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Nutritional alternatives to commercial lipid sources: Impact of the dietary inclusion of black soldier fly (*Hermetia illucens*) larvae oil on broiler chicken productivity, breast meat quality traits and caeca microbiome



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ABSTRACT

Protein production from poultry, particularly broiler chickens, is considered a key component of future global food security, due to its relatively high sustainability. However, the use of resources such as soybean oil remains a concern. Black soldier fly (*Hermetia illucens* [HI]) larvae oil represents a promising alternative due to a relatively rapid rearing cycle and ability to utilize organic waste as growth substrates. This study investigated how replacing a commercial lipid source such as soybean oil, with HI larvae oil affects broiler growth performance, meat quality traits, fatty acid (FA) profile, and caeca microbiome. A total of 552 one-d-old male Ross 308 broilers, with equal initial weights (48.89 ± 0.18 g; $P = 0.597$), were allocated to three dietary treatments with 8 replicate pens per group (23 birds/pen). All birds received the same commercial basal diet, formulated to be isoenergetic and with the same amino acid profile, differing only in the source of the supplemented oil: 100% soybean oil group (CON), 50% soybean oil + 50% HI larvae oil group (MIX), or 100% HI larvae oil group (HIO). Growth performance parameters were recorded at the end of each feeding phase (14, 28, and 42 d). At slaughter (42 d), 10 breasts (pectoralis-major muscle) and thighs (extensor-iliotibialis muscle) samples per group were collected for meat quality assessment, and caecal content samples were obtained from 8 birds/group for microbiome analysis. Growth performance metrics showed an improvement in feed conversion ratio during the starter phase for HI larvae oil-fed groups (1.54 vs. 1.45 vs. 1.46 for CON, MIX, and HIO, respectively; $P < 0.001$) and comparable performance across the trial. Meat quality traits remained within commercially acceptable ranges, with minimal effects observed, apart from variations in breast fillet redness and thigh protein oxidation. FA analysis indicated higher levels of saturated FAs in the HI groups, with a concurrent reduction in omega (n)-6 levels and a more balanced n-6 to n-3 ratio (16.47 vs. 15.18 vs. 11.60 for CON, MIX, and HIO, respectively; $P < 0.001$). The caecal microbiome revealed stable diversity across groups, with only minor shifts in relative abundance. Overall, the findings showed that HI larvae oil is an effective alternative to conventional vegetable lipid sources in poultry nutrition, with added potential to enhance growth performance during the early growth stages.

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1. Introduction

Protein from poultry, particularly meat-type broiler chickens, is projected to be a crucial component of future global food security (FAO, 2022). As the fastest-growing meat sector (Baldi et al., 2021; Windhorst, 2017), the poultry industry is tasked with balancing the increasing demand for protein while advancing sustainable

production methods (Mottet and Tempio, 2017). Although poultry production already shows higher efficiency and lower environmental impacts than other livestock systems (Leinonen and Kyriazakis, 2016; Tallentire et al., 2018), further optimisation of its sustainability is still needed. One aspect is related to resource utilisation, manufacturing, and transport of feedstuffs, as the poultry sector accounts for a major portion of the global cereal and oilseeds utilisation (Zampiga et al., 2021). Several studies advise paying special attention to the production of soybean oil, one of the most widely grown oil crops, as its cultivation, process and transportation have been associated with added environmental costs (Leinonen and Kyriazakis, 2016; Mannucci et al., 2023; Mottet and Tempio, 2017), as well as consumer concerns regarding the sustainability and ecological impact of genetically modified organisms (Zampiga et al., 2021).

In this context, the growing scientific research on insects as feed (Van Huis, 2020) underscores the potential of alternatives such as black soldier fly (*Hermetia illucens* [HI]) larvae oil to replace conventional vegetable oils in poultry diets. HI is favoured among farmed insect species due to its relatively rapid development (Hall et al., 2002) and preference for organic waste as a growth substrate, contributing to a reduced environmental footprint (Meneguz et al., 2018; Nguyen et al., 2015; Sheppard et al., 1994; Van Huis et al., 2013; Tomberlin and Van Huis, 2020). In fact, HI could be more environmentally friendly, as the range of materials used as growth substrates is limited by EU legislation due to health and safety concerns rather than by HI's utilisation capability (Van Huis and Oonincx, 2017). Furthermore, a recent study showing consumer acceptance of meat from broiler chickens fed diets containing HI, with no significant differences in flavour or texture of breast fillets, provides additional support for the use of HI in poultry feed (Roccatello et al., 2024).

Regarding the nutritional value of feedstuffs, HI larvae are valued for their high-fat content, typically reaching up to 500 g/kg. Fatty acid (FA) profile predominantly consists of saturated fatty acids (SFA), with lower amounts of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). HI larvae oil is rich in lauric acid (C12:0), palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2) as its primary FAs (Srisuksai et al., 2024; Truzzi et al., 2020). Although it differs from vegetable oils such as soybean oil, mainly composed of PUFA and MUFA accounting for about 85% of FA composition (Nil, 2016), HI larvae oil has recently been reported as a possible replacement for conventional vegetable oils in diets for fish (Herawati et al., 2023; Li et al., 2016), turkeys (Kierończyk et al., 2022), and broiler chickens (Aprianto et al., 2023; Dabbou et al., 2021; Schiavone et al., 2017). These studies have shown that HI larvae oil replacement can maintain the required high growth standards, carcass traits, and overall meat quality. The inclusion of insect-based products in monogastric livestock feeding has also been reported to impact gut microbiota composition. Insect protein meal has been reported to increase gut microbial diversity (Biasato et al., 2023), while insect oil has been associated with a positive reduction in potentially pathogenic bacteria (Dabbou et al., 2021). Creating a balanced microbial community is generally beneficial, promoting intestinal stability and enhancing resistance to pathogen colonisation (Biasato et al., 2018; Spees et al., 2013). The microbiota is also a key player in host nutrition, acting as a “metabolic organ” with various functions related to digestive performance, intestinal physiology, and intestinal immune homeostasis (Schroeder and Bäckhed, 2016; Spees et al., 2013; Yin et al., 2023). For instance, caecum, which is the intestinal segment with the highest microbial density in broiler chickens, can supply more than 10% of the host's energy demand (Rehman et al., 2007). It is therefore plausible that a balanced caecal microbiota is pivotal to the overall performance of

broilers. Considering these aspects, it was evaluated the feasibility of replacing lipids from commercial sources with HI larvae oil in broiler chicken diets by assessing growth performance indicators, breast and thigh meat quality traits, occurrence of foot pad dermatitis (FPD), and caecal metagenomic profile.

2. Materials and methods

2.1. Animal ethics statement

All experimental procedures were conducted according to the current European and Italian legislation (Dir. 2007/43/EC; Reg. 2009/1099/EC; Dir. 2010/63/EU) and approved by the Ethical Committee of the Alma Mater Studiorum–University of Bologna and by the Italian Minister of Health (No. 1145/2020).

2.2. Birds, husbandry, and growth performance traits

A total of 552 1-d-old male Ross 308 broilers, with equal initial body weight (48.89 ± 0.16 g; $P = 0.597$), were assigned to three dietary treatments (8 pens/treatment and 23 birds/pen) and reared from day of hatch until d 42. The birds were housed in randomly distributed concrete-floor pens (2.5 m² each) with wood shavings (2 kg/m²) provided as litter material. Stocking density followed the EU legislation in force (Dir. 2007/43/EC) with a maximum of 33 kg live weight/m². Each pen was provided with a circular pan feeder able to guarantee at least 2 cm of front space/bird and an independent drinking system with 1 nipple/5 birds. The environmental temperature, humidity and lighting were defined according to the management guide provided by the breeding company. Observations were conducted twice daily to record general flock condition, temperature, lighting, water, feed, litter condition and mortality. A 3-phase feeding program was used (starter: 0–14 d; grower: 15–28 d; and finisher: 29–42 d). No antibiotics or coccidiostats were included in the feed as chicks were vaccinated at the hatchery. Feed was administered in a mash form for ad-libitum consumption (water was supplied on ad-libitum basis as well). All birds were fed a commercial basal diet formulated to meet the current recommendations for fast-growing broilers (Table 1). Dry matter and ash content were determined according to the method 930.15 and 942.05, respectively (AOAC, 1990). Organic matter was then calculated as 100% minus ash. Crude protein and crude fat content were analyzed by the standard Kjeldahl copper catalyst method (method 984.13) and the Soxhlet method (method 920.39), as reported in AOAC (1990), respectively, using a Gerhardt Soxtherm (C. Gerhardt GmbH & Co., KG, Königswinter, Germany).

The diet of the control (CON) group included 100% soybean as added oil source, the diet of the MIX group included 50% soybean oil and 50% HI larvae oil, and the diet of the HIO group included 100% HI larvae as added oil source. The HI larvae oil used in this study was provided by Mutatec (Cavaillon, France). Table 2 presents the FA composition in feedstuffs across feeding phases and dietary treatments. Performance traits were recorded and calculated on a pen basis at each diet change (14 and 28 d), at slaughter (42 d) and for the overall period. The following traits were determined: body weight, weight gain, feed intake, and feed conversion ratio (FCR). Caeca content was sampled on d 21 and 42 from 8 birds/group per sampling time (1 bird/replicate pen per sampling time), following euthanasia by controlled gas (CO₂) stunning system. On d 42, birds were processed at a commercial slaughterhouse, with breast and thigh samples ($n = 10$ /group) collected for further analysis of quality traits. The incidence and severity of FPD were assessed on all birds (1 foot/bird). Severity was determined according to a 3-point evaluation scale (Ekstrand et al., 1998):

Table 1
Diet composition in groups (% as-is basis).¹

Items	Starter (0–14 d)			Grower (15–28 d)			Finisher (29–42 d)		
	CON	MIX	HIO	CON	MIX	HIO	CON	MIX	HIO
Ingredients									
Corn	34.93	34.93	34.93	33.94	33.94	33.94	35.01	35.01	35.01
Wheat	20.00	20.00	20.00	25.00	25.00	25.00	30.00	30.00	30.00
Pea	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soybean full-fat grain toasted	7.93	7.93	7.93	13.51	13.51	13.51	14.04	14.04	14.04
Soybean full-fat flower extruded	22.25	22.25	22.25	16.31	16.31	16.31	10.29	10.29	10.29
Corn gluten	3.00	3.00	3.00	0.00	0.00	0.00	0.00	0.00	0.00
Sunflower meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soybean oil	2.00	1.00	0.00	2.00	1.00	0.00	2.00	1.00	0.00
HI larvae oil	0.00	1.00	2.00	0.00	1.00	2.00	0.00	1.00	2.00
Calcium carbonate	0.45	0.45	0.45	0.58	0.58	0.58	0.82	0.82	0.82
Dicalcium phosphate	0.87	0.87	0.87	0.32	0.32	0.32	0.00	0.00	0.00
Sodium chloride	0.32	0.32	0.32	0.36	0.36	0.36	0.31	0.31	0.31
Sodium bicarbonate	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.06	0.06	0.06
Lys sulfate	0.65	0.65	0.65	0.52	0.52	0.52	0.47	0.47	0.47
L-Thr (98%)	0.18	0.18	0.18	0.16	0.16	0.16	0.13	0.13	0.13
DL-Met (99%)	0.32	0.32	0.32	0.32	0.32	0.32	0.28	0.28	0.28
Phytase	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
NSP enzyme (0.05%)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Amino acid mix (37% Arg, 31% Val, 11% Ile)	0.30	0.30	0.30	0.28	0.28	0.28	0.14	0.14	0.14
Vitamin-mineral premix ²	0.50	0.50	0.50	0.45	0.45	0.45	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Chemical composition									
Dry matter	88.47	88.38	87.95	88.46	88.41	88.09	88.69	88.73	88.55
Crude protein	22.00	21.93	21.82	20.00	19.90	20.29	18.91	19.11	19.03
Total lipid	5.58	5.51	5.17	5.63	5.65	5.67	6.63	6.25	6.11
Crude fiber	2.83	2.68	2.99	2.97	2.87	3.16	2.94	2.95	2.86
Organic matter	94.91	94.52	94.50	94.95	95.64	94.89	95.59	95.24	95.10
Thr (total)	0.98	0.98	0.98	0.88	0.88	0.88	0.76	0.76	0.76
Lys (total)	1.39	1.39	1.39	1.27	1.27	1.27	1.10	1.10	1.10
Met + Cys (total)	1.06	1.06	1.06	0.98	0.98	0.98	0.88	0.88	0.88
Ca (total)	0.68	0.68	0.68	0.58	0.58	0.58	0.54	0.54	0.54
P (total)	0.55	0.55	0.55	0.44	0.44	0.44	0.37	0.37	0.37
AME, MJ/kg	12.66	12.66	12.66	12.97	12.97	12.97	13.24	13.24	13.24

NSP = non-starch polysaccharides; AME = apparent metabolisable energy; CON = control.

¹ CON, diet included 2% soybean oil; MIX, diet included 1% soybean oil and 1% *Hermetia illucens* (HI) larvae oil; HIO, diet included 2% HI larvae oil.

² The premix provides the following per kg of feed: vitamin A (retinyl acetate), 12,500 IU; vitamin D₃, 5000 IU (i.e., cholecalciferol, 3500 IU + 25-OH D₃, 1500 IU); vitamin E (D,L- α -tocopheryl acetate), 125 mg; vitamin K (menadione sodium bisulfite), 6.75 mg; riboflavin, 9.0 mg; pantothenic acid, 22.0 mg; niacin, 75 mg; pyridoxine, 5 mg; folic acid, 3.0 mg; biotin, 0.35 mg; thiamine, 4.0 mg; vitamin B₁₂, 50 μ g; Mn, 100 mg; Zn, 102 mg; Fe, 30 mg; Cu, 15 mg; I, 2.0 mg; Se, 0.35 mg.

Table 2
Fatty acid (FA) profile in broiler chicken (% of total FA).¹

FA	Starter (0–14 d)			Grower (15–28 d)			Finisher (29–42 d)		
	CON	MIX	HIO	CON	MIX	HIO	CON	MIX	HIO
Lauric acid C12:0	0.00	6.55	14.8	0.15	7.03	13.2	0.19	5.24	12.8
Myristic acid C14:0	0.00	1.44	2.99	0.11	1.48	2.82	0.00	1.19	2.67
Palmitic acid C16:0	10.8	11.7	12.5	11.0	11.8	12.8	10.7	11.2	13.0
Palmitoleic acid C16:1	0.43	0.53	0.87	0.15	0.48	0.85	0.00	0.41	0.87
Stearic acid C18:0	3.39	3.13	2.80	3.43	3.16	2.94	3.47	3.32	3.06
Oleic acid C18:1C	25.5	23.4	20.5	25.8	23.2	20.8	26.4	24.6	21.4
Linoleic acid C18:2n6C (Ω -6)	54.2	48.3	40.9	54.0	48.0	41.7	54.8	49.3	41.5
Arachidic acid C20:0	0.38	0.30	0.28	0.29	0.27	0.26	0.29	0.30	0.24
Alpha-linolenic acid C18:3n3 (Ω -3)	4.16	3.92	3.62	4.37	3.86	4.00	3.48	3.76	3.89
Gadoleic (eicosenoic) acid C20:1	0.33	0.34	0.38	0.28	0.32	0.38	0.29	0.32	0.33
Arachidonic acid C20:4n6 (Ω -6)	0.38	0.31	0.00	0.35	0.29	0.00	0.38	0.34	0.00
Other	0.49	0.00	0.00	0.09	0.09	0.00	0.00	0.00	0.00
Total									
SFA	14.55	23.20	33.70	14.98	23.70	32.28	14.65	21.26	32.04
MUFA	26.21	24.31	21.78	26.23	24.03	22.02	26.68	25.30	22.56
PUFA	58.75	52.49	44.53	58.70	52.19	45.70	58.68	53.43	45.40
Ω -3	4.16	3.92	3.62	4.37	3.86	4.00	3.48	3.76	3.89
Ω -6	54.59	48.56	40.90	54.33	48.32	41.70	55.20	49.68	41.51
Ω -6/ Ω -3	13.12	12.37	11.30	12.44	12.51	10.43	15.86	13.22	10.68

Ω = omega; SFA = saturated fatty acids; MUFA = monounsaturated; PUFA = polyunsaturated fatty acids.

¹ CON, diet included 2% soybean oil; MIX, diet included 1% soybean oil and 1% *Hermetia illucens* (HI) larvae oil; HIO, diet included 2% HI larvae oil.

score 0 = no lesions, score 1 = mild lesions (<0.8 cm), and score 2 = severe lesions (>0.8 cm).

2.3. Fatty acids profile determination

The FA profile of feeds and breast meat was determined following the method of Christopherson and Glass (1969). Briefly, 250 µg lipids, extracted from the samples following the Folch method (Folch et al., 1957), and 500 µL methylating solution (KOH/methanol 2 N) were added to a vial, in which 5 mL hexane and 1 g anhydrous sodium sulphate were previously included. After mixing for 30 s, the vial was placed in a water bath (40 °C) for 15 min and then the sample was stirred and cooled on ice. The upper phase was collected and the fatty acid methyl esters (FAME) contained in it were separated by means of a Shimadzu GC17A gas chromatograph presenting a WP-4 Shimadzu integration system (Shimadzu Corp., Tokyo, Japan), equipped with a Varian CPSIL88 capillary column (100 m long, 0.25 mm i.d., 0.20 mm thick film; Varian Medical Systems, Inc., Walnut Creek, CA, USA) and a flame ionization detector. As for the operating conditions, the oven temperature was maintained at 170 °C for 15 min, then increased to 190 °C at 1 °C/min, and finally raised to 220 °C (5 °C/min rate) and kept for 17 min. The injector and detector temperatures were 270 and 300 °C, respectively. Helium was used as the carrier gas at a constant flow rate of 1.7 mL/min. FAs were identified using PUFA-2 FA methyl ester standards (Matreya LLC, Pleasant Gap, PA, USA) and quantified based on the concentration of the internal standard methyl nonadecanoate 98 % (C19:0) (Sigma Chemical Co., St. Louis, MO, USA), which was added prior to lipid extraction. Results were reported as a percentage of each FAME of the total FAME detected.

2.4. Meat quality traits

The ultimate pH of each pectoralis major muscle was measured at the anterior region using a portable pH-meter (HI-98163, Hanna Instruments Ltd., Leighton Buzzard, UK), previously calibrated with standard buffer solutions at pH 4.01 and 7.01. Colour was measured in triplicate at the same position using a portable colorimeter Chroma Meter CR-400 Konica Minolta Sensing device (Minolta Sensing Inc., Osaka, Japan) under standardized conditions (i.e., illuminant C and CIE 2° standard observer). Colour was then expressed as lightness (L*), redness (a*), and yellowness (b*) (CIE, 1976).

Water holding capacity in breast fillets was assessed by measuring drip and cooking losses. A parallelepiped meat cut (8 cm × 4 cm × 3 cm, weighing about 80 g) was excised from the antero-ventral position of each breast muscle. After being individually weighed, samples were placed in plastic boxes over sieved plastic racks and stored for 48 h at 4 ± 1 °C. Then, samples were blotted (to remove any surface fluid), weighed and drip loss was calculated as the percentage of weight lost during refrigerated storage (Petracci and Baéza, 2011). Each sample was individually vacuum-packed and cooked in a water bath at 75 °C until the internal temperature reached 72 °C, after which cooking loss was calculated as the percentage of weight lost during cooking.

To assess oxidative phenomena in thigh muscles, carbonyl content and thiobarbituric acid reactive substances (TBARS) were measured to determine the oxidation levels of the protein and lipid fractions, respectively (Bao and Ertbjerg, 2015; Soglia et al., 2016).

2.5. Caeca metagenome and bioinformatics

The DNA from the caecal content was extracted using the QIAmp DNA Stool Mini Kit (Qiagen S.r.l., Milan, Italy), which

utilizes a bead-beating method. To assess the DNA yield in terms of both quantity and quality, the extracted DNA from each sample was quantified using a BioSpectrometer (Eppendorf S.r.l., Milan, Italy). The total DNA was then fragmented and tagged with sequencing indexes and adapters via the Nextera XT DNA Library Preparation Kit (Illumina Inc., San Diego, CA). Shotgun metagenomic sequencing was conducted on the NextSeq500 platform (Illumina) in paired-end mode (2 × 150 bp). The sequencing process generated a total of 460.8 gigabases, corresponding to an average of 64.0 million reads per sample.

Metagenome raw sequencing data are available at the SRA-NCBI portal. Project: PRJNA1225454 (<https://dataview.ncbi.nlm.nih.gov/object/PRJNA1225454?reviewer=jsqr5h2qafjahkktkrg614hmr>). Demultiplexing and sequence adapters trimming of raw reads were performed using the Illumina bcl2fastq Conversion Software. Cleaned reads were then mapped against the corresponding host genome (galGal6, <https://hgdownload.soe.ucsc.edu/goldenPath/galGal6/bigZips/>) with bowtie2 (Langmead and Salzberg, 2012) algorithm. Unmapped reads were analyzed using the Kaiju pipeline (Menzel et al., 2016) to determine taxonomical abundance at the phylum, class, order, family, and genus by comparing metagenomics reads against the nr_euk database. The abundance of each taxon was calculated as relative abundance.

A suite of R packages was adopted to perform the downstream analysis. Alpha-diversity was calculated using the Shannon metric, implemented through the 'diversity' function in the 'vegan' package. The same package was used to compute Bray–Curtis beta distance via the 'vegdist' function. To perform principal coordinate analysis (PCoA), beta-diversity values were transformed using classical multidimensional scaling (function 'cmdscale' from the 'stats' package). A 3D projection plot was then generated using the 'rgl' utility.

2.6. Statistical analysis

All data were analyzed using IBM SPSS Statistics (version 28.0.1.1–14). Statistical models followed the structure described by Steel et al. (1997). For one-way ANOVA analyses, the model used was:

$$Y = \mu + \alpha + \varepsilon,$$

where Y is the dependent variable; μ is the overall mean; α is the fixed effect of diet; and ε is the random error. Data were tested for normal distribution using the Shapiro–Wilk test, whereas homoscedasticity was assessed by means of the Levene test. Growth performance data were analyzed using one-way ANOVA followed by Tukey's post-hoc test, considering the diet as the experimental factor and replicates as the experimental unit. Data on meat quality and fatty acid profiles were analyzed using one-way ANOVA followed by Tukey's post-hoc test, considering the diet as the experimental factor. Trend analyses (linear fit and quadratic fit) were also evaluated. FPD data were analyzed for severity using a 3-point scale (0 = none, 1 = mild, 2 = severe), with the sampled animal as the experimental unit. A 95 % confidence interval and Pearson's chi-squared test assessed significance. To detect differences in the relative abundance of caeca microbiome genera, a two-way ANOVA was performed, considering the factors of treatment groups and age, using the model:

$$Y = \mu + \alpha + \beta + \alpha\beta + \varepsilon,$$

where Y is the dependent variable; μ is the overall mean; α is the effect of diet; β is the effect of age; $\alpha\beta$ is the interaction effect; and ε is the random error. Additionally, to examine differences within

age, a one-way ANOVA was conducted. Significance level for all tests was considered at $P < 0.05$.

3. Results

3.1. Growth performance and FPD occurrence

Analysis of growth performance results revealed that the inclusion of HI larvae oil did not adversely affect overall broiler performance (Table 3). However, birds fed HI oil (MIX and HIO) diets achieved significantly improved feed efficiency during the starter phase, with lower FCR values compared to the CON group (1.45 and 1.46 vs. 1.54, respectively; $P < 0.001$). Across the grower and finisher phases, as well as at slaughter, body weight, daily weight gain, feed intake, and mortality did not differ significantly among treatments, confirming that HI oil maintained normal growth performance throughout the rearing period ($P > 0.05$).

As a trait affecting performance and welfare, the incidence and severity of FPD were also evaluated (Table 4). Chi-square test indicated no significant differences between groups, with over 95% of birds showing no lesions and less than 1% exhibiting severe lesions ($P = 0.059$).

3.2. Fatty acids (FA) profile in breast fillets

Differences in key FAs in breast meat are shown in Table 5. The level of lauric acid was significantly higher in the HIO group, being more than twice that of the MIX group and approximately 25-fold higher than in the CON group, which had the lowest level ($P < 0.001$). A similar pattern was observed for myristic acid and

myristoleic acid, with levels in the HIO group nearly double those in the MIX group and more than triple those in CON ($P < 0.001$). Palmitic acid was significantly higher in the HIO and MIX groups compared to CON ($P < 0.001$), whereas linoleic acid showed an opposite trend, with significantly lower levels in the HIO and MIX groups compared to CON ($P < 0.001$). Palmitoleic and gadoleic acids were significantly higher in the HIO group compared to CON ($P < 0.001$ and $P = 0.005$, respectively). As for total SFA levels, these were significantly higher in the HIO group, with the MIX group intermediate and the CON group showing the lowest levels ($P < 0.001$). Total MUFA levels were consistent across groups, whereas total PUFA levels opposed SFA levels, with the HIO group having the lowest levels, followed by MIX, and the highest levels in CON ($P < 0.001$). The omega (n)-6 to n-3 ratio was significantly lower in the HIO group, with the ratio being 1.3-fold higher in MIX and 1.4-fold higher in CON ($P < 0.001$). This was mainly due to the

Table 4
Foot pad dermatitis (FPD) incidence and severity in 42-d-old broilers (%).

Scores ¹	Groups ²			P-value
	CON	MIX	HIO	Chi-squared
0-No lesions	98.18	99.39	95.03	0.059
1-Mild lesions	1.21	0.00	4.35	
2-Severe lesions	0.61	0.61	0.62	

¹ Count data of FPD, with severity rate in a score of 0 (none), 1 (mild), and 2 (severe) were analyzed with Pearson's chi-squared test (confidence interval of 95%).

² CON, diet included 2% soybean oil; MIX, diet included 1% soybean oil and 1% *Hermetia illucens* (HI) larvae oil; HIO, diet included 2% HI larvae oil; $n = 165, 164$ and 161 birds in CON, MIX and HIO groups, respectively (1 foot/bird).

Table 3
Growth performance of broilers¹.

Items	Groups			SEM	P-value		
	CON	MIX	HIO		ANOVA	Linear	Quadratic
Hatchling weight, g/bird	48.98	48.68	49.02	0.157	0.597	0.700	0.354
Starter period (0–14 d)							
Body weight, g/bird	411.45	423.88	416.44	3.510	0.322	0.542	0.172
Daily weight gain, g/bird	25.82	26.72	26.42	0.271	0.370	0.352	0.290
Daily feed intake, g/bird per d	39.63	38.79	38.56	0.360	0.508	0.276	0.715
Feed intake, kg/bird	0.56	0.54	0.54	0.005	0.508	0.276	0.715
FCR	1.54 ^a	1.45 ^b	1.46 ^b	0.012	<0.001	<0.001	0.029
Mortality, %	1.09	0.54	0.54	0.020	0.799	0.568	0.741
Grower period (15–28 d)							
Body weight, g/bird	1430	1496	1465	11.670	0.094	0.220	0.066
Daily weight gain, g/bird	72.76	75.97	74.50	0.695	0.209	0.328	0.138
Daily feed intake, g/bird per d	112.41	115.49	113.00	0.910	0.279	0.761	0.122
Feed intake, kg/bird	1.57	1.62	1.58	0.013	0.279	0.761	0.122
FCR	1.55	1.52	1.52	0.013	0.534	0.305	0.263
Mortality, %	0.54	1.63	1.09	0.030	0.574	0.599	0.678
Finisher period (29–42 d)							
Body weight, g/bird	2892	2990	2959	19.7	0.105	0.150	0.109
Daily weight gain, g/bird	104.97	106.47	106.59	0.880	0.583	0.357	0.648
Daily feed intake, g/bird per d	185.44	187.43	189.31	1.110	0.372	0.166	0.982
Feed intake, kg/bird	2.60	2.62	2.65	0.020	0.372	0.166	0.982
FCR	1.77	1.76	1.78	0.010	0.921	0.791	0.766
Mortality, %	0.00	0.60	1.73	0.017	0.308	0.140	0.781
Entire trial (0–42 d)							
Body weight	2892	2990	2959	19.7	0.105	0.150	0.109
Daily weight gain	67.55	69.89	69.13	0.470	0.108	0.150	0.108
Daily feed intake, g/bird per d	110.90	111.99	111.68	0.660	0.768	0.620	0.608
Feed intake, kg/bird	4.72	4.78	4.77	0.030	0.642	0.480	0.544
FCR	1.66	1.63	1.64	0.008	0.516	0.420	0.421
Mortality, %	1.63	2.72	3.26	0.020	0.637	0.360	0.858

FCR = feed conversion ratio; CON= control; SEM = standard error of the mean.

Within a row, means without a common superscript letter differ at $P < 0.05$ by one-way ANOVA analysis. Data are presented as the mean of 8 replicate pens/group (arranged in a completely randomized block design with 23 birds/replicate pen) and corrected for mortality.

¹ CON, diet included 2% soybean oil; MIX, diet included 1% soybean oil and 1% *Hermetia illucens* (HI) larvae oil; HIO, diet included 2% HI larvae oil.

Table 5
Fatty acid (FA) profile in breast meat of 42-d-old broilers (% of total FA).

FA	Groups ¹			SEM	P-value		
	CON	MIX	HIO		ANOVA	Linear	Quadratic
Lauric acid C12:0	0.14 ^c	1.56 ^b	3.64 ^a	0.272	<0.001	<0.001	0.050
Myristic acid C14:0	0.41 ^c	1.15 ^b	2.09 ^a	0.131	<0.001	<0.001	0.192
Myristoleic acid C14:1	0.13 ^b	0.21 ^b	0.38 ^a	0.024	<0.001	<0.001	0.128
Pentadecanoic acid C15:0	0.06	0.10	0.07	0.009	0.236	0.651	0.101
Palmitic acid C16:0	19.60 ^b	21.65 ^a	21.99 ^a	0.270	<0.001	<0.001	0.053
Palmitoleic acid C16:1	4.51 ^b	5.24 ^{ab}	5.58 ^a	0.147	<0.010	<0.001	0.478
Margaric acid C17:0	0.07	0.07	0.11	0.011	0.181	0.107	0.383
Heptadecenoic acid C17:1	0.08	0.05	0.07	0.010	0.439	0.731	0.217
Stearic acid C18:0	6.43	6.22	6.28	0.153	0.853	0.690	0.687
Oleic acid C18:1C	35.28	34.93	34.42	0.377	0.655	0.363	0.924
Linoleic acid C18:2n6C (Ω-6)	27.49 ^a	23.48 ^b	19.69 ^c	0.657	<0.001	<0.001	0.876
Arachidic acid C20:0	0.05 ^a	0.01 ^b	0.02 ^{ab}	0.008	0.052	0.087	0.067
Alpha-linolenic acid C18:3n3 (Ω-3)	1.46	1.40	1.45	0.025	0.631	0.816	0.352
Gadoleic acid (eicosenoic acid) C20:1	0.21 ^b	0.34 ^{ab}	0.43 ^a	0.028	0.005	<0.010	0.773
Gamma-linolenic acid C18:3n6 (Ω-6)	0.21	0.32	0.25	0.030	0.451	0.696	0.230
Heneicosanoic acid C21:0	0.00	0.01	0.04	0.007	0.068	0.050	0.552
Eicosadienoic acid C20:2n6 (Ω-6)	0.21	0.22	0.20	0.010	0.833	0.794	0.595
Arachidonic acid C20:4n6 (Ω-6)	1.68	1.53	1.36	0.144	0.659	0.366	0.988
EPA C20:5n3 (Ω-3)	0.03	0.01	0.13	0.033	0.265	0.211	0.308
Lignoceric acid C24:0	0.39	0.33	0.39	0.040	0.801	0.960	0.509
Nervonic acid C24:1	0.10	0.06	0.08	0.014	0.462	0.635	0.249
DPA C22:5n3 (Ω-3)	0.19	0.18	0.26	0.025	0.312	0.233	0.370
DHA C22:6n3 (Ω-3)	0.14	0.04	0.09	0.021	0.197	0.374	0.111
Other	1.13	0.79	0.93	0.072	0.291	0.384	0.183
Summary							
Total SFA	27.17 ^c	31.10 ^b	34.63 ^a	0.628	<0.001	<0.001	0.758
Total MUFA	40.31	40.83	40.96	0.475	0.854	0.593	0.864
Total PUFA	31.41 ^a	27.28 ^b	23.48 ^c	0.719	<0.001	<0.001	0.850
Total Ω-3	1.82	1.73	1.93	0.069	0.483	0.501	0.328
Total Ω-6	29.59 ^a	25.55 ^b	21.49 ^c	0.714	<0.001	<0.001	0.996
Ω-6/Ω-3	16.47 ^a	15.18 ^a	11.60 ^b	0.515	<0.001	<0.001	0.145

Ω = omega, n; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; SFA= saturated fatty acids; MUFA= monounsaturated; PUFA= polyunsaturated fatty acids; SEM = standard error of the mean.

Within a row, means without a common superscript letter differ at $P < 0.05$ by one-way ANOVA analysis, $n = 10$.

¹ CON, diet included 2% soybean oil; MIX, diet included 1% soybean oil and 1% *Hermetia illucens* (HI) larvae oil; HIO, diet included 2% HI larvae oil.

significantly lower n-6 levels of the HIO group compared to MIX and CON ($P < 0.001$).

3.3. Meat quality

Results concerning the quality of both breast and thigh meat are presented in Table 6. For breast meat, ultimate pH was consistent across groups, with no significant differences observed ($P = 0.108$). Similarly, L* and yellowness b*, showed no significant variation, while a* was higher in the MIX group compared to the CON group (1.62 vs. 1.05; $P = 0.048$), with the HIO group showing an intermediate value. Drip loss and cooking loss percentages were comparable among groups, indicating similar water-holding capacity. In thigh meat, ultimate pH and colour values were not significantly different between groups. However, oxidative stability indicators showed some variation; the MIX group presented a significantly higher carbonyl content than HIO (2.89 vs. 2.12 nmol/mg, respectively; $P = 0.010$), with CON presenting an intermediate value. As for TBARS, values were not significantly different among groups ($P = 0.092$).

3.4. Caeca microbiome

The metagenome analysis examined differences in caeca microorganism genomes on 21- and 42-d-old broilers (Fig. 1). Across treatment groups and time points, only limited microbial shifts were observed, as indicated by the relative abundance (RA) heat map of the top 25 genera. A two-way ANOVA analysis was

performed to detect differences between groups and days, revealing significant differences in nine bacterial genera. However, eight were related to the effect of age. The RA was significantly higher on d 21 for *Lachnoclostridium* ($P = 0.003$), *Mediterraneibacter* ($P < 0.001$), *Clostridium* ($P = 0.006$), *Anaerotignum* ($P = 0.013$), and *Lactobacillus* ($P = 0.009$). Three had significantly higher RA on d 42: *Intestinimonas* ($P < 0.001$), *Subdoligranulum* ($P = 0.002$), and *Pseudoflavonifractor* ($P = 0.016$). Only three genera demonstrated an interaction between day and group: RA of *Agathobaculum* was significantly higher in MIX on d 42 ($P = 0.034$), RA of *Mediterraneibacter* was significantly higher in CON on d 21 ($P = 0.038$), and RA of *Acutalibacter* was significantly lower in CON on d 21 ($P = 0.003$). To exclude the age factor, which might mask differences, a one-way ANOVA analysis among dietary treatments on each day was also performed. This analysis revealed significant differences in four genera. On d 21, RA of *Mediterraneibacter* in the CON group was significantly higher than in the HIO group ($P = 0.039$), RA of *Ruminococcus* and *Anaerotignum* in the CON group was significantly higher than in the MIX group ($P = 0.026$ and $P = 0.024$), and the RA of *Acutalibacter* was significantly lower in CON compared to MIX ($P = 0.016$). Further evaluation of bacterial diversity through alpha-diversity analysis, which reflects microbial community richness, showed no significant differences between groups on d 21 and 42, as indicated by the Shannon diversity index (P -adjusted = 0.289 and = 0.807, respectively). Beta-diversity analysis followed by PCoA plots provided a clear visualisation, showing no clustering patterns between groups on d 21 and 42.

Table 6
Meat quality traits in 42-d-old broilers.

Items	Groups ¹			SEM	P-value		
	CON	MIX	HIO		ANOVA	Linear	Quadratic
Breast meat							
Ultimate pH	5.62	5.65	5.56	0.018	0.108	0.137	0.127
L*	57.38	57.30	57.55	0.396	0.969	0.865	0.854
a*	1.05 ^b	1.62 ^a	1.17 ^{ab}	0.100	0.048	0.613	0.050
b*	6.94	6.41	6.83	0.186	0.487	0.806	0.244
Drip loss, %	2.41	2.46	2.72	0.173	0.739	0.475	0.769
Cooking loss, %	27.79	28.94	29.04	0.467	0.490	0.286	0.598
Thigh meat							
Ultimate pH	6.33	6.40	6.41	0.024	0.396	0.215	0.581
L*	54.46	54.48	54.79	0.303	0.893	0.673	0.834
a*	3.67	3.36	3.51	0.161	0.752	0.702	0.519
b*	2.32	2.26	3.18	0.203	0.119	0.085	0.247
TBARS, mg MDA/kg meat ¹	1.25	1.46	1.73	0.090	0.092	0.050	0.847
Carbonyls, nmol/mg protein ¹	2.40 ^{ab}	2.89 ^a	2.12 ^b	0.109	0.010	0.251	<0.001

L* = lightness; a* = redness; b* = yellowness; TBARS = thiobarbituric acid reactive substances; MDA = malondialdehyde; SEM = standard error of the mean. Within a row, means without a common superscript letter differ at P < 0.05 by one-way ANOVA analysis, n = 10.

¹ CON, diet included 2% soybean oil; MIX, diet included 1% soybean oil and 1% *Hermetia illucens* (HI) larvae oil; HIO, diet included 2% HI larvae oil.

4. Discussion

This study investigated how replacing soybean oil affects broiler chicken growth performance, meat quality, FA profiles, and caeca microbiome. The findings indicated that dietary inclusion of HI oil modulated the FA composition of breast muscle while maintaining overall performance and meat quality traits. Additionally, limited effects on the caeca microbiome were detected. These observations corroborate the pivotal role of dietary lipid fraction in modulating the breast meat FA profile. Nevertheless, a contribution from gut microbiome-mediated effects cannot be entirely ruled out.

Growth performance indicators (i.e., body weight, weight gain, feed intake, and FCR) confirmed the expectations, showing that HI larvae oil can replace soybean oil. This aligns with previous trials conducted in fish (Li et al., 2016; Herawati et al., 2023), turkeys (Kierończyk et al., 2022), and broiler chickens (Aprianto et al., 2023; Dabbou et al., 2021; Schiavone et al., 2017). The fact that HI larvae oil contains a similar energy density to that of soybean oil provides further evidence for the assumption of comparable performance outcomes (Kierończyk et al., 2023). Nevertheless, a notable improvement in FCR was observed in the early growth phase (starter period) for broilers receiving HI larvae oil, whether partially (MIX, 50%) or fully (HIO, 100%) replacing soybean oil. This improvement may be attributed to the high levels of lauric acid (C12:0), reaching nearly 15% in the HIO group compared to negligible amounts in the CON group diet. Medium-chain FAs (6–12 carbon atoms), such as lauric acid, are more effectively absorbed and metabolised as compared to long-chain FAs (Ravindran et al., 2016; Zeitz et al., 2015), likely providing a more efficient energy source during the critical post-hatch period. Additionally, the antimicrobial properties related to medium-chain FAs, previously shown in piglets (Zentek et al., 2011), may also contribute to improved gut health and energy utilisation. Nevertheless, it is important to note that performance responses to medium-chain FAs can vary, depending on factors such as intestinal absorption site, inclusion level, and the total FA composition (Gomez-Osorio et al., 2021).

The FA profiles in feedstuffs and breast meat demonstrated a consistent pattern linked to the inclusion of HI larvae oil in broiler diets. As expected, these profiles in feeds translated into corresponding shifts in the breast muscle FA profile, with the HIO group exhibiting the highest levels of SFAs, particularly lauric, myristic, and palmitic acids. PUFAs, such as linoleic acid (C18:2, omega n-6),

exhibited an opposite trend, being significantly lower in both feedstuffs and breast filets of the HI groups compared to CON. These findings are comparable to those of other studies, such as Schiavone et al. (2017) and Dabbou et al. (2021), which reported similar effects of HI larvae oil inclusion. The n-6 to n-3 ratio also decreased in the HI groups, with the HIO group maintaining the lowest ratios across both feed and muscle. This reduction reflected the dietary composition and suggested improved balance in omega FA profiles. An adequately maintained n-6 to n-3 ratio is valuable for several biological, physiological, developmental, and beneficial health functions (Alagawany et al., 2019; Kalakuntla et al., 2017; Simopoulos, 2011). Conversely, it is important to address the higher SFA content in breast meat from birds fed HI larvae oil (particularly HIO), a somewhat expected outcome as the larvae are naturally rich in SFAs, which have been hypothesised to be able to synthesise themselves (Ewald et al., 2020). While broiler performance is maintained, the potential implications of increased SFA intake on human health should be considered. Although high SFA consumption has been associated with increased cardiovascular risk, its overall impact depends on dietary context and is generally considered non-harmful when consumed in moderation, within the recommended SFA intake limit of less than 10% of total energy (Maki et al., 2021).

Meat quality traits were only marginally affected by the dietary treatments. In detail, differences in pH and colour were negligible and of low practical importance. These findings align with previous studies by Schiavone et al. (2017) and Dabbou et al. (2021), who reported no significant effects on breast meat quality traits. Water-holding capacity, assessed through drip and cooking losses, remained unaffected, supporting the suitability of HI oil for maintaining meat texture and juiciness. For thigh meat, dietary treatment did not affect the main quality traits or the oxidative status of the lipid fraction. However, the lower carbonyl content observed in HIO may be attributed to the differences in the FA profile of thigh meat in this group, which showed a remarkable increase in SFA and a reduction in PUFA content. Recent studies by Schäfer et al. (2023a), Schäfer et al. (2023b) align with our findings of higher SFA levels and suggest that the increase in their proportion, induced by HI oil inclusion, may ultimately improve meat quality from a technological perspective. In particular, complete replacement of soybean oil with HI oil reduced the formation of cholesterol oxidation products and lipid peroxidation markers in heat-processed breast muscle, thereby enhancing oxidative stability during cooking (Schäfer et al., 2023b).

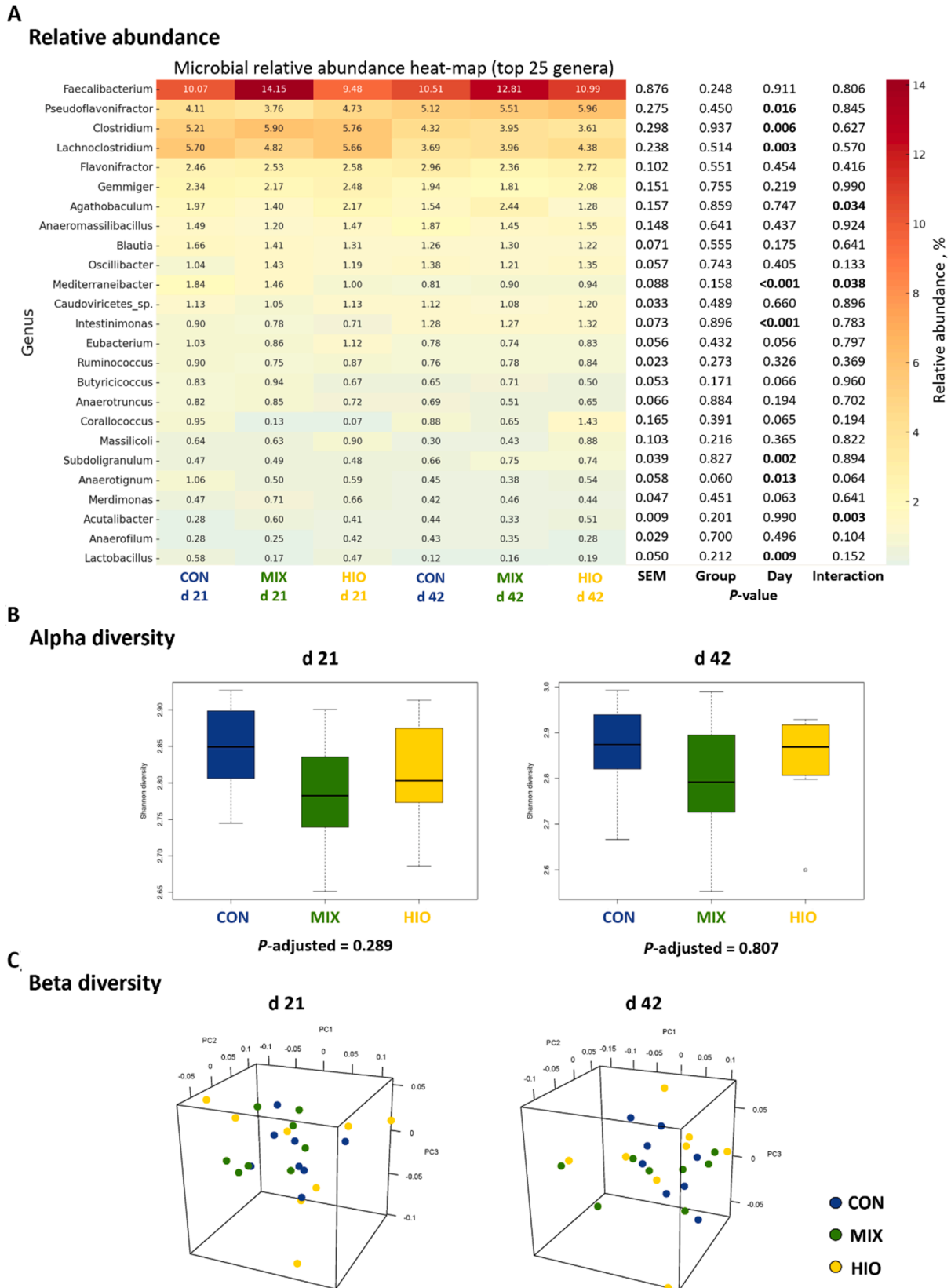


Fig. 1. Metagenome analysis in caeca samples of 21-d and 42-d old broilers. (A) Heat map of the top 25 genera based on the average relative abundance. (B) Beta-diversity is visualized by principal coordinate analysis (PCoA) plots. (C) Alpha-diversity compares the genus richness between treatments. CON, diet included 2% soybean oil; MIX, diet included 1% soybean oil and 1% *Hermetia illucens* (HI) larvae oil; HIO, diet included 2% HI larvae oil. SEM = standard error of the mean. Differences in relative abundance data between groups and days were examined by two-way ANOVA analysis ($P < 0.05$); $n = 8$.

The metagenomic analysis in this study highlighted the feasibility of using HI larvae oil as an alternative to conventional vegetable oil sources in broiler diets. The alpha- and beta-diversity analyses revealed no significant clustering patterns across groups, indicating microbial stability. Despite limited microbial shifts across groups, the overall composition and diversity of the caecal microbiome remained stable. Creating a balanced microbial community is crucial for promoting intestinal stability and enhancing resistance to pathogen colonisation (Biasato et al., 2018; Spees et al., 2013). The results are generally consistent with those of Kierończyk et al. (2022) and Dabbou et al. (2021), who reported minimal effects of HI oil inclusion on microbiome composition of turkeys and broilers, respectively. While this study did not find clear correlations between microbial enrichment and muscle FA composition, effects on microbial populations in other parts of the gastrointestinal tract cannot be ruled out.

5. Conclusion

This study demonstrated the feasibility of replacing soybean oil with HI larvae oil in broiler diets. Growth performance was maintained across dietary treatments, with a notable improvement in FCR during the starter phase for birds receiving partial (MIX, 50%) or full (HIO, 100%) inclusion of HI larvae oil. This is possibly linked to the efficient metabolism and gut health-promoting properties of medium-chain FAs such as lauric acid, which are abundant in HI larvae oil. Meat quality traits, including pH, colour, and water-holding capacity, were unaffected by the treatments, remaining commercially acceptable, with some variations in redness and protein oxidation levels. Dietary inclusion of HI larvae oil significantly influenced the breast meat FA profile, reducing n-6 to n-3 ratios while increasing SFA content. Metagenomic analysis revealed that the caecal microbiome composition and diversity remained stable among dietary treatments. Overall, the findings showed that HI larvae oil is a suitable alternative to conventional vegetable oils in poultry nutrition.

Credit Author Statement

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Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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