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Review

A Systematic Review and Metanalysis on the Use of *Hermetia illucens* and *Tenebrio molitor* in Diets for Poultry

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Simple Summary: The dietary use of insect meal is a trend to reach sustainability in animal production. *Hermetia illucens* (black soldier fly) and *Tenebrio molitor* (yellow mealworm) are the most common insect species reared for larvae production since they have the capacity to transform wasted materials and food into a high protein meal that can be included in poultry diets. However, the potential use of either *H. illucens* or *T. molitor* larvae meal and oil is not limited to the nutritional composition of its products, but it is extended to the functional effects on animal health. Recently, the functional and antimicrobial properties of dietary insect provision have also been evaluated. This manuscript brings a systematic review and a metanalysis on the use of either *H. illucens* or *T. molitor* meal and oil as ingredients or feed additives and their effects on broilers` growth performance and gut health. Insect meal and oil enhanced immune status and gut microbiota, demonstrating an opportunity for the use of products that can contribute to poultry health.

Abstract: Insect meal as a protein source has been considered a sustainable way to feed animals. *H. illucens* and *T. molitor* larvae meal are considered high protein sources for poultry, also presenting considerable amounts of fatty acids, vitamins, and minerals. However, other potential components in insect meal and insect oil have been more studied nowadays. Chitin, lauric acid, and antimicrobial peptides can present antimicrobial and prebiotic functions, indicating that low inclusions of insect meal can beneficially affect broilers` health and immune responses. This systematic review was developed to study the impact of insect products on health parameters of broilers, and a metanalysis was conducted to evaluate the effects on performance. A database was obtained based on a selection of manuscripts from January 2016 to January 2023, following the mentioned parameters. There were positive effects of both *H. illucens* and *T. molitor* meal or oil products on poultry health status, especially on ileal and cecal microbiota population, immune responses, and antimicrobial properties. The average daily gain was greater in broilers fed *T. molitor* meal compared to *H. illucens* meal ($p = 0.002$). The results suggest that low levels of insect meal were suitable for broilers, without resulting in negative effects on body weight gain and feed conversion ratio, while the insect oil can totally replace soybean oil without negative impacts.

Keywords: broiler; chitin; immune response; insect meal; insect oil; microbiota

1. Introduction

Different vegetable and animal protein sources have been evaluated in diets for poultry, which include dietary inclusions of insect products [1–3]. Most of the studies have been conducted to evaluate insect types, and also to elucidate the ideal inclusion of insect meal as an ingredient. There is a large number of insects with economic interest in animal production; however, *Hermetia illucens* (HI; black soldier fly) and *Tenebrio molitor* (TM; yellow mealworm) are the ones that can reach an industrial scale of production [4]. Meals generated from these insects contain high protein levels and sufficient quantities of fatty acids, vitamins, fiber, and minerals [5]. Additionally, *H. illucens* and *T. molitor* in either meal or oil forms are considered suitable feed ingredients for broilers as they are well accepted for poultry based on a natural behavior [9].

Feeding diets with insect products has also been considered sustainable because insects can be easily reared on a wide range of biological waste streams [6–8], using less land and water compared to other protein and energy ingredient sources [9]. Animal production systems based on reduced waste and pollution have become priority, indicating that an increased efficiency of raw material utilization is needed. However, the variability in nutrient composition, scale of production, and ingredient cost and availability are the main challenges for the poultry chain when insect products are intended to be used.

Since more attention has been given to the precision of nutrition and animal health aiming to improve the efficiency of poultry production, *H. illucens* and *T. molitor* larvae have been intensively studied to provide accurate information on nutrient composition and digestibility profiles of different products for broilers. Recently, there was an increased interest in better understanding larvae compounds that present functional, prebiotic, or antibiotic functions and antimicrobial properties, reported to benefit broiler immunity and intestinal health [10]. In this context, chitin from the exoskeleton of the insects [11,12], the fatty acid profile with high levels of lauric acid, and the defense mechanisms provided by antimicrobial peptides [13], can label the insect meal and oil also as functional ingredients for animal production. However, there is a lack of information regarding the antimicrobial properties of insect products against intestinal challenges in poultry, and there is limited information available evaluating the inclusion of low amounts of insect meal, the use of insects as functional additives, and their effects on intestinal health.

Therefore, the objective of this study was to review and group the most expressive and recent literature of either *H. illucens* or *T. molitor* meal and oil sources for poultry. This study focuses on evaluating the impact of insect products on blood parameters, immune system, gut histomorphology and microbiota of broilers, based on a systematic review. This manuscript was also outlined to present the effects of insect products on broiler performance through a metanalysis.

2. Materials and Methods

2.1. Systematic Review

A literature review was performed using the PRISMA method [14] to select the studies for the database construction. PubMed, Scopus, and Web of Science were used for the PICo framework electronic databases in the systematic review and metanalysis [15]. The following terms were searched: "poultry" or "broiler" or "broilers" or "chicken" or "chickens", in combination with, "*Hermetia illucens*" or "*Tenebrio molitor*", in addition to "chitin" or "chitosan" or "performance" or "feed conversion" or "feed conversion ratio" or "feed to gain" or "feed : gain" or "feed efficiency" or "gain : feed" or "feed intake" or "average daily gain" or "body weight gain" or "body weight" or "FCR" or "ADFI" or "ADG" or "BW" or "FI" or "health parameters" or "immune system" or "immune response" or "histomorphology" or "histology" or "morphometric" or "morphology" or "intestinal health" or "antioxidant" or "antimicrobial" or "antimicrobial peptides" or "intestinal challenge" or "challenge".

The systematic review was conducted using published manuscripts and the cited references in the manuscripts were reviewed to identify additional studies that were relevant. All the references were exported to Mendeley software (Mendley Ltd., Elsevier, 2023) and each publication was independently reviewed to be selected for the next step. All the manuscripts selected for this publication were published in scientific journals from January 2016 to January 2023, considering the objective of the systematic review and the possibility of metanalysis.

For this systematic review, manuscripts reporting mainly results on the impact of HI and TM on the intestinal health of broiler chickens were selected, which basically included the effects on intestinal microbiota, histomorphology and histochemistry measurements, and blood parameters.

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Therefore, all the main observations from the previous studies were grouped into result tables to better demonstrate the effects of dietary HI or TM products on poultry health. In the result tables, the inclusion of insect products was maintained in percentage according to each publication. Percentages of replacement were presented as inclusion levels, when possible.

2.2. Metanalysis

After selecting the manuscripts, a spreadsheet was created based on broiler growth performance, where columns indicate the authorship, feeding phase, ingredient inclusions, average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR). All the data presented in the selected manuscripts was transformed to a daily basis for feed intake and body weight gain, being expressed in g/kg, whereas the FCR was presented in g:g. The obtained information for the metanalysis also includes the type of insect and physical characteristics of the ingredient (meal or oil). Additionally, manuscripts that had live or whole larvae and treatments that did not include insect products (excluding the control diet) were excluded from the metanalysis.

To perform metanalysis, each publication received a sequential code number, which is an inter-code with a sequential code plus a number that represents the treatment. The codes were repeated to indicate different phases of the same treatment (for example, manuscript 10 and treatment 01 for the starter phase = 1001; manuscript 10 and treatment 01 for grower phase = 1001; manuscript 10 and treatment 02 for the starter phase = 1002…). This sequential code allowed us to study the effects of treatments in different growing phases, analyzing values and finding evaluated means. The feeding phases were used for categorical analyzes as: starter (1 to 21 days), grower (21 to 35 days), and finisher (35 to 42 days). Afterwards, the spreadsheet was set and ADG, ADFI, and FCR variables were calculated as a percentage to the respective control in two ways. First, the response for the given performance parameter with the control treatment in each publication, separated by feeding phase, and the control diet was considered 0 (zero). This information was presented as the variation between the control and treatments in percentage. Secondly, the greatest results for ADG and ADFI of each phase were considered 100%; however, for FCR the lowest (best or improved) result was considered 100% for analyses. Results were separated in control treatment or treatments with either HI or TM insect products in low inclusion levels (≤ 100 g/kg for meal and ≤ 25 g/kg for oil) and high inclusion levels (> 100 g/kg for meal and > 25 g/kg for oil). This procedure was used to reduce the variability among studies in the database [16].

In the present manuscript, the metanalysis was performed to combine results from several assays in the database to compare the effects of the two insect products (HI and TM meal or oil) on broiler performance. Graphical analyses are presented to show the results of each manuscript and to obtain an overview of the data heterogeneity. Still, this procedure was divided into type of insect products, meal or oil forms, and feeding phases that were included in the same graphs for better visualization. For the metanalysis, at the end, the 25 selected publications containing information regarding ADFI, ADG, and FCR were used to create 8 graphs with an exploratory analysis, demonstrating the effects of insects as ingredients on broiler performance. Also, the variation on ADFI, ADG, and FCR of broilers fed diets with either insect meal or oil and an analysis of variance to compare both insects were expressed in two tables.

2.2. Statistical Analysis

Data were submitted to analysis of variance to show the effects of inclusion levels of the insect products on growth performance. It was performed using the univariate procedure and the Shapiro-Wilk test of Minitab® Statistical Software. After, performance responses were analyzed for variance-covariance. The model included the code of the manuscript as random effect. Insect meal and oil were fixed effects. Mean differences were considered significant at $p \leq 0.05$ using the Tukey test.

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3. Results

The literature review resulted in 597 manuscripts that were identified and imported from the database as shown on the PRISMA flow (Figure 1). In the identification, a total of 508 studies were excluded due to inconsistent title, abstract, or duplicates. For the present publication a total of 60 manuscripts were eligible, presenting growth performance information and health impacts in broiler chickens. After full evaluation, 29 publications were removed from the metanalysis due the lack of information on performance data, that precluded the correction for ADFI, ADG, and FCR or were removed due to the missing information on diets description. A total of 25 studies were retained as described in Figure 1 and Table 1. The nutritional and aminoacidic composition of either HI or TM meal are presented in Figures S1–S4, and 16 studies were obtained using insects as ingredients in the diets, also presenting the nutrient composition of the meals. Data were corrected to a dry matter basis when needed.

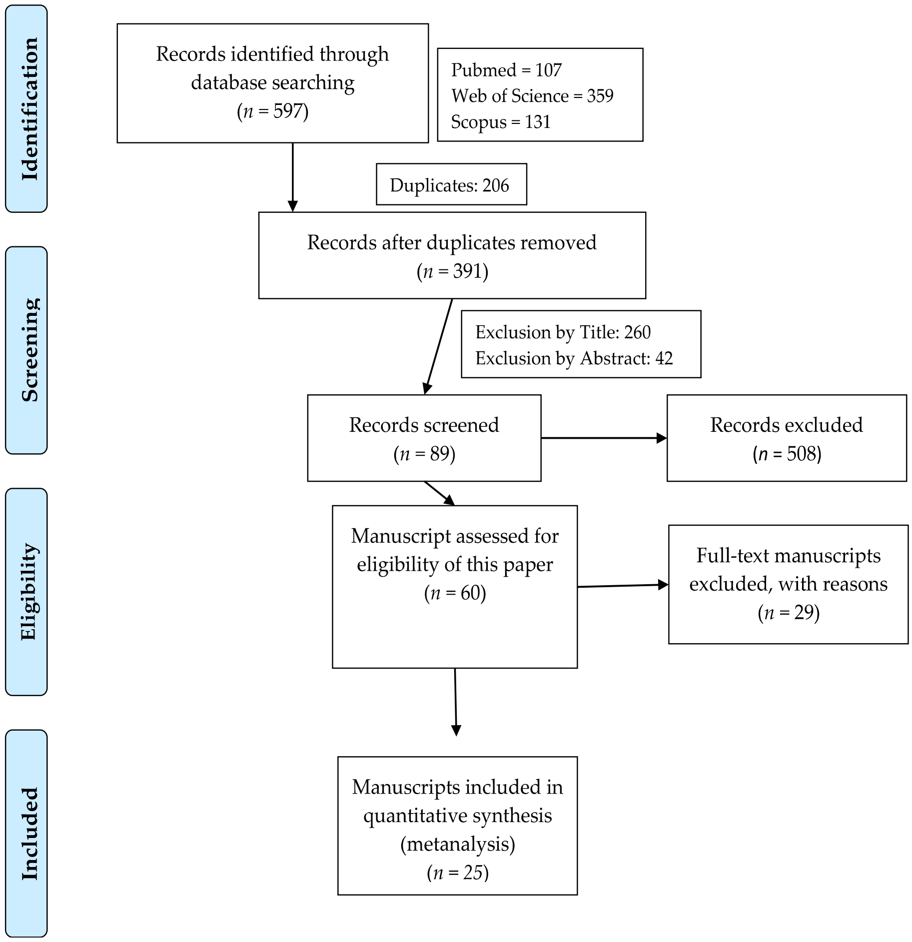

Figure 1. Flow diagram of the systematic review process.

Table 1. Summary of live production studies used in the metanalysis.

Reference	Code of publication	Insect used[1]	Feeding phases[2]	Genetic/sex[3]	Protein/oil source[4]	n^5
Mat et al. [17]	1	HI	Str, Grw, Fin	Cobb/male	FM, SBM	360
Sedgh-Gooya et al. [18]	2	TM	Str, Fin	Arbor Acres	FM, SBM	180
Kim et al. [19]	3	HI	Str, Grw	Ross/male	SBM, CGM	126
Kierończyk et al. [6]	4	HI oil	Str, Grw	Ross	SBM	960
Hartinger et al. [20]	5	HI	Str, Grw	Ross/male	SBM	432
Murawska et al. [21]	6	HI	Str, Grw, Fin	Ross/male	SBM	384
Hartinger et al. [22]	7	HI	Str, Grw	Ross/mixed	SBM	216

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Elangovan et al. [23]	8	HI	Str	Cobb/mixed	SBM	90
Kim et al. [24]	9	HI oil	Str, Grw	Ross/male	SBM, CGM	300
Pietras et al. [25]	10	TM	Fin	Ross/male	SBM, LM	64
Dabbou et al. [26]	11	HI oil	Str, Grw	Ross/male	SBM, CGM	200
Mutisya et al. [27]	12	HI	Str, Fin	Cobb/mixed	FM	120
Elahi et al. [28]	13	TM	Str, Fin	Ross/male	SBM	700
Benzertiha et al. [29]	14	TM	Str, Grw	Ross/female	SBM, RS, FM	300
Benzertiha et al. [30]	15	TM oil	Str, Grw	Ross/female	SBM	48
Khan et al. [31]	16	TM	Str, Grw	Ross	SBM, CM, GM, SM	100
Brede et al. [32]	17	HI	Str, Grw	Ross/male	SBM	240
Onsongo et al. [33]	18	HI	Str, Fin	Cobb/male	SBM, FM	288
Biasato et al. [34]	19	TM	Str, Grw, Fin	Ross/male	SBM, CGM	160
Biasato et al. [35]	20	TM	Str, Grw, Fin	Ross/female	SBM, CGM	160
Bovera et al. [36]	21	TM	Fin	Shaver Brown /male	SBM	80
Józefiak et al. [37]	22	HI and TM	Str, Grw	Ross/female	SBM, RS, rye, FM	300
Schiavone et al. [38]	23	HI oil	Str, Grw, Fin	Ross/male	SBM, GLM	150
Schiavone et al. [39]	24	HI oil	Fin	Ross/male	SBM, GLM	120
Kareem et al. [40]	25	HI	Str, Grw, Fin	Cobb/female	SBM, FM	216

[1] HI = *Hermetia illucens* and TM = *Tenebrio molitor* in meal form unless indicated as oil. [2] Feeding phases = Str: starter; Grw: grower; Fin: finisher. [3] Sex was informed whenever indicated in the manuscripts. [4] Protein sources in the control treatment; FM = fish meal; SBM = soybean meal; CGM = corn gluten meal; LM = lupine meal; RS = rapeseed meal; GM = guar meal; SM = sunflower meal; CM = cottonseed meal; GLM = gluten meal; [5] *n*: number of birds in the experiment and fed control diets and insect products.

3.1. Systematic Review

The results of the systematic review of broilers fed diets with HI and the impact on health parameters are shown in Table 2. This table presents authorship, overall information of diet and insects, inclusion levels, and the main observed effects. The publications evaluated HI meal or oil separately, with variable inclusion levels of these products. Few studies replaced soybean meal (SBM) by HI meal or soybean oil by HI oil in different percentages of replacement. On the main effects, previous publications mainly evaluated microbiota, immune responses, intestinal histomorphology, and blood parameters. Results of histomorphology and blood parameters were variable, ranging from no effects to improvements in villus height and blood triglycerides, monocytes, and red blood cell distribution. Some publications demonstrated that there is a potential of using HI meal or oil to improve the relative abundance of beneficial microorganisms and to decrease *Clostridium* in the gastrointestinal tract (GIT).

Table 2. Use of *Hermetia illucens* (black soldier fly) meal or oil as ingredients for broilers and the impacts on health parameters.

Reference	Overall[1]	Inclusion	Main effects[3]
Ipema et al. [41]	Replacement of 8% of the diet in DM with different provision of HI (meal + oil, live scattered on litter, whole dried in the feeder and whole dried scattered	5.76%	No difference was found for feather corticosterone, IgG, and IgM against keyhole limpet hemocyanin from plasma. Footpad dermatitis and litter quality were affected by the treatments

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	on litter) and impacts on broiler health and behavior		
Bongiorno et al. [42]	HI live larvae for local poultry and the effects on blood parameters	10% live larvae supplementation on the ADFI²	HI reduced leukocytes levels and increased monocytes and cholesterol levels. Higher gamma glutamyl transferase in blood samples, indicating improvement on liver health status
Mat et al. [17]	Different replacement levels of fishmeal by defatted HI meal for broilers and the effects on blood parameters	4, 8, and 12%	Corpuscular volume, monocytes, red blood cells, granulocytes, hematocrits, hemoglobin, corpuscular hemoglobin concentration, red cell distribution width, platelet volume, and lymphocytes were affected by HI meal
Kierończyk et al. [43]	HI oil replacing soybean oil and the effects on gut microbiota and immune characteristics	1.67 and 3.34%	100% replacement increased the total number of *Clostridium leptum* subgroup and *Enterobacteriaceae* and *Lactobacillus* groups in crop. No effects in jejunal microbiota. Reduced *C. perfringens* and all the other microorganisms except *Bacteroides* with 50% HI replacement. Reduced cholesterol and ALT in plasma. No effects on plasma immunoglobulins and IL
Hartinger et al. [20]	HI oil replacing 50 or 100% of soybean oil and HI meal replacing a 15% CP diet for broilers. Evaluated the effects on ileum histomorphology and caeca microbial metabolites	5.2, 4.64 and 4% of HI meal and/or 0.75, 1.5, 0.64 and 1.28% of HI oil	HI meal decreased biogenic amines Agmatine, Spermidine, Spermine, and ammonia in caeca contents. Ethanolamine was higher with the HI oil. HI oil increased the concentration of Agmatine in colon contents. HI meal and 50 or 100% oil replacement increased jejunal villus area and width compared to control diets
Kim et al. [19]	Microwave-dried HI meal replacing 25 and 50% of SBM	Starter: 7.5 and 15%. Grower: 7 and 14%. Finisher: 6.5 and 13%	SCFA in caeca were higher in HI treatments (except butyrate) especially with 50% replacement. Blood triglycerides, monocytes, and red blood cell distribution were higher 50% replacement. Decreased low-density lipoprotein was obtained
Zhang et al. [44]	Immune responses using HI larvae meal for broilers experimentally infected with IBV	1, 5, and 10%	Reduced IBV symptoms with 10% inclusion. At the tissue level presented reduced IBV infection and lesion. Increased survival rate of chicks. Improved proliferation of CD8+ lymphocytes
Hartinger et al. [22]	0, 15, and 30% of CP from SBM replaced by HI meal	0, 4, 10%	No differences on intestine morphology parameters
de Souza Vilela et al. [45]	Increasing levels of full fat HI meal for broilers and the effects on immune responses	Starter: 2.5, 5, 7.5, and 10%. Grower/Finisher: 5, 10, 15, and 20%	Decreased blood lymphocytes at 21 d and decreased cytotoxic T cell CD3+CD8+ in jejunum

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Kim et al. [46]	Replacement of 50 and 100% of soybean oil by HI oil and the effects on intestinal health and blood profile	1.5% and 3%	Higher ileum villus height with 50% replacement. Higher butyrate levels in caeca content with HI oil. Lowered the lipase levels on blood samples
Biasato et al. [47]	Use of defatted HI meal for broilers and the impact on gut health	5, 10, and 15%	HI did not affect the relative abundance of Firmicutes and Bacteroidetes and Firmicutes:Bacteroidetes ratio, but 15% HI had higher relative abundance of *Proteobacteria*. L-*Ruminococcus* (*Ruminococcus* from *Lachnospiraceae* family), *Faecalibacterium*, *Blautia*, *Clostridium*, *Bacteroides*, *Roseburia*, and *Helicobacter* genera. *Lactobacillus* and *Ruminococcus* improved in 10% HI diet. The mucin staining was not affected by HI
Kierończyk et al. [6]	Replacement 25, 50, 75 and 100% of soybean oil by HI oil and the effects on broilers' gut histomorphology	Starter: 0.83, 1.67, 2.34, and 3.34%. Finisher: 1.23, 2.46, 3.44 and 4.92%	No effects on histomorphology of duodenum, jejunum, and ileum. The HI oil reduced the jejunum and ileum weights relative to BW
Ipema et al. [48]	Use of live larvae and impacts on health and behavior of broilers	5 and 10% of ADFI[2] in DM with live larvae	No effects on hock burns, lameness, cleanliness, thigh scratches, tibia length, tibia fluctuating asymmetry, and tibia breaking strength, only reduced the tibia width with 10% replacement
Lee et al. [49]	Immune activity of broilers experimentally infected with *Salmonella* Gallinarum fed with HI larvae	1, 2, and 3%	Higher presence of CD3+CD4+ T lymphocytes in spleen. Amplified spleen lymphocyte proliferation, increased lysozyme activity in serum, and increased survival rate of chicks with 3% inclusion
Dabbou et al. [50]	HI defatted meal and impacts on blood traits, gut morphology, and histological features	5, 10, and 15%	No effects on hematological and serum parameters. Diet with 15% HI decreased villi height, crypt depth, and V:C ratio compared to the other diets
Schiavone et al. [39]	Replacement of 50 and 100% of soybean oil by HI oil for broilers (finisher phase) and effects on blood and gut morphology parameters	3.43 and 6.87%	No differences on blood and histomorphology parameters
Schiavone et al. [38]	50 and 100% of soybean oil replaced by HI oil and the effects on blood parameters	2.91 and 5.85	No differences observed in blood analyses

[1] HI = *Hermetia illucens*; SBM = soybean meal; IBV = avian infectious bronchitis virus; CP = crude protein. [2] ADFI = average daily feed intake. [3] IgG = immunoglobulin G; IgM = immunoglobulin M; SCFA = short-chain fatty acids; ALT = alanine aminotransferase; IL = interleukin; BW = body weight; V:C = villus:crypt ratio.

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Table 3 presents the results of the systematic review of broilers fed diets with *T. molitor* and the impact on health parameters. Authorship, overall information of diets and insects, inclusion levels, and the main observed effects are shown. Almost all the publications evaluated TM meal and few studies tested TM oil in broiler diets. Also, the majority of the studies was designed to evaluate different inclusions of TM meal in diets for broilers as a protein source, and few studies tested the supplementation of full fat TM meal as a feed additive. The relative abundance of microorganisms was the main studied effect in the previous publications, followed by measurements in immune responses and blood parameters. Interestingly, some publications reported that TM meal inclusion in broiler diets was effective to decrease populations of *Escherichia coli*, *Clostridium*, *Coprococcus*, and *Ruminococcus* in different trials.

Table 3. Use of *Tenebrio molitor* (yellow mealworm) meal or oil as ingredients for broilers and the effects on health parameters.

Reference	Overall[1]	Inclusion	Main effects[2]
Sedgh-Gooya et al. [18]	Effects on histomorphology of broilers fed full fat TM meal	2.5 and 5%	No effects on histomorphology
Sedgh-Gooya et al. [51]	Inclusion of TM meal in broiler diets and effects on caeca microbiota and blood parameters	2.5 and 5%	Decreased blood albumin:globulin ratio with TM diets. *Escherichia coli* reduced with 5% TM inclusion
Benzertiha et al. [29]	Full fat TM meal supplemented in broiler diets and impacts on bird immune system	0.2 and 0.3%	TM meal diets had same IgY, IgM, levels than diets with salinomycin, but increased IL-2 and TNF-α with 0.3% TM inclusion
Biasato et al. [52]	TM meal and the effects on broiler intestinal microbiota and mucin	5, 10, and 15%	Decreased relative abundance of Firmicutes:Bacteroidetes ratio and the relative abundance of *Clostridium*, *Coprococcus*, L-*Ruminococcus*, and *Ruminococcus* with 15% TM. Higher mucin staining with 5% TM
Elahi et al. [28]	Different levels of dried TM meal and usage of fresh TM meal	2, 4, 8, and 10.48% as fresh matter that correspond to 4% of dried TM inclusion	Linear increase of ALT in blood. Reduced total protein. Higher malondialdehyde and lower total antioxidant capacity. Higher uric acid in blood. Decreased levels of lysozyme with fresh TM
Józefiak et al. [53]	Full fat TM meal supplemented in broiler diets and effects on caeca microbiome	0.2 and 0.3%	Phylum: Decreased relative number of Actinobacteria with 0.2% TM and Proteobacteria in both treatments. Increased Bacteroidetes with TM addition. Class: 0.3% TM decreased Clostridia and 0.2% TM increased Clostridia and decreased Actinobacteria. Order: 0.2% TM increased Clostridiales, but there was a decrease with 0.3% TM, and 0.2% TM decreased Lactobacilales. Family: 0.3% TM reduced *Ruminococcaceae*, *Enterobacteriaceae*

			and *Bifidobacteriaceae*. Genus: 0.3% TM stimulated growth of *Ruminococcus* and *Bifidobacterium*, and 0.2% TM decreased *Lactobacillus*
Biasato et al. [54]	TM full fat meal inclusion in broiler diets and effects on caeca microbiota and health	5 and 15%	Higher inclusion of TM meal decreased relative abundance of Firmicutes phylum and Firmicutes:Bacteroidetes ratio. Higher inclusion of TM meal decreased villi mucin staining
Benzertiha et al. [30]	Full fat TM meal supplementation in diets and effects on enzyme activity and microbiota	0.2 or 0.3%	TM meal at 0.3% decreased the caeca *Bacteroides– Prevotella* cluster. Also, 0.2 and 0.3% decreased *C. perfringens*. Salinomycin and TM treatments had the lowest extracellular β-glucuronidase and higher α-glucosidase activity on caeca contents
Benzertiha et al. [55]	TM oil and effects on pancreatic enzyme and blood parameters	5%	TM oil reduced amylase activity and triglycerides of blood, also triglycerides and total cholesterol of liver
Loponte et al. [56]	Total replacement of SBM by full fat TM meal in broiler diets and the effects on caeca VFAs	100% SBM replacement	Almost double Mmol/L of all VFAs, increased % of butyrate in total VFAs and decreased the acetate, propionate, and valerianate
Biasato et al. [57]	TM meal and effects on intestinal microbiota, histomorphology, and mucin composition of free-range broilers	7.5%	Increased *Sutterella*, *Ruminococcus*, *Oscillospira*, *Clostridium*, *Coprococcus*, and Firmicutes:Bacteroidetes ratio. Increased mucin staining intensity on ileum
Biasato et al. [35]	TM meal and the effects on broilers health	5, 10, and 15%	Increased levels of erythrocytes, linear decrease in albumin levels, and quadratically decrease in gamma glutamyl transferase in blood. No differences in histomorphology using TM meal
Islam and Yang. [58]	TM probiotic supplementation for broilers experimentally challenge with *Salmonella enteritis* and *E. coli*	0.4% of TM probiotic	IgG and IgA levels were higher with the probiotic. Lower mortality of birds and lower presence of *E. coli* and *Salmonella* spp. in caeca microbiota with the probiotic

[1]TM = *Tenebrio molitor*; SBM = soybean meal; VFAs = volatile fatty acids. [2] IgY = immunoglobulin Y; IgM = immunoglobulin M; IL = interleukin; TNF-α = tumor necrosis factor alpha; ALT = alanine aminotransferase; V:C = villus:crypt ratio; IgG = immunoglobulin G; IgA = immunoglobulin A.

Table 4 shows the effects of *H. illucens* and *T. molitor* in broiler diets and the impact on health characteristics based on a systematic review. There are less studies evaluating either HI or TM for broilers in the same experiment compared to experiments evaluating only one insect species. Two studies were developed to evaluate microbiota with different provision of HI or TM products and one publication had supplemented HI or TM. Until the date of this systematic review, there were no found publications evaluating both HI and TM in the same diet.

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Table 4. Use of *Hermetia illucens* and *Tenebrio molitor* as ingredients for broilers and their impacts on health parameters.

Reference	Overall[1]	Inclusion	Main effects
Bellezza Oddon et al. [59]	Live TM and HI larvae and impacts on health	5% of ADFI[2] with larvae of TM or HI	Gut histomorphology index and histopathological alterations were not influenced by HI and TM larvae. No effects on hematological and serum parameters
Colombino et al. [60]	Live TM and HI larvae for broilers and effects on mucin, immune response, and caeca microbiota	5% of the expected ADFI	TM diets had lower interleukin-2 expression compared to HI diets. Mucin was not affected by live larvae. HI and TM influenced the relative abundance of *Victivillaceae* family. *Saccharibacteria* and *Clostridium* increased HI, *Collinsella* was more abundant in TM treatment, and *Eubacterium* increased in both diets
Józefiak et al. [37]	TM and HI full fat meal supplementation and impact on microbiota	0.2% supplemented	Crop digesta: HI decreased *C. leptum* and increased *C. coccoides–Eubacterium rectale* cluster. *Lactobacillus* spp. and *Enterococcus* increased in TM and HI groups, while TM reduced *Bacteroides–Prevotella*. Ileal digesta: both insects increased the number of C. *coccoides*–E. *rectale*, *Lactobacillus* spp. *Enterococcus* spp. counts decreased in HI and increased in TM groups. Caeca digesta: *Bacteroides–Prevotella*, *Streptococcus* spp./*Lactococcus* spp., *C. coccoides–E. rectale* cluster, and *Lactobacillus* spp./*Enterococcus* spp. count increased with HI

[1] HI = *Hermetia illucens*; TM = *Tenebrio molitor*. [2] ADFI = average daily feed intake.

3.2. Metanalysis

The exploratory graphical analyses are shown in Figures 2–7. The ADFI, ADG, and FCR of broilers fed HI or TM larvae meal are presented in Figures 2, 3 and 4, respectively. The figures allowed us to observe the biological coherence between HI or TM larvae meal inclusion and growth performance parameters of broilers. Also, with the metanalysis it was possible to obtain a general view of the data consistency and heterogeneity as well as to observe data distribution regarding dietary insect levels. Since the feeding phases are within the same graphs in the present manuscript, we can observe the results of different phases observing points that are closer or farther to the Y axis in the graph of each publication.

Figure 2 shows the ADFI from 0 to 200 grams with the HI or TM larvae meal inclusion ranging from 0 to 300 g/kg. It can be seen a decrease in ADFI with the increasing levels of HI meal in 4 manuscripts [19,22,24,72]. On the other hand, increasing inclusions of TM meal does not seem to change ADFI.

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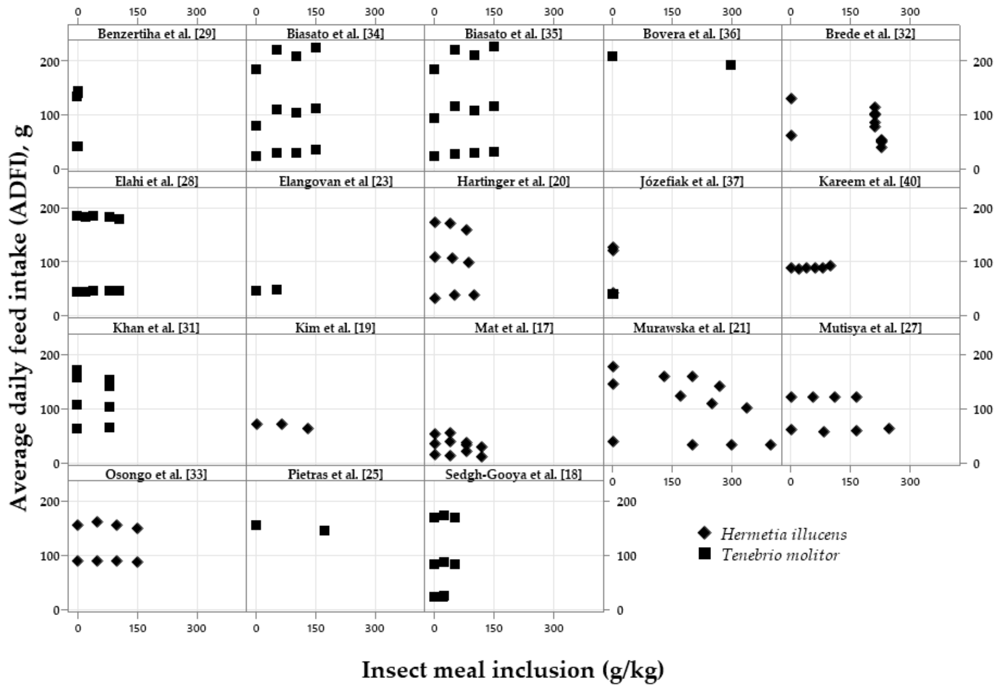

Figure 2. Exploratory graphs with the effects of increasing dietary inclusions of *Hermetia illucens* or *Tenebrio molitor* larvae meal on the average daily feed intake (ADFI) of broilers.

The effects of insect meal inclusions on broiler ADG is shown in Figure 3. For this parameter, the ADG does not seem to change with increasing levels for both insects, but there is a publication [35] demonstrating an increase on ADG in the finisher phase.

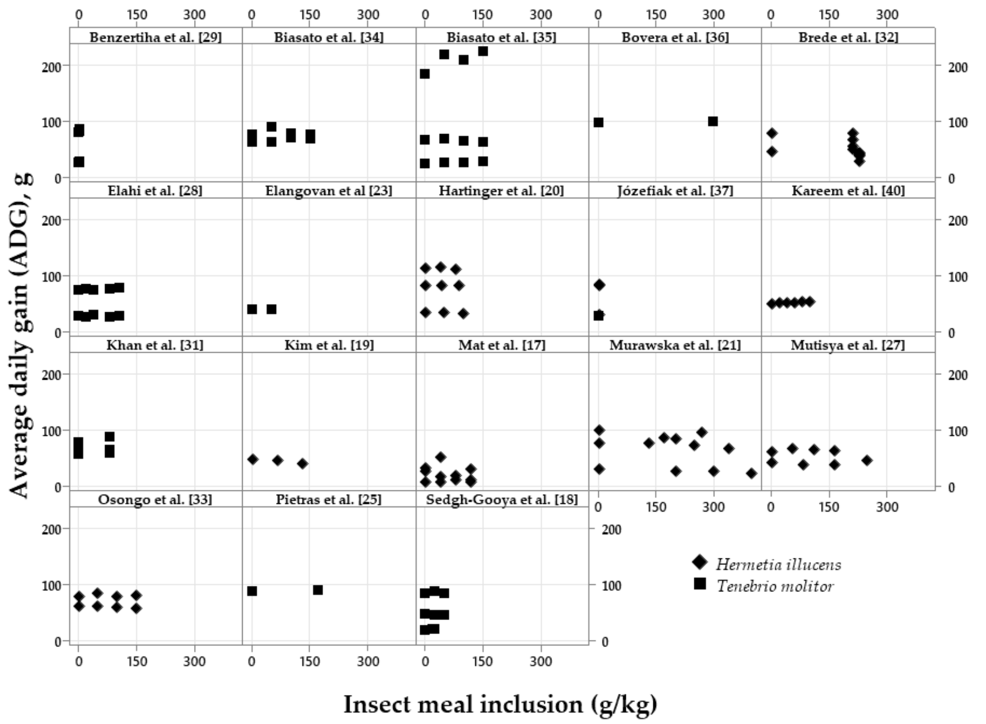

Figure 3. Exploratory graphs with the effects of increasing dietary inclusions of *Hermetia illucens* or *Tenebrio molitor* larvae meal on the average daily gain (ADG) of broilers.

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The FCR of broilers according to the insect meal inclusion is presented in Figure 4. This shows that no changes on FCR between the insect sources were observed. The FCR results were, moving from increased FCR using increasing levels of TM meal [34,35] to improvements on FCR [31]. With HI meal inclusions, an increase on FCR was also reported [25,51].

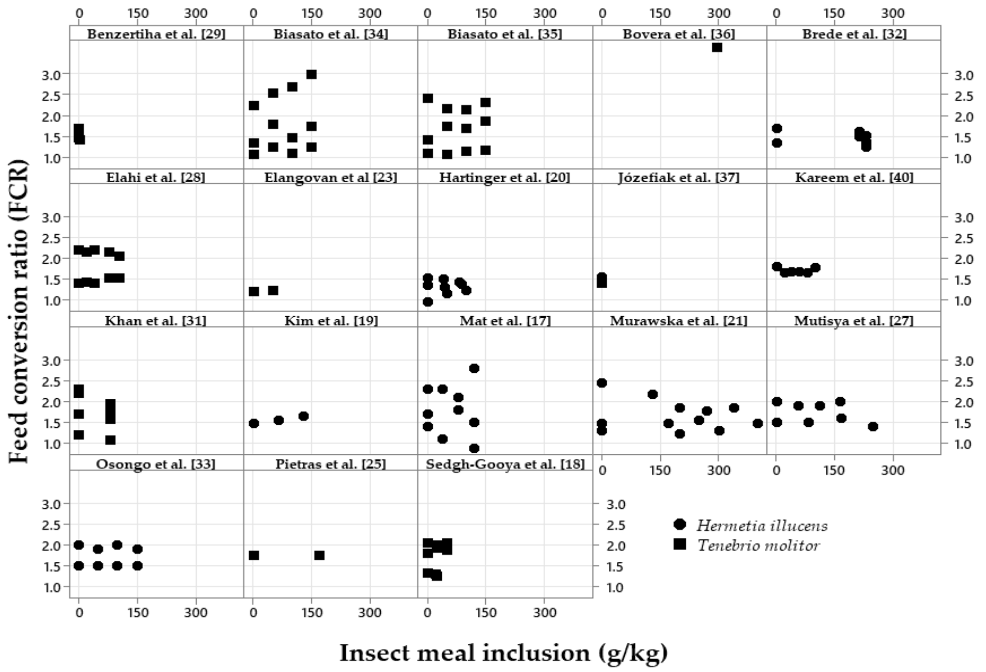

Figure 4. Exploratory graphs with the effects of increasing dietary inclusions of *Hermetia illucens* or *Tenebrio molitor* larvae meal on the feed conversion ratio (FCR) of broilers.

The effects of increasing levels of HI or TM oil on broiler performance are described in Figures 5 to 7. Figure 5 shows the ADFI from 0 to 200 g when broilers were fed HI or TM larvae oil ranging from 0 to 50 g/kg. Increasing levels of insect oil did not affect ADFI of broiler chickens. One publication was found evaluating TM oil for broilers in floor pens [55] and other in metabolic cages [61].

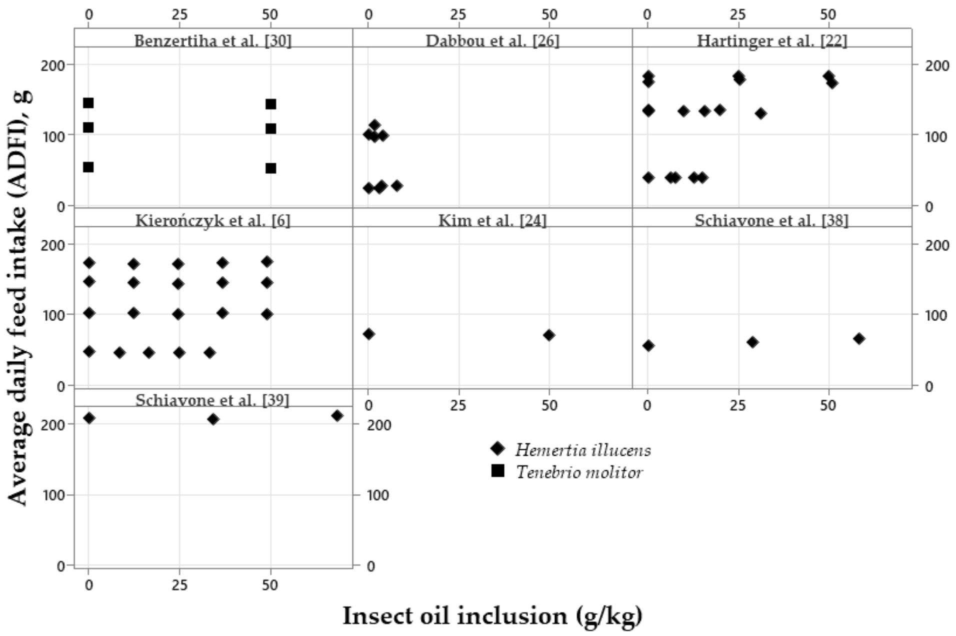

Figure 5. Exploratory graphs with the effects of increasing dietary inclusions of *Hermetia illucens* or *Tenebrio molitor* oil on the average daily feed intake (ADFI) of broilers.

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In a similar manner to the ADFI results, the ADG of broilers was not affected by increasing levels of either HI or TM oil (Figure 6).

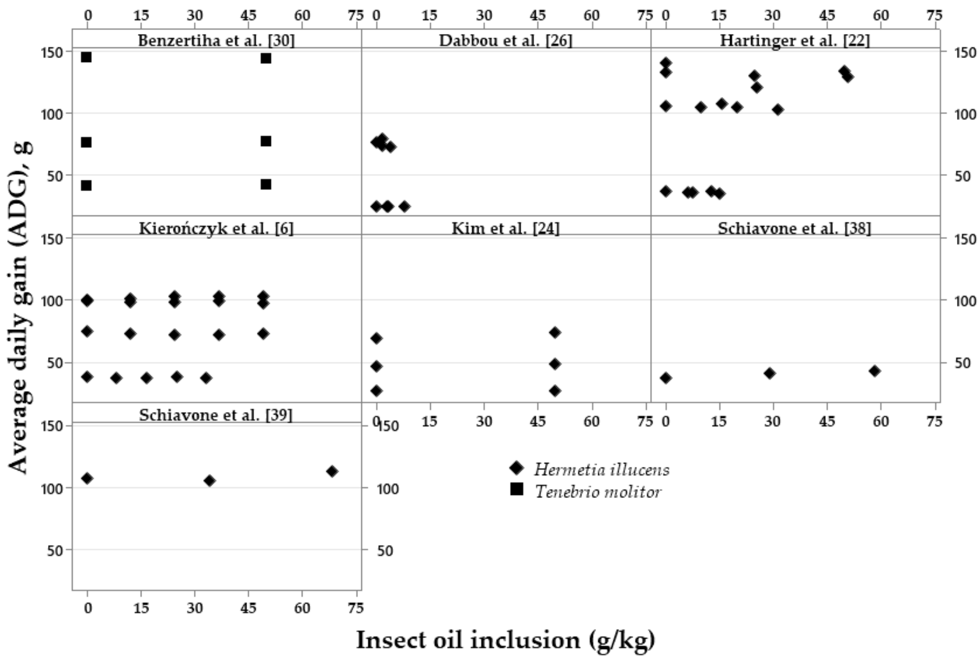

Figure 6. Exploratory graphs with the effects of increasing dietary inclusions of *Hermetia illucens* or *Tenebrio molitor* oil on the average daily gain (ADG) of broilers.

Figure 7 shows the FCR from 1.0 to 2.5 g:g with HI or TM oil ranging from 0 to 50 g/kg. The insect oils, independent of the source, do not seem to have any effect on this parameter.

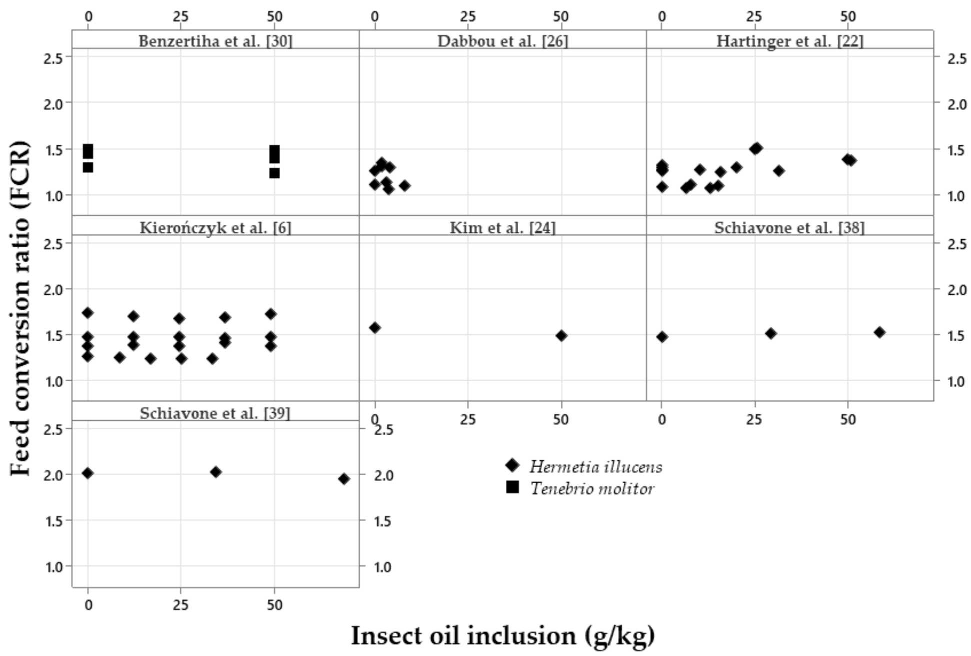

Figure 7. Exploratory graphs with the effects of increasing dietary inclusions of *Hermetia illucens* or *Tenebrio molitor* oil on the feed conversion ratio (FCR) of broilers.

Since the feeding phases of each publication were included in the already presented graphs, this can imply in a high heterogeneity of results. The ADFI tend to decrease with the inclusion of insect

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meal in most of the manuscripts, while ADG or FCR were stable throughout the inclusion of insect sources. Using insect oil, the majority of broiler performance results was similar among inclusions.

The dispersion graphs below (Figures 8–10) show the percentage of variation in ADFI, ADG, and FCR of broilers fed either HI or TM meal and oil related to the control diets (which is zero in the Y axis) using all selected publications.

Figure 8 presents the variation in ADFI between treatments with inclusion of insect products and the control diet for broilers. The variation between control and treatment diets with HI larvae meal is high and negative. For HI oil, TM meal, and TM oil, the ADFI did not vary from the insect inclusion to the control diet in most of the publications.

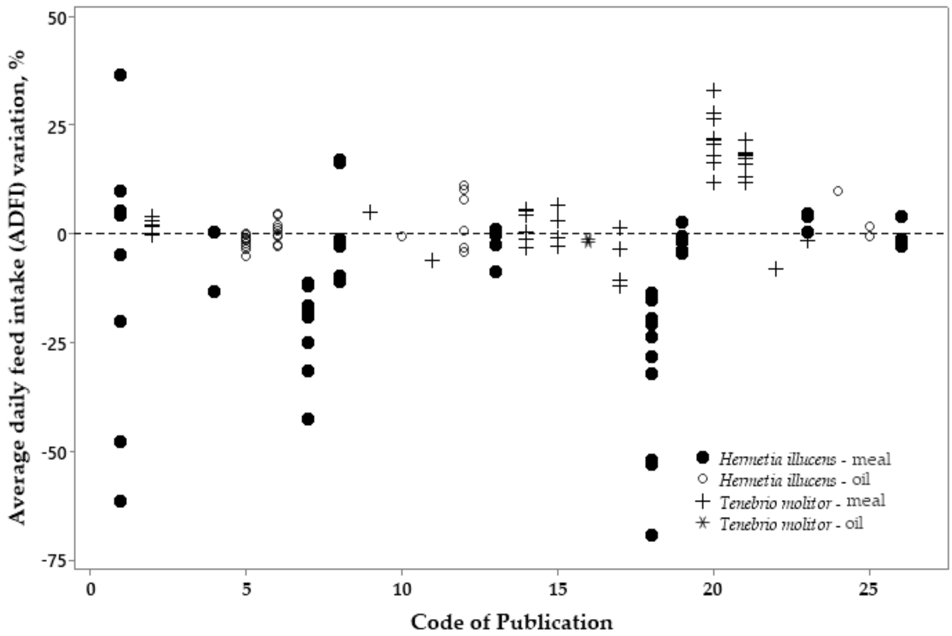

Figure 8. Variation (%) in the average daily feed intake (ADFI) responses between broilers fed control diets (zero in the Y axis) or diets with *Hermetia illucens* or *Tenebrio molitor* sources.

The ADG variation between control diets and diets with insect meal sources (Figure 9) indicate high variation. Negative values seem to be more present when TM meal was used. There was no observed variation between the use of insect oil or TM meal compared to the control diets.

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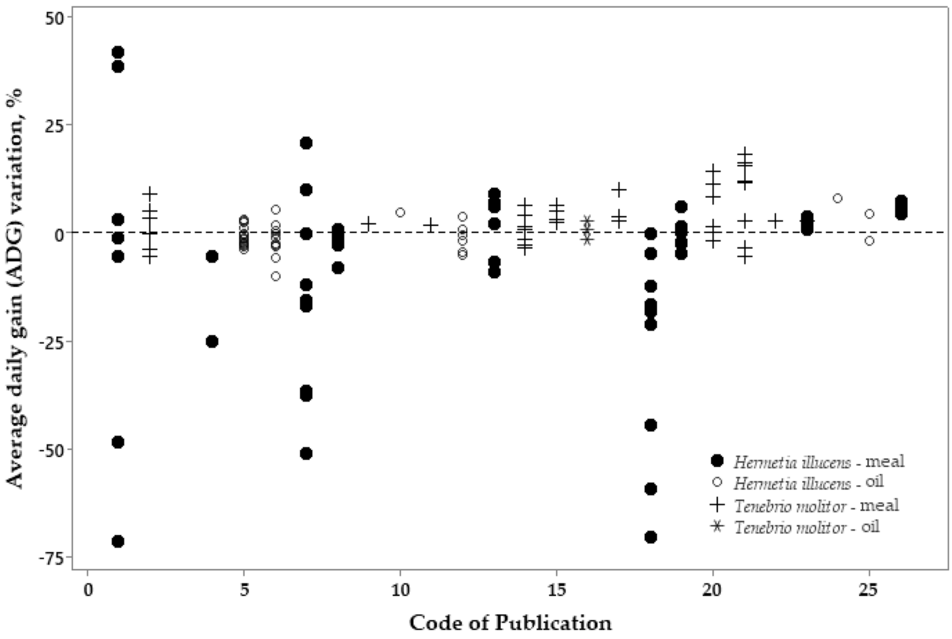

Figure 9. Variation (%) in the average daily gain (ADG) responses between broilers fed control diets (zero in the Y axis) or diets with *Hermetia illucens* or *Tenebrio molitor* sources.

Figure 10 shows the variation in FCR of broilers fed diets containing different levels of insect meal or oil compared to control diets. This parameter followed ADFI and ADG results, with most negative variation values for HI larvae meal and higher variation for TM meal. The oil sources did not affect the variation in FCR.

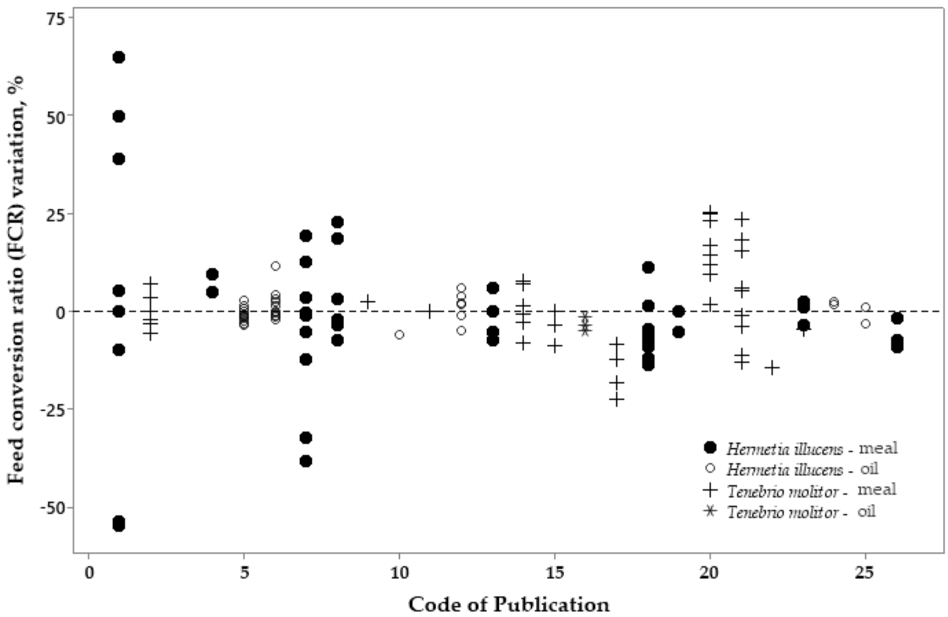

Figure 10. Variation (%) in the feed conversion ratio (FCR) responses between broilers fed control diets (zero in the Y axis) or diets with *Hermetia illucens* or *Tenebrio molitor* sources.

The variance-covariance analysis comparing both insect sources is presented in Tables 5 and 6. Table 5 shows the variation in percentage of HI and TM inclusion against the control diets and a comparison between the two insects. Significant differences on variance of performance results were observed in ADFI, where there was a difference of -10.70 in ADFI using HI meal compared to TM

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meal that had +7.20 variance in the meal form. The ADG followed the same premises, as -7.92 was obtained for HI meal and +4.05 for TM meal. For oil sources, there were differences ($p \leq 0.05$) in ADFI and FCR. Then, the variance in ADFI was +0.60 for HI oil and -4.25 for TM oil, while +0.41 was found in the FCR for HI oil and -3.54 for TM oil.

Table 5. Variation between control diets and dietary inclusions of HI or TM meal or oil for broilers.

Variation, %[1]	Meal		*p*-value[4]	Oil		*p*-value
	HI[2]	TM[3]		HI	TM	
ADFI	-10.70	7.20	<0.001	0.61	-4.25	0.036
ADG	-7.92	4.05	0.002	-0.51	0.81	0.888
FCR	-1.49	2.46	0.241	0.41	-3.56	0.049

[1] Variation in percentage between control diets (zero) and treatment diets with insect products in meal or oil forms for all feeding phases; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio. [2] HI = *Hermetia illucens*. [3] TM = *Tenebrio molitor*. [4] Probability of treatment effects, differences were considered significant at $p \leq 0.05$.

Table 6 shows the effects of low or high inclusions of either insect meal or oil, without separating the insect species. For the insect meal, high inclusion of the insect meal decreased the variation of ADFI and ADG of broilers ($p < 0.001$), without effects on FCR. For the insect oil inclusion, no differences were found.

Table 6. Effects of insect inclusion on broiler performance.

Variation, %[1]	Meal[2]			*p*-value[4]	Oil[3]			*p*-value
	Control	Low	High		Control	Low	High	
ADFI	94.42[a]	95.98[a]	85.62[b]	<0.001	98.44	97.85	97.17	0.620
ADG	92.46[a]	94.70[a]	83.41[b]	<0.001	97.12	97.83	96.85	0.545
FCR	107.1	109.7	107.9	0.647	102.4	101.8	101.7	0.576

[a,b] Differences were considered significant at $p \leq 0.05$. [1]Variation in percentage of each performance parameter with the best performance result; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio. [2] Low (≤ 100 g/kg) or high (> 100 g/kg) inclusion of insect meal. [3] Low (≤ 25 g/kg) or high (> 25 g/kg) inclusion of insect oil. [4] Probability of treatment effects.

4. Discussion

4.1. Impacts of dietary inclusions of insect products on poultry health

Insect-based products are receiving great attention as potential ingredient sources for animals. However, it is difficult to obtain an evidence-based view from the large volume and diversity of information that has been increasingly generated. For this reason, a systematic review with a metanalysis can be a useful method to summarize the findings of all the previous studies. Thus, this manuscript contains all searched studies that explored insect products either in meal or oil forms used in diets for broilers and their effects on growth performance and health parameters.

Insect meals have been evaluated and utilized in broiler diets mainly as dietary protein sources and as functional feed additives. Most of the publications have tested inclusion levels of *H. illucens* (black soldier fly) and *T. molitor* (yellow mealworm) to partially replace soybean meal, whereas the studies evaluating insect oil are often designed to replace soybean oil. However, feeding insect products for broilers still generates variable results due to the variability in their composition, the different tested inclusion levels, and production systems as well as the lack of information on digestibility and functional properties of insect products.

The commercial poultry production demands high amounts of ingredients; however, the practical and economical approach of replacing soybean meal by insect meal is still a challenge. For this reason, the benefits of low inclusions of insect products have been recently addressed. Therefore, more data is needed to better understand if insect meal or oil sources can beneficially impact intestinal

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health, immune system, and antimicrobial functions of broilers. Considering these premises, it is also important to evaluate the effects of insect products in the health status of broilers reared under enteric challenge conditions, which is something that was not already published in the literature. The only published material found through the systematic review had broilers fed HI meal and reared under a *Salmonella enteritidis* Gallinarum challenge [49].

As an avian species, broiler chickens searching and ingesting insects is a natural behavior. The utilization of scattered larva have enhanced the natural behavior, affecting leg health and welfare of chickens [41,48]. Since poultry have this behavior through their evolution, it has been highlighted that acid chitinases are present on the glandular stomach [62]. Chitin is one important component that constitutes the exoskeleton of the insects and it has been associated to a decrease in nutrient digestibility when present in high inclusions in diets for poultry [57]. However, chitin is also known as a natural antimicrobial component [63,64], and besides chitin, the presence of different antimicrobial peptides identified in both *H. illucens* and *T. molitor* [65] can contribute to poultry health. For this reason, chitin composition in different insect meal products and the possible beneficial properties of chitin and antimicrobial peptides on poultry health should be more studied in the future.

The evaluation of poultry health has variable and different approaches and the present review was developed to better understand the effects of the main insect meals and insect oils available for poultry diets. The present systematic review intended to demonstrate the effects of insect products that were already reported on broiler microbiota, gut histomorphology, and blood parameters. The importance of poultry microbiota in gut health is related to its capability to ferment carbohydrates and dispose energy and fatty acids as substrates for the GIT [66]. The most predominant genera of microorganisms in the GIT of broilers are *Clostridium*, *Ruminococcus*, *Lactobacillus*, and *Bacteroides* [67], which have different relative abundance in each intestine segment [68]. In the current study, it was observed that the population of beneficial bacteria is affected by the dietary inclusion of either insect meal or oil for broilers. The presence of lauric acid in insect meal can be one reason that influences bacteria population in the GIT since this fatty acid affects the proliferation of potentially pathogenic bacteria. Kierończyk et al. [43] reported that increasing levels of butyric and lactic bacteria were observed using the insect oil, which also decreased crop pH and helped to control pathogenic bacteria that usually do not tolerate acidic pH. However, the authors observed increased proliferation of *Bacteroides–Prevotella* cluster, *Enterobacteriaceae* and *C. perfringens*, probably due to the feed retention that was correlated to the antimicrobial effect and the availability of lauric acid in the crop.

Ruminococcaceae and *Lachnospiraceae* families have been reported to produce butyric acid [69], which is used as an energy source for enterocytes in the gut [70]. Chitin from insects can affect the production of butyric acid [56] and a previous study indicated that low levels of insect full fat meal (0.2%) reduced pathogens as *C. perfringens* [30]. On the other hand, high doses of HI meal improved the volatile fatty acid profile of the caeca content of broilers [19]; however, the authors only described the correlation between the fatty acid profile of the HI meal in the caeca, without relating chitin with the prebiotic effects. Prebiotic functions provided by chitin from insects were previously described as favorable to improve gut health [71]. The mucin dynamics, when using insect meal, was suggested to beneficially improve histological parameters that were correlated to microbiota due to production of volatile fatty acids [47].

Protein and amino acids that escape from digestion and absorption in the hindgut can be fermented in the caeca being used by bacteria [68]. This can extend the nutritional functions of feeding protein sources once they can be used by the microbiota, impacting the gut health of animals. The caeca microbiome as affected by *H. illucens* and *T. molitor* meals was already described [52,54]. Indirect approaches using metabolites can be used as demonstrated by Hartinger et al. [20] and through a principal component analyses [53]. In the present study, it was observed that insect meal affected gut microbiota especially Firmicutes, *Lactobacilli,* and Bacteroides populations. Positive impacts of insect products in the microbiota can be indicated since lower rates of Bacteroidetes and higher *Lactobacilli* are correlated with good gut health [47,53]. The *Ruminococcaceae* family is also important for the intestinal health and it was affected by insect meal [47]. Based on the results, HI

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and TM probably have different paths in microbiota modulation and the effects differed from each other [37,60]. Also, the region of GIT where microbiota populations were evaluated is variable among the peer-reviewed publications, which can influence the results.

In the present study, we observed that histomorphology findings were not consistent with the inclusion of insect meal or oil in diets for broilers. Some publications that enhanced this affirmation used 50% to 100% replacement of soybean oil by HI oil and did not observe alterations in gut histomorphology [6,32]. However, there are reports using 50% of soybean oil replaced by insect oil where increased villus height of ileum was observed [46], while similar results on jejunum villus height were reported using 50 and 100% oil replacement [20]. There is a publication [34] indicating that 15% inclusion of TM meal decreased villus, deepened crypts, and decreased villus:crypt ratio compared to 5 and 10% for broilers, whereas Biasato et al. [35], evaluating the same TM meal, did not observe any differences on histomorphology parameters.

Higher monocytes and hemoglobin were reported in broilers fed HI meal [17] replacing fish meal. Serum lipids and cholesterol were investigated using HI meal, and decreased cholesterol was found in blood samples, which was associated to the presence of chitin [19], indicating that the chitin could decrease lipid absorption by binding to lipids, and as a result, the plasma cholesterol decreased. Using TM meal for broilers increased albumin:globulin ratio and serum globulin levels [51]. These parameters were also related to the chitin levels that could work as prebiotic and boost the immune response. Increased erythrocytes and decreased albumin and gamma glutamyl transferase (GGT) using TM meal were reported by Biasato et al. [35]. These findings were seen as good results since high GGT levels indicate liver and bile flow disorders.

The inclusion of HI oil as a functional ingredient for poultry diets has been increased because the lauric acid presents antimicrobial properties [6,43]. The impacts of 100% replacement of soybean oil with insect oil were not negative according to our systematic review. Low levels of cholesterol in blood samples demand low bile synthesis, indicating that the animal had a highly efficient fat utilization. Additionally, this result together with low levels of alanine aminotransferase can be related to good liver health. Serum triglycerides reduced when TM oil was compared to poultry fat [55]. The authors also indicated that the high levels of polyunsaturated fatty acid of TM oil could reduce the conversion of acetyl-Coa to triglycerides in the liver, affecting blood triglycerides in broilers.

The use of insect meal has been demonstrated to potentially improve the immunity status in poultry, crustaceans, and fish [72–74]. Even though publications with other animal species were not selected in the systematic review, pigs fed HI meal had decreased diarrhea on weaning age and improved intestine homeostasis under the presence of *Escherichia coli*, by promoting levels of mucin-1 and mucin-2, while regulated the expression of antimicrobial peptides through TLR2-NF-κB/MAKP signaling pathway [75]. Also, the authors observed decreased pro-inflammatory and increased anti-inflammatory cytokines at finishing ages using HI meal on diets [76]. As for dogs and cats, there are reports indicating improvements in the antioxidant status [77], with reduced TNF-α and increased concentrations of superoxide dismutase and glutathione peroxidase in serum of dogs fed diets with HI meal [78]. However more studies are needed to better understand the effects in anti-inflammatory and immunity parameters [79] in companion animals but also in poultry. For the immunology effects in broilers, it shows a decrease in white cells and improved performance [45]. Also, under a viral challenge, there was a reduction in the load of avian infectious bronchitis virus (IBV) of trachea and kidney using 10% HI meal [44], and an increase in the survival rate of broilers with less damage to the trachea tissues. The authors summarized that interferon-gamma (IFN-γ) was activated in mRNA levels and this was involved in the activation of T-cells. Still, the interleukin-2 levels increased and promoted CD8+ lymphocytes responses. Finally, major histocompatibility complex-I (MHC-I) molecules that control CD8+ increased, and with the CD8+ lymphocytes promotion, the elimination of the avian IBV increased when diets had HI meal.

An experimental challenge with *Salmonella enteritidis* Gallinarum was conducted in broiler chickens fed HI meal [49]. The authors observed an increase on birds' survival rate followed by a decrease on Gram negative colonies on liver, spleen, bursa of Fabricius, and caeca. However, the mechanism of HI meal against S. *enteritidis* Gallinarum was not clear. Evaluating a fermented probiotic from TM meal for broilers under *Salmonella* and *Escherichia coli* challenge, Islam and Yang [58] reported decreased mortality and improved levels of immunoglobulin (Ig) IgA and IgG. This study showed reduction in the caeca *E. coli* and *Salmonella* counts, indicating that the combination of antimicrobial effects of chitin and antimicrobial peptides from larvae could contribute to reduce pathogenic bacteria. The results highlight the possibility of evaluating the effects of insect products at low levels on pathogenic bacteria when broilers are reared under intestinal challenges.

4.2. Metanalysis: exploratory analyses of insect products on broiler performance

Regarding the growth performance of broilers fed diets with the inclusion of insect products, a metanalysis was previous published [80], but did not discriminate the insect products. By the meantime this manuscript was written, another metanalysis was published to predict the metabolizable energy of the HI meal and the effects on performance [81]. In the current manuscript, the metanalysis was conducted to compare results of performance parameters such as ADFI, ADG, and FCR between the insect sources.

The database utilized for the present metanalysis consisted of manuscripts published in scientific journals between January 2016 and January 2023, as presented in the Table 1. Different sources of protein as main ingredients, variable broiler ages and genetics, and insect products were reported, and this could be a reason to the heterogeneity of results reported in Figures 2–7. Regardless, the Figures 8–10 indicate a greater growth performance of broilers fed TM meal compared to HI meal, and this was confirmed in Table 5 for ADFI ($p < 0.001$) and ADG ($p = 0.002$). A different outcome was observed for the oil sources, probably due to the lower number of available publications with TM oil compared to TM meal and HI oil. During the construction of this database, no manuscripts were found evaluating, in the same study, both insects as ingredients on broiler performance. For this reason, further experiments can be performed using different insect sources in the same diet since the availability of insect products can be variable worldwide.

Most of the publications regarding the inclusion of insect products for broilers have been conducted using insect meal to replace soybean meal. The levels of inclusion or replacement of HI or TM meal usually ranged from 5 to 15%. However, this can be not cost-effective for the commercial broiler productions and, for this reason, evaluating lower inclusion of insect products can be highlighted. In the present study, the insect meal at the low inclusion levels had similar growth performance to the control treatment in broilers without any intestinal challenge. No negative effects on performance were reported evaluating low levels of insect meal for broilers [18,21,22,34]. Dietary inclusions of insect meal with levels higher than 1% and lower than 10% should be more studied since this can result in different effects compared to very low or high levels. This evaluation can improve the information on the effects of low inclusions of insect meal on broiler nutrition and health, also allowing results that can be more cost-effective. For the insect oil, no differences were found with low or high inclusion levels, also without presenting any negative effects on broiler performance [38]. No data on full soybean oil replacement with TM meal was found in the literature.

In conclusion, the use of insect products in poultry diets affected broilers` health, demonstrating positive effects in the modulation of microbiota. This can be noticed through the different effects of various phylum, class, family, and genus of bacteria reported in this review that are mostly from caeca content. The reduction in pathogenic bacteria and the enhanced production of short-chain fatty acids are also characteristics of insect inclusions in poultry diets. Nevertheless, the immunology system is indeed improved, and reduced mortality was observed in challenged animals, with positive impacts on immunoglobulins, interleukins, and lymphocytes. To better understand the microbiota mechanism, future studies should be conducted focus on the inclusion levels of insect products and different intestinal challenges that can be found in production systems. For the performance data, *T. molitor* had better results if compared to *H. illucens*, also high meal inclusion (>100 g/kg) of insects decreased broiler performance as already reported in some manuscripts. The dietary utilization of insect oil did not affect any performance parameters and it seems that *H. illucens* oil can be used to completely replace soybean oil, whereas more studies are needed using *T. molitor* oil. Also, more data is needed to evaluate the effects of insect oil on poultry health aiming to provide more robust conclusions in the presence of an enteric challenge.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1. Nutritional composition of different *Hermetia illucens* meal. Figure S2. Nutritional composition of different *Tenebrio molitor* meal. Figure S3. Total amino acids (%) of *Hermetia illucens* larvae meal. Figure S4. Total amino acids (%) of *Tenebrio molitor* larvae meal.

Author Contributions: Conceptualization, Y.K.D. and C.S.; methodology, Y.K.D. and C.H.F.; formal analysis, Y.K.D.; data curation, Y.K.D.; writing—original draft preparation, Y.K.D. and C.S.; writing—review and editing, Y.K.D. and C.S. All authors have read and agreed to the published version of the manuscript.

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References

1. Veldkamp, T.; van Duinkerken, G.; van Huis, A.; Lakemond, C.M.M.; Ottevanger, E.; G.;, B.; van Boekel, M. a. J.S. Insects as a Sustainable Feed Ingredient in Pig and Poultry Diets - a Feasibility Study. *Wageningen UR Livest. Res.* **2012**, *(report 63*, 62.
2. Józefiak, D.; Józefiak, A.; Kierończyk, B.; Rawski, M.; Świątkiewicz, S.; Długosz, J.; Engberg, R.M. Insects - A Natural Nutrient Source for Poultry - A Review. *Ann. Anim. Sci.* **2016**, *16*, 297–313. https://doi.org/10.1515/aoas-2016-0010.
3. Allegretti, G.; Talamini, E.; Schmidt, V.; Bogorni, P.C.; Ortega, E. Insect as Feed: An Emergy Assessment of Insect Meal as a Sustainable Protein Source for the Brazilian Poultry Industry. *J. Clean. Prod.* **2018**, *171*, 403–412. https://doi.org/10.1016/j.jclepro.2017.09.244.
4. Thrastardottir, R.; Olafsdottir, H.T.; Thorarinsdottir, R.I. Yellow Mealworm and Black Soldier Fly Larvae for Feed and Food Production in Europe, with Emphasis on Iceland. *Foods* **2021**, *10*, 1–35. https://doi.org/10.3390/foods10112744.
5. Józefiak, D.; Józefiak, A.; Kierończyk, B.; Rawski, M.; Świątkiewicz, S.; Długosz, J.; Engberg, R.M. Insects - A Natural Nutrient Source for Poultry - A Reviewa. *Ann. Anim. Sci.* **2016**, *16*, 297–313. https://doi.org/10.1515/aoas-2016-0010.
6. Kierończyk, B.; Sypniewski, J.; Rawski, M.; Czekała, W.; Swiatkiewicz, S.; Józefiak, D. From Waste to Sustainable Feed Material: The Effect of Hermetia Illucens Oil on the Growth Performance, Nutrient Digestibility, and Gastrointestinal Tract Morphometry of Broiler Chickens. *Ann. Anim. Sci.* **2020**, *20*, 157–177. https://doi.org/10.2478/aoas-2019-0066.
7. Moula, N.; Scippo, M.L.; Douny, C.; Degand, G.; Dawans, E.; Cabaraux, J.F.; Hornick, J.L.; Medigo, R.C.; Leroy, P.; Francis, F.; et al. Performances of Local Poultry Breed Fed Black Soldier Fly Larvae Reared on Horse Manure. *Anim. Nutr.* **2018**, *4*, 73–78. https://doi.org/10.1016/j.aninu.2017.10.002.
8. Ramos-Elorduy, J.; González, E.A.; Hernández, A.R.; Pino, J.M. Use of Tenebrio Molitor (Coleoptera: Tenebrionidae) to Recycle Organic Wastes and as Feed for Broiler Chickens. *J. Econ. Entomol.* **2002**, *95*, 214–220. https://doi.org/10.1603/0022-0493-95.1.214.
9. van Huis, A.; Oonincx, D.G.A.B. The Environmental Sustainability of Insects as Food and Feed. A Review. *Agron. Sustain. Dev.* **2017**, *37*. https://doi.org/10.1007/s13593-017-0452-8.
10. Koutsos, E.; Modica, B.; Freel, T. Immunomodulatory Potential of Black Soldier Fly Larvae: Applications beyond Nutrition in Animal Feeding Programs. *Transl. Anim. Sci.* **2022**, *6*, 1–9. https://doi.org/10.1093/tas/txac084.

11. Zeitz, J.O.; Fennhoff, J.; Kluge, H.; Stangl, G.I.; Eder, K. Effects of Dietary Fats Rich in Lauric and Myristic Acid on Performance, Intestinal Morphology, Gut Microbes, and Meat Quality in Broilers. *Poult. Sci.* **2015**, *94*, 2404–2413. https://doi.org/10.3382/ps/pev191.
12. Shin, C.S.; Kim, D.Y.; Shin, W.S. Characterization of Chitosan Extracted from Mealworm Beetle (Tenebrio Molitor, Zophobas Morio) and Rhinoceros Beetle (Allomyrina Dichotoma) and Their Antibacterial Activities. *Int. J. Biol. Macromol.* **2019**, *125*, 72–77. https://doi.org/10.1016/j.ijbiomac.2018.11.242.
13. Józefiak, A.; Engberg, R.M. Insect Proteins as a Potential Source of Antimicrobial Peptides in Livestock Production. A Review. *J. Anim. Feed Sci.* **2017**, *26*, 87–99. https://doi.org/10.22358/jafs/69998/2017.
14. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G.; Antes, G.; Atkins, D.; Barbour, V.; Barrowman, N.; Berlin, J.A.; Clark, J.; et al. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med.* **2009**, *6*. https://doi.org/10.1371/journal.pmed.1000097.
15. Richardson WS, Wilson MC, Nishikawa J, Hayward RS. The well-built clinical question: a key to evidence-based decisions. ACP J Club. 1995, Nov-Dec;123(3):A12-3. PMID: 7582737..
16. Van Milgen, J.; Gloaguen, M.; Le Floc'H, N.; Brossard, L.; Primot, Y.; Corrent, E. Meta-Analysis of the Response of Growing Pigs to the Isoleucine Concentration in the Diet. *Animal* **2012**, *6*, 1601–1608. https://doi.org/10.1017/S1751731112000420.
17. Mat, K.; Abdul Kari, Z.; Rusli, N.D.; Rahman, M.M.; Che Harun, H.; Al-Amsyar, S.M.; Mohd Nor, M.F.; Dawood, M.A.O.; Hassan, A.M. Effects of the Inclusion of Black Soldier Fly Larvae (Hermetia Illucens) Meal on Growth Performance and Blood Plasma Constituents in Broiler Chicken (Gallus Gallus Domesticus) Production. *Saudi J. Biol. Sci.* **2022**, *29*, 809–815. https://doi.org/10.1016/j.sjbs.2021.10.027.
18. Sedgh-Gooya, S.; Torki, M.; Darbemamieh, M.; Khamisabadi, H.; Abdolmohamadi, A. Growth Performance and Intestinal Morphometric Features of Broiler Chickens Fed on Dietary Inclusion of Yellow Mealworm (Tenebrio Molitor) Larvae Powder. *Vet. Med. Sci.* **2022**, 2050–2058. https://doi.org/10.1002/vms3.881.
19. Kim, B.; Kim, H.R.; Baek, Y.C.; Ryu, C.H.; Ji, S.Y.; Jeong, J.Y.; Kim, M.; Jung, H.; Park, S.H. Evaluation of Microwave-Dried Black Soldier Fly (Hermetia Illucens) Larvae Meal as a Dietary Protein Source in Broiler Chicken Diets. **2022**, *8*, 977–987. https://doi.org/10.3920/JIFF2021.0113.
20. Hartinger, K.; Fröschl, K.; Ebbing, M.A.; Bruschek-Pfleger, B.; Schedle, K.; Schwarz, C.; Gierus, M. Suitability of Hermetia Illucens Larvae Meal and Fat in Broiler Diets: Effects on Animal Performance, Apparent Ileal Digestibility, Gut Histology, and Microbial Metabolites. *J. Anim. Sci. Biotechnol.* **2022**, *13*, 1–16. https://doi.org/10.1186/s40104-022-00701-7.
21. Murawska, D.; Daszkiewicz, T.; Sobotka, W.; Gesek, M.; Witkowska, D.; Matusevičius, P.; Bakuła, T. Partial and Total Replacement of Soybean Meal with Full-Fat Black Soldier Fly (Hermetia Illucens l.) Larvae Meal in Broiler Chicken Diets: Impact on Growth Performance, Carcass Quality and Meat Quality. *Animals* **2021**, *11*. https://doi.org/10.3390/ani11092715.
22. Hartinger, K.; Greinix, J.; Thaler, N.; Ebbing, M.A.; Yacoubi, N.; Schedle, K.; Gierus, M. Effect of Graded Substitution of Soybean Meal by Hermetia Illucens Larvae Meal on Animal Performance, Apparent Ileal Digestibility, Gut Histology and Microbial Metabolites of Broilers. *Animals* **2021**, *11*, 1–16. https://doi.org/10.3390/ani11061628.
23. Elangovan, A.V.; Udayakumar, A.; Saravanakumar, M.; Awachat, V.B.; Mohan, M.; Yandigeri, M.S.; Krishnan, S.; Mech, A.; Rao, S.B.N.; Giridhar, K.; et al. Effect of Black Soldier Fly, Hermetia Illucens (Linnaeus) Prepupae Meal on Growth Performance and Gut Development in Broiler Chicken. *Int. J. Trop. Insect Sci.* **2021**, *41*, 2077–2082. https://doi.org/10.1007/s42690-020-00377-4.
24. Kim, Y.B.; Kim, D.H.; Jeong, S.B.; Lee, J.W.; Kim, T.H.; Lee, H.G.; Lee, K.W. Black Soldier Fly Larvae Oil as an Alternative Fat Source in Broiler Nutrition. *Poult. Sci.* **2020**, *99*, 3133–3143. https://doi.org/10.1016/j.psj.2020.01.018.
25. Pietras, M.; Orczewska-Dudek, S.; Szczurek, W.; Pieszka, M. Effect of Dietary Lupine Seeds (Lupinus Luteus L.) and Different Insect Larvae Meals as Protein Sources in Broiler Chicken Diet on Growth Performance, Carcass, and Meat Quality. *Livest. Sci.* **2021**, *250*, 104537. https://doi.org/10.1016/j.livsci.2021.104537.
26. Dabbou, S.; Lauwaerts, A.; Ferrocino, I.; Biasato, I.; Sirri, F.; Zampiga, M.; Bergagna, S.; Pagliasso, G.; Gariglio, M.; Colombino, E.; et al. Modified Black Soldier Fly Larva Fat in Broiler Diet: Effects on Performance, Carcass Traits, Blood Parameters, Histomorphological Features and Gut Microbiota. *Animals* **2021**, *11*. https://doi.org/10.3390/ani11061837.

27. Mutisya, M.M.; Agbodzavu, M.K.; Kinyuru, J.N.; Tanga, C.M.; Gicheha, M.; Hailu, G.; Salifu, D.; Khan, Z.; Niassy, S. Can Black Soldier Fly Desmodium Intortum Larvae-Based Diets Enhance the Performance of Cobb500 Broiler Chickens and Smallholder Farmers' Profit in Kenya? *Poult. Sci.* **2021**, *100*, 420–430. https://doi.org/10.1016/j.psj.2020.11.021.
28. Elahi, U.; Wang, J.; Ma, Y.B.; Wu, S.G.; Wu, J.; Qi, G.H.; Zhang, H.J. Evaluation of Yellow Mealworm Meal as a Protein Feedstuff in the Diet of Broiler Chicks. *Animals* **2020**, *10*, 1–15. https://doi.org/10.3390/ani10020224.
29. Benzertiha, A.; Kierończyk, B.; Kołodziejski, P.; Pruszyńska–Oszmałek, E.; Rawski, M.; Józefiak, D.; Józefiak, A. Tenebrio Molitor and Zophobas Morio Full-Fat Meals as Functional Feed Additives Affect Broiler Chickens' Growth Performance and Immune System Traits. *Poult. Sci.* **2020**, *99*, 196–206. https://doi.org/10.3382/ps/pez450.
30. Benzertiha A; Rawski, M.; Józefiak, A.; K., K.; Jankowski J.; Józefiak D. Tenebrio Molitor and Zophobas Morio Full-Fat Meals in Broiler Chicken Diets: Effects on Nutrients Digestibility, Digestive Enzyme Activities, and Cecal Microbiome. *Animals* **2019**, *5*, 248–253.
31. Khan, S.; Khan, R.U.; Alam, W.; Sultan, A. Evaluating the Nutritive Profile of Three Insect Meals and Their Effects to Replace Soya Bean in Broiler Diet. *J. Anim. Physiol. Anim. Nutr. (Berl).* **2018**, *102*, e662–e668. https://doi.org/10.1111/jpn.12809.
32. Brede, A.; Wecke, C.; Liebert, F. Does the Optimal Dietary Methionine to Cysteine Ratio in Diets for Growing Chickens Respond to High Inclusion Rates of Insect Meal from Hermetia Illucens? *Animals* **2018**, *8*, 1–16. https://doi.org/10.3390/ani8110187.
33. Onsongo, V.O.; Osuga, I.M.; Gachuiri, C.K.; Wachira, A.M.; Miano, D.M.; Tanga, C.M.; Ekesi, S.; Nakimbugwe, D.; Fiaboe, K.K.M. Insects for Income Generation through Animal Feed: Effect of Dietary Replacement of Soybean and Fish Meal with Black Soldier Fly Meal on Broiler Growth and Economic Performance. *J. Econ. Entomol.* **2018**, *111*, 1966–1973. https://doi.org/10.1093/jee/toy118.
34. Biasato, I.; Gasco, L.; De Marco, M.; Renna, M.; Rotolo, L.; Dabbou, S.; Capucchio, M.T.; Biasibetti, E.; Tarantola, M.; Sterpone, L.; et al. Yellow Mealworm Larvae (Tenebrio Molitor) Inclusion in Diets for Male Broiler Chickens: Effects on Growth Performance, Gut Morphology, and Histological Findings. *Poult. Sci.* **2018**, *97*, 540–548. https://doi.org/10.3382/ps/pex308.
35. Biasato, I.; Gasco, L.; De Marco, M.; Renna, M.; Rotolo, L.; Dabbou, S.; Capucchio, M.T.; Biasibetti, E.; Tarantola, M.; Bianchi, C.; et al. Effects of Yellow Mealworm Larvae (Tenebrio Molitor) Inclusion in Diets for Female Broiler Chickens: Implications for Animal Health and Gut Histology. *Anim. Feed Sci. Technol.* **2017**, *234*, 253–263. https://doi.org/10.1016/j.anifeedsci.2017.09.014.
36. Bovera, F.; Loponte, R.; Marono, S.; Piccolo, G.; Parisi, G.; Iaconisi, V.; Gasco, L.; Nizza, A. Use of Tenebrio Molitor Larvae Meal as Protein Source in Broiler Diet: Effect on Growth Performance, Nutrient Digestibility, and Carcass and Meat Traits. *J. Anim. Sci.* **2016**, *94*, 639–647. https://doi.org/10.2527/jas.2015-9201.
37. Józefiak, A.; Kierończyk, B.; Rawski, M.; Mazurkiewicz, J.; Benzertiha, A.; Gobbi, P.; Nogales-Mérida, S.; Świątkiewicz, S.; Józefiak, D. Full-Fat Insect Meals as Feed Additive – the Effect on Broiler Chicken Growth Performance and Gastrointestinal Tract Microbiota. *J. Anim. Feed Sci.* **2018**, *27*, 131–139. https://doi.org/10.22358/jafs/91967/2018.
38. Schiavone, A.; Cullere, M.; De Marco, M.; Meneguz, M.; Biasato, I.; Bergagna, S.; Dezzutto, D.; Gai, F.; Dabbou, S.; Gasco, L.; et al. Partial or Total Replacement of Soybean Oil by Black Soldier Fly Larvae (Hermetia Illucens L.) Fat in Broiler Diets: Effect on Growth Performances, Feed-Choice, Blood Traits, Carcass Characteristics and Meat Quality. *Ital. J. Anim. Sci.* **2017**, *16*, 93–100. https://doi.org/10.1080/1828051X.2016.1249968.
39. Schiavone, A.; Dabbou, S.; De Marco, M.; Cullere, M.; Biasato, I.; Biasibetti, E.; Capucchio, M.T.; Bergagna, S.; Dezzutto, D.; Meneguz, M.; et al. Black Soldier Fly Larva Fat Inclusion in Finisher Broiler Chicken Diet as an Alternative Fat Source. *Animal* **2018**, *12*, 2032–2039. https://doi.org/10.1017/S1751731117003743.
40. Kareem, K.Y.; Abdulla, N.R.; Foo, H.L.; Zamri, A.N.M.; Shazali, N.; Loh, T.C.; Alshelmani, M.I. Effect of Feeding Larvae Meal in the Diets on Growth Performance, Nutrient Digestibility and Meat Quality in Broiler Chicken. *Indian J. Anim. Sci.* **2018**, *88*, 1180–1185. https://doi.org/10.56093/ijans.v88i10.84155.
41. Ipema, A.F.; Bokkers, E.A.M.; Gerrits, W.J.J.; Kemp, B.; Elizabeth Bolhuis, J. Provision of Black Soldier Fly Larvae (Hermetia Illucens) in Different Ways Benefits Broiler Welfare and Performance, with Largest

Effects of Scattering Live Larvae. *Physiol. Behav.* **2022**, *257*, 113999. https://doi.org/10.1016/j.physbeh.2022.113999.

42. Bongiorno, V.; Gariglio, M.; Zambotto, V.; Cappone, E.E.; Biasato, I.; Renna, M.; Forte, C.; Coudron, C.; Bergagna, S.; Gai, F.; et al. Black Soldier Fly Larvae Used for Environmental Enrichment Purposes: Can They Affect the Growth, Slaughter Performance, and Blood Chemistry of Medium-Growing Chickens? *Front. Vet. Sci.* **2022**, *9*. https://doi.org/10.3389/fvets.2022.1064017.
43. Kierończyk, B.; Rawski, M.; Mikołajczak, Z.; Leciejewska, N.; Józefiak, D. Hermetia Illucens Fat Affects the Gastrointestinal Tract Selected Microbial Populations, Their Activity, and the Immune Status of Broiler Chickens. *Ann. Anim. Sci.* **2022**, *22*, 663–675. https://doi.org/10.2478/aoas-2021-0071.
44. Zhang, Y.; Yang, C.Y.; Li, C.; Xu, Z.; Peng, P.; Xue, C.; Tomberlin, J.K.; Hu, W.; Cao, Y. Black Soldier Fly (Hermetia Illucens L.) Larval Diet Improves CD8+ Lymphocytes Proliferation to Eliminate Chicken Coronavirus at an Early Infection Stage. *Vet. Microbiol.* **2021**, *260*, 109151. https://doi.org/10.1016/j.vetmic.2021.109151.
45. de Souza Vilela, J.; Andronicos, N.M.; Kolakshyapati, M.; Hilliar, M.; Sibanda, T.Z.; Andrew, N.R.; Swick, R.A.; Wilkinson, S.; Ruhnke, I. Black Soldier Fly Larvae in Broiler Diets Improve Broiler Performance and Modulate the Immune System. *Anim. Nutr.* **2021**, *7*, 695–706. https://doi.org/10.1016/j.aninu.2020.08.014.
46. Kim, B.; Bang, H.T.; Jeong, J.Y.; Kim, M.; Kim, K.H.; Chun, J.L.; Ji, S.Y. Effects of Dietary Supplementation of Black Soldier Fly (Hermetia Illucens) Larvae Oil on Broiler Health. *J. Poult. Sci.* **2021**, *58*, 222–229. https://doi.org/10.2141/jpsa.0200070.
47. Biasato, I.; Ferrocino, I.; Dabbou, S.; Evangelista, R.; Gai, F.; Gasco, L.; Cocolin, L.; Capucchio, M.T.; Schiavone, A. Black Soldier Fly and Gut Health in Broiler Chickens: Insights into the Relationship between Cecal Microbiota and Intestinal Mucin Composition. *J. Anim. Sci. Biotechnol.* **2020**, *11*, 1–12. https://doi.org/10.1186/s40104-019-0413-y.
48. Ipema, A.F.; Bokkers, E.A.M.; Gerrits, W.J.J.; Kemp, B.; Bolhuis, J.E. Long-Term Access to Live Black Soldier Fly Larvae (Hermetia Illucens) Stimulates Activity and Reduces Fearfulness of Broilers, without Affecting Health. *Sci. Rep.* **2020**, *10*, 1–13. https://doi.org/10.1038/s41598-020-74514-x.
49. Lee, J.; Kim, Y.M.; Park, Y.K.; Yang, Y.C.; Jung, B.G.; Lee, B.J. Black Soldier Fly (Hermetia Illucens) Larvae Enhances Immune Activities and Increases Survivability of Broiler Chicks against Experimental Infection of Salmonella Gallinarum. *J. Vet. Med. Sci.* **2018**, *80*, 736–740. https://doi.org/10.1292/jvms.17-0236.
50. Dabbou S.; Gai F.; Biasato I.; Capucchio M. T.; Biasibetti E.; Dezzutto D.; Meneguz M.; Plachà I.; Gasco L.; Schiavone A.; Black Soldier Fly Deffated Meal as a Dietary Protein Source for Broiler Chickens: Effects on Growth Performance, Blood Tratis, Gut Mophology and Histological Features. J. Anim. Sci Bio. 2018, 9, 49. https://doi.org/10.1186/s40104-018-0266-9.
51. Sedgh-Gooya, S.; Torki, M.; Darbemamieh, M.; Khamisabadi, H.; Karimi Torshizi, M.A.; Abdolmohamadi, A. Yellow Mealworm, Tenebrio Molitor (Col: Tenebrionidae), Larvae Powder as Dietary Protein Sources for Broiler Chickens: Effects on Growth Performance, Carcass Traits, Selected Intestinal Microbiota and Blood Parameters. *J. Anim. Physiol. Anim. Nutr. (Berl).* **2021**, *105*, 119–128. https://doi.org/10.1111/jpn.13434.
52. Biasato, I.; Ferrocino, I.; Grego, E.; Dabbou, S.; Gai, F.; Gasco, L.; Cocolin, L.; Capucchio, M.T.; Schiavone, A. Yellow Mealworm Inclusion in Diets for Heavy-Size Broiler Chickens: Implications for Intestinal Microbiota and Mucin Dynamics. *Animals* **2020**, *10*, 1–13. https://doi.org/10.3390/ani10101909.
53. Józefiak, A.; Benzertiha, A.; Kierończyk, B.; Łukomska, A.; Wesołowska, I.; Rawski, M. Improvement of Cecal Commensal Microbiome Following the Insect Additive into Chicken Diet. *Animals* **2020**, *10*. https://doi.org/10.3390/ani10040577.
54. Biasato, I.; Ferrocino, I.; Grego, E.; Dabbou, S.; Gai, F.; Gasco, L.; Cocolin, L.; Capucchio, M.T.; Schiavone, A. Gut Microbiota and Mucin Composition in Female Broiler Chickens Fed Diets Including Yellow. *Animals* **2019**.
55. Benzertiha, A.; Kierończyk, B.; Rawski, M.; Kołodziejski, P.; Bryszak, M.; Józefiak, D. Insect Oil as an Alternative to Palm Oil and Poultry Fat in Broiler Chicken Nutrition. *Animals* **2019**, *9*, 1–18. https://doi.org/10.3390/ani9030116.
56. Loponte, R.; Bovera, F.; Piccolo, G.; Gasco, L.; Secci, G.; Iaconisi, V.; Parisi, G. Fatty Acid Profile of Lipids and Caeca Volatile Fatty Acid Production of Broilers Fed a Full Fat Meal from Tenebrio Molitor Larvae. *Ital. J. Anim. Sci.* **2019**, *18*, 168–173. https://doi.org/10.1080/1828051X.2018.1502053.
57. Biasato, I.; Ferrocino, I.; Biasibetti, E.; Grego, E.; Dabbou, S.; Sereno, A.; Gai, F.; Gasco, L.; Schiavone, A.; Cocolin, L.; et al. Modulation of Intestinal Microbiota, Morphology and Mucin Composition by Dietary

Insect Meal Inclusion in Free-Range Chickens. *BMC Vet. Res.* **2018**, *14*, 1–15. https://doi.org/10.1186/s12917-018-1690-y.
58. Islam, M.M.; Yang, C.J. Efficacy of Mealworm and Super Mealworm Larvae Probiotics as an Alternative to Antibiotics Challenged Orally with Salmonella and E. Coli Infection in Broiler Chicks. *Poult. Sci.* **2017**, *96*, 27–34. https://doi.org/10.3382/ps/pew220.
59. Bellezza Oddon, S.; Biasato, I.; Imarisio, A.; Pipan, M.; Dekleva, D.; Colombino, E.; Capucchio, M.T.; Meneguz, M.; Bergagna, S.; Barbero, R.; et al. Black Soldier Fly and Yellow Mealworm Live Larvae for Broiler Chickens: Effects on Bird Performance and Health Status. *18* **2021**, *105*, 10–18. https://doi.org/10.1111/jpn.13567.
60. Colombino, E.; Biasato, I.; Ferrocino, I.; Oddon, S.B.; Caimi, C.; Gariglio, M.; Dabbou, S.; Caramori, M.; Battisti, E.; Zanet, S.; et al. Effect of Insect Live Larvae as Environmental Enrichment on Poultry Gut Health: Gut Mucin Composition, Microbiota and Local Immune Response Evaluation. **2021**.
61. Kierończyk, B.; Rawski, M.; Józefiak, A.; Mazurkiewicz, J.; Świątkiewicz, S.; Siwek, M.; Bednarczyk, M.; Szumacher-Strabel, M.; Cieślak, A.; Benzertiha, A.; et al. Effects of Replacing Soybean Oil with Selected Insect Fats on Broilers. *Anim. Feed Sci. Technol.* **2018**, *240*, 170–183. https://doi.org/10.1016/j.anifeedsci.2018.04.002.
62. Tabata, E.; Kashimura, A.; Wakita, S.; Ohno, M.; Sakaguchi, M.; Sugahara, Y.; Kino, Y.; Matoska, V.; Bauer, P.O.; Oyama, F. Gastric and Intestinal Proteases Resistance of Chicken Acidic Chitinase Nominates Chitin-Containing Organisms for Alternative Whole Edible Diets for Poultry. *Sci. Rep.* **2017**, *7*, 1–11. https://doi.org/10.1038/s41598-017-07146-3.
63. Vogel, H.; Müller, A.; Heckel, D.G.; Gutzeit, H.; Vilcinskas, A. Nutritional Immunology: Diversification and Diet-Dependent Expression of Antimicrobial Peptides in the Black Soldier Fly Hermetia Illucens. *Dev. Comp. Immunol.* **2018**, *78*, 141–148. https://doi.org/10.1016/j.dci.2017.09.008.
64. Saviane, A.; Tassoni, L.; Naviglio, D.; Lupi, D.; Savoldelli, S.; Bianchi, G.; Cortellino, G.; Bondioli, P.; Folegatti, L.; Casartelli, M.; et al. Mechanical Processing of Hermetia Illucens Larvae and Bombyx Mori Pupae Produces Oils with Antimicrobial Activity. *Animals* **2021**, *11*, 1–17. https://doi.org/10.3390/ani11030783.
65. Manniello, M.D.; Moretta, A.; Salvia, R.; Scieuzo, C.; Lucchetti, D.; Vogel, H.; Sgambato, A.; Falabella, P. Insect Antimicrobial Peptides: Potential Weapons to Counteract the Antibiotic Resistance. *Cell. Mol. Life Sci.* **2021**, *78*, 4259–4282. https://doi.org/10.1007/s00018-021-03784-z.
66. Oakley, B.B.; Lillehoj, H.S.; Kogut, M.H.; Kim, W.K.; Maurer, J.J.; Pedroso, A.; Lee, M.D.; Collett, S.R.; Johnson, T.J.; Cox, N.A. The Chicken Gastrointestinal Microbiome. *FEMS Microbiol. Lett.* **2014**, *360*, 100–112. https://doi.org/10.1111/1574-6968.12608.
67. Wei, S.; Morrison, M.; Yu, Z. Bacterial Census of Poultry Intestinal Microbiome. *Poult. Sci.* **2013**, *92*, 671–683. https://doi.org/10.3382/ps.2012-02822.
68. Apajalahti, J.; Vienola, K. Interaction between Chicken Intestinal Microbiota and Protein Digestion. *Anim. Feed Sci. Technol.* **2016**, *221*, 323–330. https://doi.org/10.1016/j.anifeedsci.2016.05.004.
69. Li, Z.; Wang, W.; Liu, D.; Guo, Y. Effects of Lactobacillus Acidophilus on Gut Microbiota Composition in Broilers Challenged with Clostridium Perfringens. *PLoS One* **2017**, *12*, 1–16. https://doi.org/10.1371/journal.pone.0188634.
70. Jung, T.H.; Park, J.H.; Jeon, W.M.; Han, K.S. Butyrate Modulates Bacterial Adherence on LS174T Human Colorectal Cells by Stimulating Mucin Secretion and MAPK Signaling Pathway. *Nutr. Res. Pract.* **2015**, *9*, 343–349. https://doi.org/10.4162/nrp.2015.9.4.343.
71. Borrelli, L.; Coretti, L.; Dipineto, L.; Bovera, F.; Menna, F.; Chiariotti, L.; Nizza, A.; Lembo, F.; Fioretti, A. Insect-Based Diet, a Promising Nutritional Source, Modulates Gut Microbiota Composition and SCFAs Production in Laying Hens. *Sci. Rep.* **2017**, *7*, 1–11. https://doi.org/10.1038/s41598-017-16560-6.
72. Foysal, M.J.; Fotedar, R.; Tay, C.Y.; Gupta, S.K. Dietary Supplementation of Black Soldier Fly (Hermetica Illucens) Meal Modulates Gut Microbiota, Innate Immune Response and Health Status of Marron (Cherax Cainii, Austin 2002) Fed Poultry-by-Product and Fishmeal Based Diets. *PeerJ* **2019**, *2019*. https://doi.org/10.7717/peerj.6891.
73. Li, Y.; Kortner, T.M.; Chikwati, E.M.; Munang'andu, H.M.; Lock, E.J.; Krogdahl, Å. Gut Health and Vaccination Response in Pre-Smolt Atlantic Salmon (Salmo Salar) Fed Black Soldier Fly (Hermetia Illucens) Larvae Meal. *Fish Shellfish Immunol.* **2019**, *86*, 1106–1113. https://doi.org/10.1016/j.fsi.2018.12.057.

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74. Su, J.; Gong, Y.; Cao, S.; Lu, F.; Han, D.; Liu, H.; Jin, J.; Yang, Y.; Zhu, X.; Xie, S. Effects of Dietary Tenebrio Molitor Meal on the Growth Performance, Immune Response and Disease Resistance of Yellow Catfish (Pelteobagrus Fulvidraco). *Fish Shellfish Immunol.* **2017**, *69*, 59–66. https://doi.org/10.1016/j.fsi.2017.08.008.
75. Tang, Q.; Xu, E.; Wang, Z.; Xiao, M.; Cao, S.; Hu, S.; Wu, Q.; Xiong, Y.; Jiang, Z.; Wang, F.; et al. Dietary Hermetia Illucens Larvae Meal Improves Growth Performance and Intestinal Barrier Function of Weaned Pigs Under the Environment of Enterotoxigenic Escherichia Coli K88. *Front. Nutr.* **2022**, *8*, 1–18. https://doi.org/10.3389/fnut.2021.812011.
76. Yu, M.; Li, Z.; Chen, W.; Rong, T.; Wang, G.; Ma, X. 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.* **2019**, *10*, 1–16. https://doi.org/10.1186/s40104-019-0358-1.
77. Valdés, F.; Villanueva, V.; Durán, E.; Campos, F.; Avendaño, C.; Sánchez, M.; Domingoz-araujo, C.; Valenzuela, C. Insects as Feed for Companion and Exotic Pets: A Current Trend. *Animals* **2022**, *12*. https://doi.org/10.3390/ani12111450.
78. Lei, X.J.; Kim, T.H.; Park, J.H.; Kim, I.H. Evaluation of Supplementation of Defatted Black Soldier Fly (Hermetia Illucens) Larvae Meal in Beagle Dogs. *Ann. Anim. Sci.* **2019**, *19*, 767–777. https://doi.org/10.2478/aoas-2019-0021.
79. Bosch, G.; Swanson, K.S. Effect of Using Insects as Feed on Animals: Pet Dogs and Cats. *J. Insects as Food Feed* **2021**, *7*, 795–805. https://doi.org/10.3920/JIFF2020.0084.
80. Moula, N.; Detilleux, J. A Meta-Analysis of the Effects of Insects in Feed on Poultry Growth Performances. *Animals* **2019**, *9*, 1–13. https://doi.org/10.3390/ani9050201.
81. Martínez Marín, A.L.; Gariglio, M.; Biasato, I.; Gasco, L.; Schiavone, A. Meta-Analysis of the Effect of Black Soldier Fly Larvae Meal in Diet on Broiler Performance and Prediction of Its Metabolisable Energy Value. *Ital. J. Anim. Sci.* **2023**, *22*, 379–387. https://doi.org/10.1080/1828051X.2023.2194909.
82. Mahmoud, A.E.; Morel, P.C.H.; Potter, M.A.; Ravindran, V. The Apparent Metabolisable Energy and Ileal Amino Digestibility of Black Soldier Fly (Hermetia Illucens) Larvae Meal for Broiler Chickens. **2023**. https://doi.org/10.1080/00071668.2022.2161873.
83. Dalmoro, Y.K.; Adams, C.B.; Haetinger, V.S.; Bairros, L.; Yacoubi, N.; Stefanello, C. Energy Values of Tenebrio Molitor Larvae Meal and Tilapia Byproduct Meal for Broiler Chickens Determined Using the Regression Method. *Anim. Feed Sci. Technol.* **2021**, *272*, 114784. https://doi.org/10.1016/j.anifeedsci.2020.114784.