinal segment	Main findings	Reference
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Partially defatted HI larva meal	10%, 20%, and 30%	Whole intestine (mucosa)	<ul style="list-style-type: none"> • ≠alpha-diversity (↓Shannon and ↑Simpson indices; 20 and 30%) • ↑Tenericutes and ↓Proteobacteria (20 and 30% vs. control diet) • ↓<i>Shewanella</i>, <i>Citrobacter</i>, <i>Kluyvera</i>, and <i>Deefgea</i> (20% vs. control diet) • ↓<i>Aeromonas</i> (30% vs. control diet) 	Rimoldi et al. (2019)
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Partially defatted HI prepupa meal	10%, 20%, and 30%	Whole intestine (mucosa)	<ul style="list-style-type: none"> • ↑alpha-diversity (PD and Chao1, Shannon and Simpson indices and number of observed species) • ↑Actinobacteria • ↓Proteobacteria (20 and 30% vs. control diet) • ↑<i>Facklamia</i>, <i>Enterococcus</i>, <i>Lactobacillus</i> and <i>Pediococcus</i> 	Terova et al. (2019)
Gilthead seabream (<i>Sparus aurata</i>)	Full-fat HI and TM larva meal	10.9% and 18% (HI), 18% (TM)	Whole intestine (digesta)	<ul style="list-style-type: none"> • =alpha diversity • ↓<i>Weissella confusa</i>, <i>Streptococcus dysgalactiae</i>, and <i>Peptostreptococcus russellii</i> 	Fabrikov et al. (2021)
European seabass (<i>Dicentrarchus labrax</i>) and Gilthead seabream (<i>Sparus aurata</i>)	Full-fat HI, TM, and MD larva meal	19.5%	Midgut (mucosa)	<ul style="list-style-type: none"> • ↓alpha-diversity (Chao1 index; MD, seabream) • ↑Firmicutes to Bacteroidetes and ↓Proteobacteria to Bacteroidetes ratios (HI and MD, seabass) • ↑Firmicutes • ↑Actinobacteria (seabass) or ↓Actinobacteria (seabream) • ↑glycan biosynthesis and metabolism (HI and MD, seabass) • ↑carbohydrate metabolism (seabass) • ↑AA metabolism (TM, seabass) • ↑xenobiotics biodegradation and metabolism (HI and MD, seabream) • ↓energy metabolism (TM and MD, seabream) • ↓metabolism of cofactors and vitamins (TM and HI, seabream) • ↓lipid metabolism (HI, seabream) 	Panteli et al. (2021)
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Partially defatted HI larva meal	15%	Whole intestine (mucosa and digesta)	<ul style="list-style-type: none"> • ↑alpha-diversity (number of observed species) • ↑Firmicutes • ↓Proteobacteria • ↑<i>Lactobacillus</i> and <i>Bacillus</i> • ↓<i>Aeromonas</i> 	Rimoldi et al. (2021)
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Partially defatted TM larva meal	20%	Whole intestine (mucosa)	<ul style="list-style-type: none"> • =alpha-diversity • ↓Proteobacteria • ↓<i>Deefgea</i> 	Terova et al. (2021)
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Partially defatted HI larva meal	8%, 16%, and 32%	Posterior intestine (digesta)	<ul style="list-style-type: none"> • ≠alpha-diversity (↑Chao1 and ↓Shannon indices) • ↑Actinobacteria • ↑<i>Bacillus</i>, <i>Actinomyces</i>, <i>Staphylococcus</i>, <i>Enterococcus</i>, and <i>Oceanobacillus</i> • ↓<i>Campylobacter</i>, <i>Listeria</i>, <i>Vagococcus</i>, <i>Lactococcus</i>, <i>Lactobacillus</i>, <i>Pediococcus</i>, <i>Leuconostoc</i>, and <i>Weissella</i> 	Biasato et al. (2022)
Atlantic salmon (<i>Salmo salar</i>)	Partially defatted HI larva meal	60%	Anterior and posterior intestine (mucosa and digesta)	<ul style="list-style-type: none"> • ≠alpha-diversity (↓PD in digesta; ↑PD and ↓Shannon indices in mucosa) • ↑Bacillaceae 	Li et al. (2022)
European seabass (<i>Dicentrarchus labrax</i>)	Partially defatted HI and TM larva meal, and HI exuvia meal	25%	Whole intestine (mucosa and digesta)	<ul style="list-style-type: none"> • =alpha-diversity • ↑Firmicutes (HI, digesta) • ↑<i>Paenibacillus</i> (HI exuvia, digesta) • ↑chitinase ChiA-encoding genes 	Rangel et al. (2022)
Atlantic salmon (<i>Salmo salar</i>)	Full-fat, defatted, and de-chitinized HI larva meal, and HI oil	20.36%, 14.89% and 24.53%, and 6.24%	Posterior intestine (digesta)	<ul style="list-style-type: none"> • ≠alpha-diversity (↑PD and ↓Shannon index; full-fat vs control diet); • ↑Firmicutes • ↓Proteobacteria • ↑lactic acid bacteria and <i>Actinomyces</i> (meals) • ↑Bacillaceae (meals and oil) • ↑mucin O-glycan degradation (full-fat) • ↓lipopolysaccharide biosynthesis (full-fat vs control diet) • ↓vitamin metabolism (full-fat) 	Weththasinghe et al. (2022)

Table 4. Main intestinal microbiota and microbiome findings in insect-fed monogastric rabbits

Rabbit strain	Insect species, stage, and form	Inclusion levels	Intestinal segment	Main findings	Reference
Weaned rabbits	HI and TM fat	1.5%	Cecum(digesta)	<ul style="list-style-type: none"> • =alpha-diversity • ↑<i>Akkermansia</i> and <i>Ruminococcus</i> • =SCFAs 	Dabbou et al. (2021)

Clostridium, *Faecalibacterium*, *Blautia*, *Coprococcus*, *Eubacterium*, *Prevotella*, *Ruminococcus* and *Staphylococcus* in pigs (Yu et al., 2019; Biasato et al., 2020a; Meyer et al., 2020; Jin et al., 2021; Kar et al., 2021), and *Akkermansia* and *Ruminococcus* in rabbits (Dabbou et al., 2020). Chitin seems to be the preferred source of microbial fermentations, since it acts as a prebiotic and promotes, in turn, the selection of beneficial taxa in the gut microbiome. Indeed, at the lowest taxonomic level, *Alkaliphilus transvaalensis*, *Christensenella minuta*, and *Flavonifractor plautii* have previously been identified in the intestinal microbiome of HI-fed laying hens in correlation with the highest levels of KEGG genes responsible for the chitin degradation (β -N-acetylhexosaminidases [K01207] and N-acetylglucosamine 6-phosphate deacetylase [K01443]), which boosted the production of butyrate and propionate (Borrelli et al., 2017). Similarly, the intestinal microbiome of insect-fed European seabass has recently displayed a *Paenibacillus*-related increase in chitinase ChiA-encoding genes (Rangel et al., 2022), while increased SCFAs production (mainly butyrate, isobutyrate, valeric, and isovaleric) has also been reported in pigs fed insect-based diets (Yu et al., 2019; Meyer et al., 2020). The production of SCFAs—especially butyrate—is considered beneficial for the gut, as they can enhance the intestinal epithelial cell barrier function by acting as energy source for the enterocytes and stimulating goblet cell differentiation and mucus production, as well as reducing the enteric pathogens because of their antimicrobial properties (Biasato et al., 2018).

Reduction in pathogens

The administration of insect-based diets to monogastric species may also cause a reduction in pathogens in their gut microbiota, even if less research studies have reported this outcome when compared to the identification of the SCFAs-producing bacteria. Such a positive effect seems to be exclusively related to the use of HI (either the meal or the fat), as a consequence of the synergic activity of its three nutraceutical components—lauric acid, chitin, and AMPs—, which all concur with its antimicrobial properties (Biasato et al., 2022). In particular, a decrease in potentially pathogenic bacteria (such as *Corynebacterium*, *Streptococcus*, *Sarcina*, *Treponema*, *Aeromonas*, *Deefgea*, *Vagococcus*, and *Lactococcus*) can be highlighted in broiler chickens (Dabbou et al., 2021), pigs (Yu et al., 2019; Meyer et al., 2020; Jin et al., 2021; Kar et al., 2021) and rainbow trout (Rimoldi et al., 2019, 2021; Fabrikov et al., 2021; Terova et al., 2021; Biasato et al., 2022). Interestingly, a reduction in selected foodborne pathogens (such as *Listeria* and *Campylobacter*) has also recently been observed in HI-fed rainbow trout (Biasato et al., 2022). However, further studies

performing bacterial or parasitic challenges on monogastric species-fed insect-based products are mandatory to confirm this hypothesis.

Decrease in nutrient digestibility

Even if the use of insect-based products in monogastric species is mainly associated with positive outcomes in terms of intestinal microbiota modulation, some “side effects” can be pointed out as well, especially in poultry. Indeed, reduced alpha diversity, increased Proteobacteria, decreased Firmicutes and Firmicutes to Bacteroidetes ratios, selection of *Helicobacter*, and decreased SCFAs-producing bacteria (such as *Clostridium*, *Coprococcus*, and *Ruminococcus*) have been reported in broiler chickens fed HI- and TM-based diets (Biasato et al., 2019, 2020b, 2020c), with a reduction in *Lactobacillus* and *Bifidobacterium* being also observed in HI-fed laying hens (Kawasaki et al., 2019). On the one hand, these negative outcomes can reasonably be attributed to the chitin-related reduction in CP digestibility (Biasato et al., 2020b); on the other, the use of full-fat meals rather than defatted ones (mainly HI) may determine itself a reduction in CP digestibility (75.8% vs. 87.2% [Basto et al., 2021]). In both situations, the nondigested protein increases at the ileal level, thus leading to hindgut protein fermentation and, in turn, formation of toxic compounds potentially capable of creating a non-healthy gut environment (Biasato et al., 2020b).

Species-Specific Response

Even if the different insect species seem to exert a similar influence on the gut microbiota of monogastric species, it is possible to underline a different response of fish, pigs, and poultry to the administration of the insect-based products. As already mentioned before, while fish and pigs usually respond well to both low (5–10%) and high ($\geq 15\%$) inclusion levels of insect meals—with only very few minor negative effects being highlighted—, poultry species are less predisposed to eat diets containing more than 15% of insect meals. Indeed, chitin- or fat-related reduction in CP digestibility does not only negatively affect the bird intestinal microbiota (as previously discussed in “Decrease in nutrient digestibility” section), but also other gut health parameters, such as morphology (in terms of low villus height, high crypt depth, and reduced villus height to crypt depth ratio) and mucin dynamics (in terms of reduced mucin staining intensity) (Biasato et al., 2020b, 2020c). Interestingly, the negative modulation of the health status of the intestine seems to have direct repercussions on bird growth performance, as worsening in feed efficiency is also commonly observed (Biasato et al., 2020b, 2020c). Differently, gut microbiota of

selected fish species (sea bream, sea bass, rainbow trout, and Atlantic salmon) and pigs may occasionally display a reduction in selected alpha diversity metrics (Chao1 [Panteli et al., 2021] and Shannon [Rimoldi et al., 2019; Biasato et al., 2022; Li et al., 2022; Weththasinghe et al., 2022] indices, and PD [Li et al., 2022]) and SCFAs-producing bacteria (*Lactobacillus* [Håkenåsen et al., 2021] and *Coproccoccus* [Meyer et al., 2020]), and selection of potential pathogens such as *Chlamydia* (Biasato et al., 2020a). However, these negative findings have always been observed along with predominant, positive outcomes, as well as unaffected gut morphology and mucin dynamics, and preserved overall health status and animal growth performance (Biasato et al., 2020a, 2022). Despite the highest chitinase activity among the omnivorous monogastric species having previously been reported in chicken stomachs (Tabata et al., 2018), such dichotomy between fish-pigs and poultry can potentially be explained by the remarkable genetic selection broiler chickens and laying hens have faced in the last decades, which has made the current strains reasonably more prone to utilize the commercial feeds and progressively less used to consume insects in their feeding regime.

Future Perspectives

Insects are highly complex organisms and constitute a pool of potential, beneficial bioactive compounds that can support animal health and supply nutrients. In the present review, the authors pointed out how insect-based products can affect the gut microbiota composition in monogastric species. However, most of the studies are only focusing on bacterial composition, without considering fungi and virus, or the insect-related shift in their functions. The application of multi-omics study is needed to decipher how insects interact with the total microbiome and the effects on the host. The application of shotgun metagenomic sequencing, including culturomics study, to explore the potential mechanism of the gut microbiota interaction with insects is still an unexplored area. It is also fundamental to generate more information about the functional relevance of specific microbiota changes at a strain level, and this is something that has barely started to be explored. So far, the “-omics” approach has allowed identifying an increase in chitin digestion-related genes (Borrelli et al., 2017; Rangel et al., 2022), different regulations of carbohydrate, amino acid, lipid, vitamin, energy, and xenobiotics degradation metabolism genes (Borrelli et al., 2017; Yu et al., 2019; Meyer et al., 2020; Panteli et al., 2021; Rimoldi et al., 2021; Weththasinghe et al., 2022), and an upregulation in mucin degradation genes (Weththasinghe et al., 2022). As a second aspect to consider, as an high overlap between the microbiota associated with gut and feed has also previously been observed in fish-fed insect-based diets (Li et al., 2022; Weththasinghe et al., 2022), following the microbiomes in the different phases of the insect-based supply chains could help to explore how the insect-based feed microbiome (resulting from the interaction among rearing substrate, larvae, and derived products) may contribute to shape animal gut microbiome, in order to ensure the food safety, and to exclude a feed microbiome-related confounding

factor and identify the impact of the feed composition alone on the microbiome (Li et al., 2022). Furthermore, since insects could potentially be reared on different waste materials, monitoring of microbial hazards along the supply chain is still required. Within this scenario, next-generation sequencing can help obtaining a quite rapid overview of potential microbial hazards that can be transferred from the rearing substrate to the insects, the insect-fed animals and, lately, the final consumers. Potential risks associated with the horizontal gene transfer, including antimicrobial resistance genes and multigene islands, along the insect-based supply chain must also be considered, as the choice of the rearing substrate can create a selective pressure for microbes to acquire specific genes or functions. Lastly, considering that specific assemblages of microbes may potentially influence growth outcomes in insect-fed animals, it should be interesting to identify specific microbiome markers—especially at a strain level—that can be related to an increase or a decrease in the growth performance.

Conclusions

In conclusion, the present review critically analyzed the existing literature about the intestinal microbiota and microbiome in monogastric animals (fish, poultry, pigs, and rabbits) fed insect-based diets, identifying four different modes of action of insects in the animal gut (increase in microbiota alpha diversity, selection of SCFAs-producing bacteria, reduction in pathogens, and decrease in nutrient digestibility), as well as a different response in fish and pigs when compared to poultry (as a reasonable consequence of the genetic selection of the latter). The authors herein also suggest undertaking three different research pathways to improve the existing knowledge about the binomial “gut microbiome-insect-based feeds”: 1) to perform multi-omics studies (especially metagenomics and metabolomics on the intestinal content), 2) to characterize the whole impact of the insect-based feed microbiome on gut microbiome structure and functions (by adopting the Bayesian Source and/or strain tracking approach), and 3) to explore the relationship between microbiome and animal growth performance (by performing correlation and regression analyses).

References

- Basto, A., J. Caldach-Giner, B. Oliveira, L. Petit, T. Sá, M.R.G. Maia, S.C. Fonseca, E. Matos, J. Pérez-Sánchez, and L.M.P. Valente. 2021. The use of defatted *Tenebrio molitor* larvae meal as a main protein source is supported in European sea bass (*Dicentrarchus labrax*) by data on growth performance, lipid metabolism, and flesh quality. *Front. Physiol.* 12:659567. doi: 10.3389/fphys.2021.659567
- Berg, G., D. Rybakova, D. Fischer, T. Cernava, M.C. Vergès, T. Charles, X. Chen, L. Cocolin, K. Eversole, G.H. Corral, et al. 2020. Microbiome definition re-visited: old concepts and new challenges. *Microbiome* 8(1):103. doi: 10.1186/s40168-020-00875-0
- Biasato, I., G. Chemello, S. Bellezza Oddon, I. Ferrocino, M.R. Corvaglia, C. Caimi, A. Resconi, A. Paul, M. van Spankeren, M.T. Capucchio, et al. 2022. *Hermetia illucens* meal inclusion in low-fishmeal diets for rainbow trout (*Oncorhynchus mykiss*): effects on the growth performance,

- nutrient digestibility coefficients, selected gut health traits, and health status indices. *Anim. Feed Sci. Technol.* 290:115341. doi: [10.1016/j.anifeedsci.2022.115341](https://doi.org/10.1016/j.anifeedsci.2022.115341)
- Biasato, I., I. Ferrocino, E. Biasibetti, E. Grego, S. Dabbou, A. Sereno, F. Gai, L. Gasco, A. Schiavone, L. Cocolin, et al. 2018. Modulation of intestinal microbiota, morphology and mucin composition by dietary insect meal inclusion in free-range chickens. *BMC Vet. Res.* 14(1):383. doi: [10.1186/s12917-018-1690-y](https://doi.org/10.1186/s12917-018-1690-y)
- Biasato, I., I. Ferrocino, E. Colombino, F. Gai, A. Schiavone, L. Cocolin, V. Vincenti, M.T. Capucchio, and L. Gasco. 2020a. Effects of dietary *Hermetia illucens* meal inclusion on cecal microbiota and small intestinal mucin dynamics and infiltration with immune cells of weaned piglets. *J. Anim. Sci. Biotechnol.* 11:64. doi: [10.1186/s40104-020-00466-x](https://doi.org/10.1186/s40104-020-00466-x)
- Biasato, I., I. Ferrocino, S. Dabbou, R. Evangelista, F. Gai, L. Gasco, L. Cocolin, M.T. Capucchio, and A. Schiavone. 2020b. Black soldier fly and gut health in broiler chickens: insights into the relationship between cecal microbiota and intestinal mucin composition. *J. Anim. Sci. Biotechnol.* 11:11. doi: [10.1186/s40104-019-0413-y](https://doi.org/10.1186/s40104-019-0413-y)
- Biasato, I., I. Ferrocino, E. Grego, S. Dabbou, F. Gai, L. Gasco, L. Cocolin, M.T. Capucchio, and A. Schiavone. 2019. Gut microbiota and mucin composition in female broiler chickens fed diets including yellow mealworm (*Tenebrio molitor*, L.). *Animals.* 9(5):213. doi: [10.3390/ani9050213](https://doi.org/10.3390/ani9050213)
- Biasato, I., I. Ferrocino, E. Grego, S. Dabbou, F. Gai, L. Gasco, L. Cocolin, M.T. Capucchio, and A. Schiavone. 2020c. Yellow mealworm inclusion in diets for heavy-size broiler chickens: implications for intestinal microbiota and mucin dynamics. *Animals.* 10(10):1909. doi: [10.3390/ani10101909](https://doi.org/10.3390/ani10101909)
- Borrelli, L., L. Coretti, L. Dipineto, F. Bovera, F. Menna, L. Chiariotti, A. Nizza, F. Lembo, and A. Fioretti. 2017. Insect-based diet, a promising nutritional source, modulates gut microbiota composition and SCFAs production in laying hens. *Sci. Rep.* 7(1):16269. doi: [10.1038/s41598-017-16560-6](https://doi.org/10.1038/s41598-017-16560-6)
- Colombino, E., I. Biasato, I. Ferrocino, S. Bellezza Oddon, C. Caimi, M. Gariglio, S. Dabbou, M. Caramori, E. Battisti, S. Zanet, et al. 2021. Effect of insect live larvae as environmental enrichment on poultry gut health: gut mucin composition, microbiota and local immune response evaluation. *Animals.* 11(10):2819. doi: [10.3390/ani11102819](https://doi.org/10.3390/ani11102819)
- Dabbou, S., I. Ferrocino, L. Gasco, A. Schiavone, A. Trocino, G. Xiccato, A.C. Barroeta, S. Maione, D. Soglia, I. Biasato, et al. 2020. Antimicrobial effects of black soldier fly and yellow mealworm fats and their impact on gut microbiota of growing rabbits. *Animals.* 10(8):1292. doi: [10.3390/ani10081292](https://doi.org/10.3390/ani10081292)
- Dabbou, S., A. Lauwaerts, I. Ferrocino, I. Biasato, F. Sirri, M. Zampiga, S. Bergagna, G. Pagliasso, M. Gariglio, E. Colombino, et al. 2021. Modified black soldier fly larva fat in broiler diet: effects on performance, carcass traits, blood parameters, histomorphological features and gut microbiota. *Animals.* 11(6):1837. doi: [10.3390/ani11061837](https://doi.org/10.3390/ani11061837)
- Fabrikov, D., M.D.C. Vargas-García, F.G. Barroso, M.J. Sánchez-Muros, S.M. Cacia Ortíz, A.E. Morales, G. Cardenete, C. Tomás-Almenar, and F. Melenchón. 2021. Effect on intermediary metabolism and digestive parameters of the high substitution of fishmeal with insect meal in *sparus aurata* feed. *Insects.* 12(11):965. doi: [10.3390/insects12110965](https://doi.org/10.3390/insects12110965)
- Håkenåsen, I.M., G.H. Grepperud, J.O. Hansen, M. Øverland, R.M. Ånestad, and L.T. Mydland. 2021. Full-fat insect meal in pelleted diets for weaned piglets: effects on growth performance, nutrient digestibility, gastrointestinal function, and microbiota. *Anim. Feed Sci. Technol.* 281:115086. doi: [10.1016/j.anifeedsci.2021.115086](https://doi.org/10.1016/j.anifeedsci.2021.115086)
- Jin, X., B. Yuan, M. Liu, M. Zhu, X. Zhang, G. Xie, W. Wu, Z. Wang, H. Xu, Y. Lv, et al. 2021. Dietary *hermetia illucens* larvae replacement alleviates diarrhea and improves intestinal barrier function in weaned piglets challenged with enterotoxigenic *escherichia coli* K88. *Front. Vet. Sci.* 8:746224. doi: [10.3389/fvets.2021.746224](https://doi.org/10.3389/fvets.2021.746224)
- Kar, S.K., D. Schokker, A.C. Harms, L. Kruijft, M.A. Smits, and A.J.M. Jansman. 2021. Local intestinal microbiota response and systemic effects of feeding black soldier fly larvae to replace soybean meal in growing pigs. *Sci. Rep.* 11(1):15088. doi: [10.1038/s41598-021-94604-8](https://doi.org/10.1038/s41598-021-94604-8)
- Kawasaki, K., Y. Hashimoto, A. Hori, T. Kawasaki, H. Hirayasu, S.I. Iwase, A. Hashizume, A. Ido, C. Miura, T. Miura, et al. 2019. Evaluation of black soldier fly (*Hermetia illucens*) larvae and pre-pupae raised on household organic waste, as potential ingredients for poultry feed. *Animals.* 9(3):98. doi: [10.3390/ani9030098](https://doi.org/10.3390/ani9030098)
- Kers, J.G., and E. Saccenti E. 2022. The power of microbiome studies: some considerations on which alpha and beta metrics to use and how to report results. *Front. Microbiol.* 12:796025. doi: [10.3389/fmicb.2021.796025](https://doi.org/10.3389/fmicb.2021.796025)
- Li, Y., K. Gajardo, A. Jaramillo-Torres, T.M. Kortner, and A. Krogdahl. 2022. Consistent changes in the intestinal microbiota of Atlantic salmon fed insect meal diets. *Anim. Microbiome* 4(1):8. doi: [10.1186/s42523-021-00159-4](https://doi.org/10.1186/s42523-021-00159-4)
- Meyer, S., D.K. Gessner, G. Maheshwari, J. Röhrig, T. Friedhoff, E. Most, H. Zorn, R. Ringseis, and K. Eder. 2020. *Tenebrio molitor* larvae meal affects the cecal microbiota of growing pigs. *Animals.* 10(7):1151. doi: [10.3390/ani10071151](https://doi.org/10.3390/ani10071151)
- Panteli, N., M. Mastoraki, M. Lazarina, S. Chatzifotis, E. Mente, K.A. Kormas, and E. Antonopoulou. 2021. Configuration of gut microbiota structure and potential functionality in two teleosts under the influence of dietary insect meals. *Microorganisms.* 9(4):699. doi: [10.3390/microorganisms9040699](https://doi.org/10.3390/microorganisms9040699)
- Rangel, F., P. Enes, L. Gasco, F. Gai, B. Hausmann, D. Berry, A. Oliva-Teles, C.R. Serra, C.R.F.C. Pereira. 2022. Differential modulation of the european sea bass gut microbiota by distinct insect meals. *Front. Microbiol.* 13:831034. doi: [10.3389/fmicb.2022.831034](https://doi.org/10.3389/fmicb.2022.831034)
- Refael, G., H.T. Riess, C.S. Levi, F. Magzal, S. Tamir, O. Koren, and U. Lesmes. 2022. Responses of the human gut microbiota to physiologically digested insect powders or isolated chitin thereof. *Future Foods.* 6:100197. doi: [10.1016/j.fufo.2022.100197](https://doi.org/10.1016/j.fufo.2022.100197)
- Rimoldi, S., M. Antonini, L. Gasco, F. Moroni, and G. Terova. 2021. Intestinal microbial communities of rainbow trout (*Oncorhynchus mykiss*) may be improved by feeding a *Hermetia illucens* meal/low-fishmeal diet. *Fish Physiol. Biochem.* 47(2):365–380. doi: [10.1007/s10695-020-00918-1](https://doi.org/10.1007/s10695-020-00918-1)
- Rimoldi, S., E. Gini, F. Iannini, L. Gasco, and G. Terova. 2019. The effects of dietary insect meal from *hermetia illucens* prepupae on autochthonous gut microbiota of rainbow trout (*oncorhynchus mykiss*). *Animals.* 9(4):143. doi: [10.3390/ani9040143](https://doi.org/10.3390/ani9040143)
- Tabata, E., A. Kashimura, A. Kikuchi, H. Masuda, R. Miyahara, Y. Hiruma, S. Wakita, M. Ohno, M. Sakaguchi, Y. Sugahara, et al. 2018. Chitin digestibility is dependent on feeding behaviors, which determine acidic chitinase mRNA levels in mammalian and poultry stomachs. *Sci. Rep.* 8(1):1461. doi: [10.1038/s41598-018-19940-8](https://doi.org/10.1038/s41598-018-19940-8)
- Terova, G., E. Gini, L. Gasco, F. Moroni, M. Antonini, and S. Rimoldi. 2021. Effects of full replacement of dietary fishmeal with insect meal from *Tenebrio molitor* on rainbow trout gut and skin microbiota. *J. Anim. Sci. Biotechnol.* 12(1):30. doi: [10.1186/s40104-021-00551-9](https://doi.org/10.1186/s40104-021-00551-9)
- Terova, G., S. Rimoldi, C. Ascione, E. Gini, C. Ceccotti, and L. Gasco. 2019. Rainbow trout (*Oncorhynchus mykiss*) gut microbiota is modulated by insect meal from *Hermetia illucens* prepupae in the diet. *Rev. Fish Biol. Fish.* 29:465–486. doi: [10.1007/s11160-019-09558-y](https://doi.org/10.1007/s11160-019-09558-y)
- Wethasinghe, P., S.D.C. Rocha, O. Øyås, L. Lagos, J.O. Hansen, L.T. Mydland, and M. Øverland. 2022. Modulation of Atlantic salmon (*Salmo salar*) gut microbiota composition and predicted metabolic capacity by feeding diets with processed black soldier fly (*Hermetia illucens*) larvae meals and fractions. *Anim. Microbiome* 4(1):9. doi: [10.1186/s42523-021-00161-w](https://doi.org/10.1186/s42523-021-00161-w)
- Yu, M., Z. Li, W. Chen, T. Rong, G. Wang, and X. Ma. 2019. *Hermetia illucens* larvae as a potential dietary protein source altered the microbiota and modulated mucosal immune status in the colon of finishing pigs. *J. Anim. Sci. Biotechnol.* 10:50. doi: [10.1186/s40104-019-0358-1](https://doi.org/10.1186/s40104-019-0358-1)

About the Authors



Iliaria Biasato is a Researcher of the Animal Production Unit at the Department of Agricultural, Forest, and Food Sciences of the University of Turin, Italy. She graduated in Veterinary Medicine and got her PhD studying the impact of insect meal in poultry feeding at the Department of Veterinary Sciences of the University of Turin, Italy. Since 2014, her research activity has been focused on farming and nutrition of fish and poultry, dealing with the impact of insect-derived products (live larvae, meals, and fats) on the productive performance, gut health features (especially gut microbiota), health and welfare, and product quality of the insect-fed animals. Since 2020, she has also been working on the effects of different byproducts on the production cycles of *Hermetia illucens* (black soldier fly) and *Tenebrio molitor* (yellow mealworm). Corresponding author: ilaria.biasato@unito.it

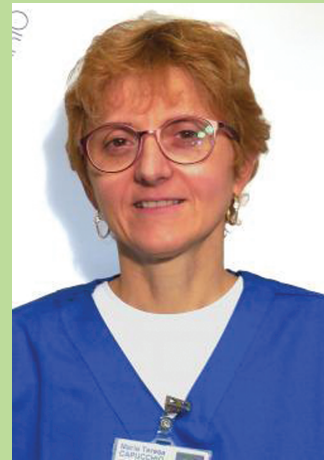


Laura Gasco is a Full Professor at the Department of Agricultural, Forest, and Food Sciences of the University of Turin, Italy. She earned her Agricultural Engineering degree at the University of Louvain-la-Neuve, in Belgium. She completed her PhD in Animal Sciences at the University of Turin and got a second degree in Agricultural Sciences. Laura focuses her research on fish, rabbit, and poultry farming and nutrition, assessing their productive performance and the impact of alternative ingredients on product quality. Since 2012, her research has been focused on the use of insect meals as innovative ingredients in animal nutrition and on the optimization of insect rearing to produce high-quality raw materials. In particular, she has a well-recognized experience with the black soldier fly (*Hermetia illucens*) and the yellow mealworm (*Tenebrio molitor*).



Achille Schiavone is a Full Professor of Poultry Science at the Department of Veterinary Sciences of the University of Turin, Italy. Since he obtained his PhD in Poultry Science, the prime focus of his research career was addressed various aspects of poultry science, especially nutrition, welfare, and biodiversity preservation. He has also dedicated considerable time and energy to numerous projects carried out in developing countries and in association with the industrial sector, with the primary scope of skill transfer. His desire to interact with the scientific community in international contexts has led him to attend a multitude of congresses and complete several research secondments in France and Spain. Since

2002, he has been teaching bachelor, master, and PhD courses in animal nutrition and poultry science. Since 2014, his research activity focuses on the impact of insect-derived products on the productive performance and welfare of the insect-fed poultry species.



Maria Teresa Capucchio is an Associate Professor of the General Pathology and Anatomic Pathology Unit at the Department of Veterinary Sciences of the University of Turin, Italy. She graduated in Veterinary Medicine and got her PhD in neuropathology at the University of Turin, Italy. Her main research activities are focused on the evaluation of gut health/systemic morphological alterations following the use of new dietary supplements (natural antioxidants, immunomodulators, antimicrobial peptides, or probiotics) and on host-pathogens interaction applying morphometry, histopathology, immunohistochemistry, and molecular biology techniques. In particular, since 2014, her research has been focused on the impact of insect-derived products on gut health with special attention for histomorphological changes, including mucins production and tight junctions/cytokins expression.



Ilario Ferrocino is an Associate Professor of the Food Technologies and Agricultural Microbiology Unit at the Department of Veterinary Sciences of the University of Turin, Italy. He graduated in Food Science and Technology and got his PhD in Science and Technology of Agri-food Productions at the University of Naples "Federico II", Italy. His main research activities are focused on the description of the structure and evolution of the microbiome in different ecosystems. His research areas are: i) Development, optimization, and application of molecular methods for microbial detection and characterization, ii) Study of microbial ecology in fermented foods (mainly sausages, cheeses, and vegetables) by using culture-independent and dependent methods, iii) Bioprotection: molecular characterization of bacteriocin production and its study in vitro and in situ, iv) Study of the human and animal gut microbiome.