



Research Article



Ground pupal exuviae from *Hermetia illucens* as a functional feed additive for laying hens: Effects on performance, post-mortem traits, egg quality, nutrient digestibility and caecal short-chain fatty acid production

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ABSTRACT

The present study evaluated the effects of purified chitin and ground pupal exuviae from *Hermetia illucens* as functional feed additives in laying hens. A total of 120 ISA Brown hens (25 weeks of age at the beginning of the trial; 28–39 weeks during the experimental period) were allocated to three dietary treatments for 11 weeks: a control diet (CTRL), a diet supplemented with purified chitin (CHIT; 7 g/kg), and a diet supplemented with ground exuviae (EXU; 28 g/kg); both supplemented diets were formulated to provide approximately 1 g of chitin/hen/day. Productive performance, egg quality, apparent ileal digestibility, abdominal fat deposition, organ traits, and caecal short-chain fatty acid (SCFA) concentrations were evaluated. The EXU group showed a lower feed conversion ratio compared with the control group, whereas feed intake and oviposition rate did not differ significantly among treatments. Both CHIT and EXU reduced crude protein digestibility compared with CTRL ($P < 0.01$), with no differences between chitin sources. Exuviae supplementation increased abdominal fat deposition ($P < 0.05$) and significantly enhanced total SCFA production. Egg quality traits were largely unaffected by dietary treatments. In conclusion, while purified chitin and exuviae exerted similar effects on protein digestibility, ground pupal exuviae induced a stronger fermentative response, likely reflecting the contribution of the whole exuviae matrix rather than chitin alone. These findings support the potential of *H. illucens* exuviae as a functional ingredient in laying hen nutrition.

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1. Introduction

In recent years, increasing attention has been directed toward the use of sustainable feed ingredients in animal nutrition. In laying hens, nutritional strategies combining feed processing and functional supplementation have been shown to enhance egg production while also reducing the environmental impact of the production system (Attia et al., 2025a). Insects, and in particular *Hermetia illucens*, also known as Black Soldier Fly (BSF), have emerged as a promising alternative source of protein, due to its ability to efficiently bioconvert a wide range of organic substrates into high-value biomass (Addeo et al., 2021; Spranghers et al., 2016; Scala et al., 2020; Scieuzo et al., 2023; Franco et al., 2022). Moreover, BSF performance and physiological traits can be influenced by rearing conditions (e.g., age and feeding level), which may contribute to variability in insect-derived materials (Li et al., 2024). Beyond nutritional value, the safety profile of BSF-derived products depends on the rearing substrate, as mineral composition and potentially toxic elements may vary and be transferred to larvae-derived materials (Addeo et al., 2024b). The larvae are characterized by a high protein content, ~40–45%, and a balanced amino acid profile (Spranghers et al., 2016; Scala et al., 2020; Scieuzo et al., 2023), making them a promising alternative to conventional protein sources in monogastric and ruminant nutrition (Jayanegara et al., 2017). In addition to proteins and lipids, *H. illucens* biomass contains chitin, a polysaccharide composed of β -(1–4)-linked N-acetyl-D-glucosamine units, primarily located in the cuticle (Koide, 1998; Hahn et al., 2018). Chitin is the main component of the exoskeleton of arthropods, and its global annual production has been estimated at levels comparable to those of cellulose, exceeding 10 billion tons (Iber et al., 2022). Although it is of animal origin, chitin shares with dietary plant-derived fibers the property of being a polysaccharide that cannot be digested by the enzymes of the mammalian digestive system (Šimůnek and Bartoňová, 2005). Chitin and its derivatives, of which the main one is chitosan, exhibit antimicrobial, anti-inflammatory, and immunostimulatory properties, which can improve growth performance and health in poultry. The benefits of introducing these polymers into poultry feed include enhanced body weight gain and conversion ratios (Świątkiewicz et al., 2015). Chitin-containing feed formulations have been associated with improved digestion and absorption, reduced fat and cholesterol intake, and enhanced immunity in chickens, contributing to healthier growth (Razdan and Pettersson, 1994). Chitin, indeed, has been recognized as functional component with prebiotic properties, capable of modulating gut microbiota composition, enhancing short-chain fatty acid (SCFA) production in the gastrointestinal tract, and influencing immune responses in vertebrates, thereby improving nutrient absorption and overall performance (Sanchez-Esteban et al., 2001; Xu et al., 2020; San-karganesh et al., 2025). The chemical composition of BSF-derived products, including lipid quality, is influenced by the rearing substrate, which can affect the nutritional and functional value of insect-derived ingredients (Addeo et al., 2024a). In poultry, dietary chitin has been reported to affect growth performance of broiler chickens, influence gut morphology, microbiota composition, nutrient digestibility, and immune response (Nuengjamnong and Angkanaporn, 2018; Józefiak et al., 2018; Gasco et al., 2019). Indeed, dietary compounds, including those derived from insects, can modulate the host immune system by stimulating immune cells such as host defense peptides (HDPs), including defensins and cathelicidins, which possess antimicrobial and immunomodulatory activities (Schauber et al., 2003; Zeng et al., 2013). Such peptides are considered promising candidates as alternatives to conventional antibiotics and potential biomarkers for genetic selection (Zeng et al., 2013). In particular, butyrate induces HDP expression, with peak activity in chicken cells (Sunkara et al., 2011, 2012). It has been reported that insect-based diets, and consequently chitin intake, in laying hens are associated with increased concentrations of short-chain fatty acids (SCFAs), particularly butyrate, through modulation of the gut microbiota (Deng et al., 2022). Similar responses in caecal SCFA production and intestinal morphometry have been observed in laying birds receiving insect-derived ingredients at low inclusion levels (Addeo et al., 2022). This supports the hypothesis that hens fed with chitin additive could enhance butyrate production and consequently upregulate HDP expression, offering a novel antibiotic-free approach to controlling and preventing infectious diseases (Belhadj Slimen et al., 2023). While several studies have explored the effects of chitin and its deacetylated derivative, chitosan, in broilers and aquaculture species (Kroeckel et al., 2012; Lopez-Santamarina et al., 2020), limited information is available on the impact of insect-derived chitin in laying hens, particularly regarding different sources such as raw pupal exuviae. Evidence from laying Japanese quail indicates that low dietary inclusion levels of insect meals can influence *in vivo* performance, ileal digestibility, and egg physicochemical traits, supporting the potential relevance of insect-derived functional ingredients in laying birds (Secci et al., 2021). The valorization of *H. illucens* pupal exuviae, an abundant by-product of insect rearing, embraces the circular economic principles and offers an opportunity to reduce waste while providing a functional feed component without the use of chemicals to obtain purified chitin. Therefore, the aim of the present study was to evaluate the effects of dietary supplementation with ground *H. illucens* pupal exuviae and purified chitin extracted from pupal exuviae on *in vivo* performance, egg quality, nutrient digestibility, and caecal SCFA production in laying hens. By directly comparing these two chitin sources, this study provides novel insights into the role of insect-derived chitin as functional feed ingredients in poultry production.

2. Material and methods

2.1. Animals and diets

A total of 120 ISA Brown laying hens (25 weeks of age), were reared in a cage system in accordance with Directive 1999/74/EC. At the beginning of the trial, birds were individually weighed and assigned to three dietary treatments to obtain homogeneous initial body weight among the experimental groups. A stratified randomization procedure was used to ensure that the mean body weight and its variance were statistically similar across all treatments. Each treatment consisted of 8 replicates of 5 hens (40 hens per treatment). Each replicate was housed in a separate cage with free access to feed and water and to the cage environmental enrichments (nest, nail scraper, perch, and scratching mat). Each hen had approximately 1000 cm² of walkable surface and cages were 45 cm in height. The cages were located in a closed house with controlled environmental conditions (22 °C and 60% relative humidity). The trial started

after a 1-week adaptation period. All hens were fed a standard corn–soybean meal layer diet formulated according to [NRC \(1994\)](#) requirements and ISA Brown management guidelines. The control group (CTRL) received the basal diet without supplementation. The experimental treatments were obtained by supplementing the basal diet (at the expense of corn) with either ground pupal exuviae from *Hermetia illucens* (EXU, 28 g/kg of diet) or purified chitin extracted from pupal exuviae (CHIT, 7 g/kg of diet). During the first week of the adaptation period, feed intake was monitored daily to determine the average intake per hen, which was 140 g/day. Pupal exuviae were analyzed for moisture and chitin content (see next section), showing a chitin concentration of 25.0% on a dry matter basis. Based on these values, the EXU diet was prepared by adding 28 g of ground exuviae per kg of diet, which provided approximately 1 g of total chitin per hen per day. The CHIT diet was prepared by supplementing the basal diet with purified chitin at approximately 7 g/kg of diet, in order to provide an intake of about 1 g of chitin per hen per day, based on the measured average daily feed intake. The experimental design aimed to compare the functional effects of purified chitin and the whole exuviae matrix, with both supplemented diets formulated to provide approximately 1 g of chitin per hen per day. The trial started on May 15 and ended on July 30, 2024 (11 weeks; from 28 to 39 weeks of age) and the experiment was carried out in a commercial laying hen farm in the province of Caserta (Italy).

2.2. Insect rearing and sample collection

Hermetia illucens pupal exuviae were supplied by Xflies s.r.l. (Potenza, Italy) and Protix (Dongen, Netherlands). The larvae were reared under controlled conditions, at 27 ± 1 °C, 70% relative humidity, on spent grain provided by Bikes beer (Rivello, PZ, Italy). Pupal exuviae were collected after adult emergence and then were washed with distilled water, dried for 10 min using an industrial microwave, and ground into a fine powder using a laboratory grinder (Waring Commercial, Stamford, USA).

2.3. Chitin extraction from pupal exuviae

Chitin was extracted from ground *H. illucens* pupal exuviae through chemical extraction, as reported in [Triunfo et al., \(2022\)](#). For demineralization, the powdered samples were treated with 0.5 M formic acid (CH_2O_2) (Merck, Darmstadt, Germany) in a 1:10 w/v ratio and stirred for 1 h at room temperature (RT). The demineralized biomass was then filtered, washed to neutrality with distilled water, and dried at 60 °C overnight in oven. The deproteinization step involved treating the demineralized powder at a 1:10 w/v ratio with a 2 M NaOH solution at 80 °C for 2 h. After the reaction, the obtained unbleached chitin was again filtered, thoroughly washed with distilled water, until reaching a neutral pH, and then oven-dried at 60 °C overnight.

2.4. Fourier-transform infrared spectroscopy (FTIR) analysis

Chitin samples obtained from pupal exuviae were analyzed by Fourier-Transform Infrared (FTIR) spectroscopy using a Jasco 460Plus IR spectrometer. Commercial chitin, from crustacean source, was used as control. Dried, pulverized chitin was mixed with potassium bromide (KBr) and compressed into 1 cm diameter tablets. Spectra were acquired in transmission mode in the 4000–400 cm^{-1} range, with a resolution of 4 cm^{-1} and 100 scans per sample. The resulting spectra were processed using JASCO Spectra Manager software.

2.5. In vivo and post-mortem performance

During the trial the live weight and the feed intake of animals were measured per replicate. Daily, the laid eggs were counted and weighed per replicate. Per each replicate of each group, egg mass was calculated by multiplying egg weight by egg production percentage, and feed conversion ratio (FCR) was calculated as gram of feed consumption per day divided by gram of egg weight per day. At the end of the trial, 2 hens per replicate (16 per group, in total 48 hens) were slaughtered. To ensure representative sampling, hens were chosen based on the average body weight of their respective pen (within $\pm 5\%$ of the pen mean) and were free from any clinical signs of disease. Selected birds were individually tagged, fasted for 12 h with continuous access to water, and then humanely transported to the processing facility (a specialized slaughterhouse). At the slaughterhouse, hens were rendered unconscious using electrical stunning (minimum 150 mA, 300 Hz) in a water bath, followed immediately by euthanasia via exsanguination through a unilateral neck cut of the carotid artery and jugular vein. Exsanguination was allowed to proceed for at least 3 min to ensure complete death before tissue sampling. After exsanguination, the live weight of each carcass was recorded, and all chickens were dissected under sterile conditions. The gastrointestinal tract was removed, separated into its main anatomical portions, and carefully emptied of its contents before measurements as reported by [Borrelli et al. \(2017\), \(2023\)](#). Subsequently, the weight of the entire gastrointestinal tract and of the individual compartments (proventricle, duodenum, jejunum, ileum, cecum, colon, and cloaca), as well as the weight of the liver, spleen, and abdominal fat, were determined and expressed as a percentage of live weight. The lengths of the various intestinal segments and portions of the oviduct (infundibulum, magnum, isthmus, and uterus) were measured after gentle tissue distension, without applying tension, in order to assess morphometric characteristics of the internal organs in relation to the different dietary treatments. For statistical purposes, measurements from the two hens within each replicate were averaged, and the replicate (cage) was used as the experimental unit.

2.6. Nutrient digestibility

During the last two weeks of the trial, experimental diets were supplemented with 1% Celite as a source of acid-insoluble ash, which was used as an internal marker for estimating apparent ileal digestibility. At the end of the trial, during slaughter, ileal contents were collected from each sacrificed hen. Samples were processed and analyzed individually, and digestibility coefficients were calculated for each experimental unit. Digestibility obtained from the two hens sampled within each replicate was averaged, and the replicate (cage) was used as the experimental unit for statistical analysis. The chemical composition of ileal digesta and the corresponding feed samples were analyzed for dry matter, organic matter, crude protein, and ether extract according to AOAC (2005) methods. Apparent ileal digestibility coefficients were measured by the acid-insoluble ash (AIA) method (Vogtmann et al., 1975) and estimated using the following equation: $100 - 100 \times [(\% \text{ AIA in diet} / \% \text{ AIA in ileal content}) \times (\% \text{ nutrient in ileal content} / \% \text{ nutrient in diet})]$ (Cuttrignelli et al., 2018). The replicate (cage) was considered the experimental unit for statistical analysis.

2.7. Egg physical analyses

In the last week of the experimental trial, a total of 144 eggs (6 eggs per replicate per group) were collected for three consecutive days, immediately refrigerated (4 °C) after collection and then moved under refrigeration conditions from the experimental facility to the laboratories of the University of Messina for subsequent analysis. Once arrived at the laboratories (1 day of travel), the eggs were immediately submitted to visual and structural quality checks and stored at + 4°C to ensure their integrity until analysis. For each dietary group (CTRL, CHIT, EXU), 18 eggs were randomly selected (totaling 54 samples) and subjected to the following assessments; eggshell breaking strength (N); shell deformation (mm); shell thickness (mm); albumen height (mm); Haugh units; vitelline membrane strength (N); yolk height (mm); yolk index; yolk diameter (mm); air cell height (mm). All measurements were performed using a TA.XTPlusC texture analyzer (Stable Micro Systems, Godalming, UK), which allows precise and reproducible assessment of egg physical properties. Analyses were carried out using the Exponent Lite Express software, equipped with specific modules for egg quality assessment, allowing for both selection and customization of measurement parameters. The individual weight of each egg and eggshell was determined separately using an electronic balance (Mettler-Toledo PL 3000, International Inc., Greifensee, Switzerland, accuracy 0.1 g). Additional measurements included: weight of the individual egg components (albumen, yolk, shell) using an electronic scale (Mettler PL 3000, accuracy 0.1 g); egg height and equatorial diameter using a digital caliper (Stainless Hardened, accuracy 1 mm); and egg circumference using a measuring tape (Kerbl). The Shell index was also calculated for each egg, according to Rodriguez-Navarro et al. (2002): Shell index (g/cm²): (shell weight) / (egg length × egg width). Egg shape was evaluated using the shape index, as proposed by Zanon et al. (2006): Egg shape index (%): (equatorial diameter / egg height) × 100. Yolk color was determined using the DSM color fan, also known as the “Yolk Color Fan” or “Roche scale.” This scale consists of 15 plastic cards numbered from 1 to 15, each corresponding to a standard yolk color. During evaluation, the cards were visually compared with the yolk to identify the number that most accurately matched the observed color. After physical measurements, egg components (albumen and yolk) were separated and used for chemical analyses.

2.8. Chemical analysis of diets and eggs

The chemical composition of the three experimental diets was analyzed in triplicate using procedures outlined by the Association of Official Analytical Chemists (AOAC, 2019): for moisture (method n. 930.15), for ash (method n. 942.05), for crude protein (method n. 2001.11) by means of the Kjeldahl procedure using a Kjeltac system (FOSS, Padua, Italy), for crude fiber (method n. 978.10) using a Fibertec™ 2010 (FOSS, Padua, Italy), and for lipid content (method n. 920.39) by using the Soxtec™ 8000 Extraction Systems and Hydrotec™ 8000 Hydrolysis Systems supplied from FOSS (Padua, Italy). Total starch content was determined using a Total Starch Assay Kit (Megazyme®, NEOGEN, Lansing, MI, USA), following the AOAC Official Method 996.1 (AOAC, 2019), which involves enzymatic hydrolysis with α-amylase and amyloglucosidase. Spectrophotometric measurements were performed using a UV-2600 UV-visible spectrophotometer (Shimadzu, Milan, Italy). Lipid extracts from diets were analyzed following oil extraction and conversion into fatty acid methyl esters (FAMES) using the method described by Christie (1993). FAMES were then examined via Gas Chromatography (Gas Chromatography-FID, TRACE 1310, Thermo Fisher Scientific, Milan, Italy) using an Omegawax 250 capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Supelco, Bellefonte, PA, USA), as described by Oteri et al. (2021). Peak identification was performed by comparing the retention times of sample FAMES with those from a certified standard mixture (mix 37 FAMES, Supelco, Inc.) analyzed under the same conditions. Chromeleon™ Data System Software (Version 7.2.9, Thermo Fisher Scientific, Milan, Italy) was used for data acquisition. Fatty acid concentrations were expressed in g/100 g of total identified FAMES. Nutritional indices including the atherogenic (AI) and thrombogenic (TI) indices (Ulbricht and Southgate, 1991), the hypocholesterolemic/hypercholesterolemic ratio (H/H) (Santos-Silva et al., 2002), and the peroxidation index (PI) (Luciano et al., 2013) were calculated according to the following equations:

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / (\sum MUFA + \sum PUFA)$$

$$TI = (C14:0 + C16:0 + C18:0) / [0.5 \times \sum MUFA + 0.5 \times \sum n-6 + 3 \times \sum n-3 + (n-3/n-6)]$$

$$HH = (\sum C18:1 + \sum PUFA) / (C14:0 + C16:0)$$

$$PI = (0.025 \times \% \text{monoenes}) + (1 \times \% \text{dienes}) + (2 \times \% \text{trienes}) + (4 \times \% \text{tetraenes}) + (6 \times \% \text{pentaenes}) + (8 \times \% \text{hexaenes})$$

Metabolizable energy of diets was calculated according to NRC (1994) procedure of estimation.

The true metabolizable energy of the diets was calculated from their chemical composition according to the following equation developed by Sibbald et al. (1980):

Table 1
Chemical characteristics and fatty acid profile of diets.

	Dietary treatments		
	Control	Extracted chitin	Exuviae
Ingredients, g/kg			
Corn	565.0	558.0	537.0
Soybean meal	262.0	262.0	262.0
Soybean oil	20.0	20.0	20.0
Calcium carbonate	130.0	130.0	130.0
Dicalcium phosphate	15.0	15.0	15.0
Salt	3.5	3.5	3.5
MinVit*	3.5	3.5	3.5
L-Lysine HCl	1.0	1.0	1.0
Extracted chitin	-	7.0	-
Ground Pupal Exuviae	-	-	28.0
Chemical-nutritional characteristics			
Dry matter (%)	89.36	89.70	89.51
Ash, % DM	13.33	13.92	14.25
Crude Fats, % DM	4.27	4.12	4.21
Crude Protein, %DM	17.55	17.74	18.18
Crude Fiber, % DM	4.20	4.30	4.45
Ca, g/kg	39.5	39.6	40.2
P, g/kg	5.8	5.8	6.1
Methionine, g/kg	4.1	4.1	4.2
Lysine, g/kg	8.8	8.8	9.1
TME, kcal/kg	3266	3227	3195
Fatty acids profile, g/100g of total identified FAMES			
C12:0	0.02	0.06	0.29
C14:0	0.08	0.11	0.16
C16:0	13.15	14.72	13.95
C16:1	0.10	0.11	0.12
C17:0	0.08	0.11	0.10
C17:1	0.04	0.05	0.04
C18:0	2.86	3.27	3.30
C18:1n9	27.92	27.01	26.48
C18:1n7	0.97	1.16	1.02
C18:2n6	50.51	48.71	50.10
C18:3n3	2.80	3.15	3.00
C20:0	0.44	0.45	0.43
C20:1n9	0.32	0.29	0.29
C20:2	0.03	0.04	0.04
C22:0	0.36	0.44	0.38
C23:0	0.06	0.09	0.07
C24:0	0.27	0.28	0.27
SFA	17.31	19.51	18.93
MUFA	29.35	28.62	27.94
PUFA	53.33	51.89	53.14
UFA	82.68	80.51	81.08
UFA/SFA	4.78	4.13	4.30
PUFA/SFA	3.08	2.66	2.82
n3	2.80	3.15	3.00
n6	50.51	48.71	50.10
n3/n6	0.06	0.06	0.06
AI	0.16	0.19	0.18
TI	0.33	0.38	0.36
H/H	6.14	5.32	5.66
PI	56.86	55.75	56.84
n6/n3	18.09	15.52	16.78

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; UFA/SFA, ratio of unsaturated to saturated fatty acids; PUFA/SFA, ratio of polyunsaturated to saturated fatty acids; n3, omega-3 fatty acids; n6, omega-6 fatty acids; n3/n6, omega-3 to omega-6 ratio; n6/n3, omega-6 to omega-3 ratio; AI, atherogenic index; TI, thrombogenic index; H/H, hypocholesterolemic/hypercholesterolemic ratio; PI, peroxidation index; TME, true metabolizable energy. *Provided per kilogram: vitamin A (retinyl acetate), 9000 IU; vitamin D3 (cholecalciferol), 1350 IU; 25-hydroxycholecalciferol, 1350 IU; vitamin E (all-rac- α -tocopheryl acetate), 32.4 mg; choline chloride, 600 mg; Cu (copper(II) sulfate pentahydrate), 9.0 mg; I (anhydrous calcium iodate), 1.08 mg; Fe (iron(II) sulfate monohydrate), 36.0 mg; Mn, 72.0 mg [36.0 mg from manganese(II) oxide and 36.0 mg from manganese sulfate monohydrate]; Se (sodium selenite), 0.14 mg; and Zn (zinc oxide), 45.0 mg.

$$\text{TME (kcal/kg)} = 3951 + 54.4 \times \text{EE} - 87.7 \times \text{CF} - 40.8 \times \text{Ash}$$

The ingredients and chemical-nutritional characteristics of the diets are reported in Table 1.

The chemical composition of eggs was analyzed using procedures outlined by the Association of Official Analytical Chemists (AOAC, 2019): moisture (method n. 930.15), ash (method n. 942.05), and crude protein (method n. 2001.11) were determined in whole egg and albumen. Lipid content (method n. 920.39) and fatty acid profile were determined only in yolk samples, as the lipid fraction is predominantly located in the yolk. Before lipid extraction, yolk samples were hydrolyzed with HCl (4 N). Quantification and identification of fatty acid methyl esters (FAMES) and calculation of nutritional indices were performed as described for diets.

2.9. Short-chain fatty acids (SCFAs)

From each carcass the caeca were tied at both ends, separated by sterile instruments from the rest of the gastrointestinal tract, placed in plastic bags in a pre-warmed thermos (37 °C), and transported in about 1 h to the laboratory. For each hen, the content of the two caeca were mixed and divided into two aliquots (~ML each), used as individual replicate to obtain an accurate measure of the SCFAs production. The samples were diluted with 0.06 M oxalic acid (1:1, v/v) and SCFA were measured by Gas Chromatograph model TRACE 1310 (Thermo Scientific SpA, Rodano, Milan, Italy) equipped with NUKOL fused silica capillary column 30 m x 0.25 mm x 0.25-micron film thickness SUPELCO, SpA, Bellefonte, PA, USA). The operating conditions were FID detector temperature: 200 °C, oven temperature: isotherm 135°C, carrier: helium flow: 1.5 mL/min. Each sample was quantified comparing samples peaks area of each SCFA with the corresponding of an in-house prepared external standard mixture composed of acetate, propionate, butyrate, isobutyrate, valerate and iso-valerate.

2.10. Statistical analysis

Data were analyzed using one-way ANOVA with the GLM procedure of SAS software, version 9.4 (SAS Institute Inc., 2013). Normality of residuals was assessed using the Shapiro–Wilk test (Shapiro and Wilk, 1965), and homogeneity of variances was verified using Levene's test (Levene, 1960). The following model was applied:

$$Y_{ij} = \mu + G_i + e_{ij}$$

where Y_{ij} is the observation, μ is the overall mean, G_i is the fixed effect of dietary treatment (Control, Extracted Chitin, Exuviae), and e_{ij} is the residual error. The replicate (cage) was considered the experimental unit for all measured variables. For post-mortem traits and caecal short-chain fatty acid concentrations, data from the two hens sampled within each cage were averaged before statistical analysis. When the treatment effect was significant, means were separated using Tukey's multiple comparison test. Statistical significance was declared at $P < 0.05$, and highly significant differences were declared at $P < 0.01$. In tables, different superscript letters indicate significant differences among treatments (lowercase letters: $p < 0.05$; uppercase letters: $p < 0.01$). RMSE represents the root mean square error.

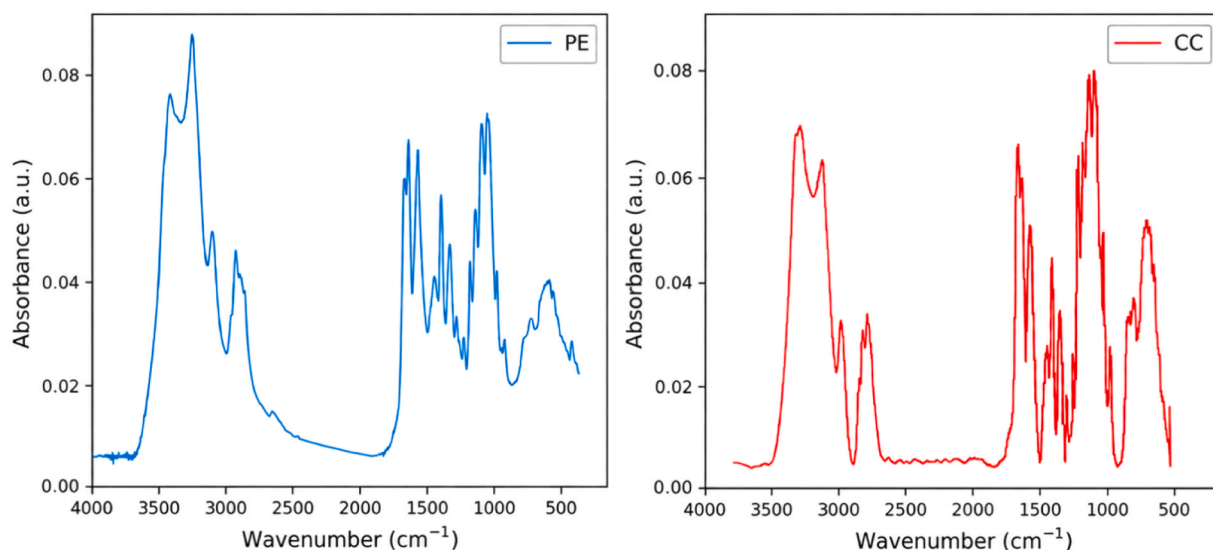


Fig. 1. FTIR spectra of purified chitin extracted from *Hermetia illucens* pupal exuviae (PE) compared with commercial chitin (CC).

3. Results

3.1. Characterization of chitin by Fourier-transformed infrared spectroscopy

The FTIR spectrum of unbleached chitin extracted from *H. illucens* pupal exuviae is shown in Fig. 1. All the characteristic absorption bands of chitin were clearly identifiable at their specific wavenumbers: 1310–1320 cm^{-1} (C–N stretching, amide III), 1550–1560 cm^{-1} (N–H bending, amide II), 1650–1655 cm^{-1} (C=O stretching, amide I), 3100–3110 cm^{-1} (N–H symmetric stretching), 3255–3270 cm^{-1} (N–H asymmetric stretching), and 3430–3450 cm^{-1} (O–H stretching). The spectral profile exhibited a close structural similarity to that of commercial chitin and the α -form was confirmed by the presence of the typical Amide I at approximately 1620 and 1650 cm^{-1} , observing the split of the amide I band into two peaks (Kumirska et al., 2010; Purkayastha and Sarkar, 2020).

Throughout the experiment, all birds remained clinically healthy, with no mortality, diarrhoea, or signs of illness observed in any group. The effect of the chitin source on laying hens' *in vivo* performance is represented in Table 2. No significant differences were observed for most parameters, except for FCR, more favorable in the EXU group than in the CTRL group ($P < 0.05$).

The apparent ileal digestibility of nutrients in laying hens fed diets containing different chitin sources is reported in Table 3. No significant differences among treatments were observed for dry matter and organic matter digestibility and for ether extract digestibility. Conversely, the apparent ileal digestibility of crude protein was significantly affected by dietary treatment ($P < 0.01$), with hens fed the control diet showing higher values compared with those receiving diets containing extracted chitin or exuviae, which did not differ from each other.

Table 4 shows post-mortem characteristics of carcasses. The use of ground exuviae or extracted chitin affected some characteristics of hen's inner organs. The weight of the proventriculus, expressed as percentage of live weight, was lower ($P < 0.01$) in the treated groups compared to the control. The jejunal weight progressively decreased ($P < 0.05$) from Control to CHIT and EXU group. Ileal length was significantly lower in the CHIT group than in both CTRL and EXU groups ($P < 0.05$), whereas ileal weight was significantly lower in CHIT than in CTRL, with EXU showing intermediate values. Jejunal weight differed significantly among all three dietary treatments, progressively decreasing from CTRL to CHIT and EXU. Conversely, abdominal fat percentage was significantly higher in the EXU group compared with the control group, while the CHIT group showed intermediate values. Regarding the oviduct, the treated groups showed a higher length of the infundibulum than the control group. The highest uterus length ($P < 0.05$) was recorded in the EXU group.

Data in Table 5 show the effect of raw or purified chitin on short-chain fatty acid (SCFA) production in the caecal content of laying hens. Total SCFA concentration, expressed as mmol/L, was significantly affected by dietary treatment ($P < 0.0001$), being highest in EXU. When expressed as percentage of total SCFA the situation appears quite different. In fact, the percentage of Acetate was lower ($P < 0.01$) in the control compared to EXU group; the propionate and valeric acid percentages were higher ($P < 0.01$ and $P < 0.05$, respectively) in the control compared to the CHIT group; isobutyrate and isovaleric acid were higher in the control compared to both EXU and CHIT groups; butyric acid was the highest in the EXU group, followed by CHIT and Control groups ($P < 0.01$).

Table 6 shows the physical characteristics of the eggs according to the diverse dietary treatments. Only shell deformation, vitelline membrane strength, and yolk height were significantly affected. In particular, shell deformation was higher in the EXU group than in the CTRL group ($P < 0.05$); the vitelline membrane strength was higher in the CHIT group compared to both Control and EXU groups ($P < 0.01$); the yolk height measured in the control group was higher than that observed in the EXU group ($P < 0.05$).

The chemical characteristics of whole eggs and albumen were reported in Tables 7 and 8, respectively: none of the parameters were significantly affected by dietary treatment.

Table 9 reports the chemical characteristics and fatty acid profile of the yolk. C14:1 showed higher values in control than in the CHIT group. C16:1 was higher ($P < 0.01$) in control than in both CHIT and EXU groups, while C17:0 was higher in the CHIT group than in the CTRL group ($P < 0.05$). Finally, the MUFA content was higher in the CTRL group than in the CHIT group ($P < 0.05$).

4. Discussion

From our results, the “crude” form of chitin associated with pupal exuviae produced different responses compared with purified chitin. Although both treatments included chitin as a functional dietary component, the EXU diet supplied it within a complex natural matrix, whereas the CHIT group received purified chitin. Both experimental diets were formulated to provide approximately 1 g of

Table 2
Effect of chitin source on *in vivo* performance of laying hens.

	Control	Extracted chitin	Exuviae	RMSE	P-value
Initial weight, kg	1.79	1.78	1.80	0.12	0.9576
Final weight, kg	1.74	1.78	1.81	0.05	0.1354
Feed intake, g/d	146.7	142.4	138.4	8.18	0.2460
Oviposition rate, %	76.4	79.9	82.8	4.88	0.1132
Egg weight, g	61.3	61.0	62.17	2.68	0.7488
Egg mass, g	46.93	48.74	51.41	3.99	0.1800
FCR, g/g	3.15 ^a	2.93 ^{ab}	2.70 ^b	0.24	0.0206

Values are means. Within a row, means with different superscript letters differ significantly (a–b: $P < 0.05$). RMSE, root mean square error. FCR: feed conversion ratio.

Table 3

Nutrient digestibility of laying hens as affected by chitin source included in the diet.

	Control	Extracted chitin	Exuviae	RMSE	P-value
Dry matter, %	82.25	80.41	80.93	4.12	0.5531
Organic matter, %	84.33	81.62	83.21	5.09	0.4416
Crude protein, %	87.23A	81.06B	82.15B	2.54	0.0058
Ether extract, %	92.15	91.03	91.87	3.85	0.6743

Values are means. Within a row, means with different superscript letters differ significantly (A–B: $P < 0.01$). RMSE, root mean square error.**Table 4**

Post-mortem characteristics of carcasses of layers feeding on different sources of chitin.

	Control	Extracted chitin	Exuviae	RMSE	P-value
Live weight, g	1744	1767	1792	52.35	0.147
Gastro-intestinal tracts, % of live weight					
Weight of GIT	8.25	7.51	7.19	0.82	0.0835
Proventriculus weight	2.23 ^A	2.04 ^B	1.96 ^B	0.17	0.0043
Gizzard weight	0.62	0.65	0.64	0.06	0.4392
Duodenal weight	0.82	0.84	0.87	0.10	0.8436
Duodenal length	1.65	1.61	1.46	0.15	0.4438
Jejunal weight	1.84 ^a	1.54 ^b	1.32 ^c	0.19	0.0108
Jejunal length	3.55	3.76	3.23	0.39	0.4200
Ileal weight	1.09 ^a	0.76 ^b	0.81 ^{ab}	0.08	0.0297
Ileal length	2.20 ^a	1.88 ^b	2.15 ^a	0.13	0.0175
Caeca weight	0.72	0.69	0.68	0.06	0.8040
Caeca length	1.88	1.66	1.75	0.11	0.2844
Colon weight	0.52	0.59	0.56	0.04	0.1061
Colon length	0.64	0.63	0.60	0.06	0.4274
Cloaca weight	0.63	0.67	0.60	0.05	0.2023
Cloaca length	0.24	0.25	0.25	0.02	0.9218
Liver weight	1.83	1.81	1.80	0.15	0.9375
Spleen weight	0.10	0.11	0.11	0.02	0.7932
Abdominal fat weight	0.88 ^b	0.96 ^{ab}	1.13 ^a	0.09	0.0322
Reproductive tract, % of live weight					
Oviduct weight	3.29	3.35	3.53	0.32	0.4164
Infundibulum length	0.42 ^b	0.50 ^a	0.52 ^a	0.06	0.0058
Magnum length	1.79	1.82	1.96	0.20	0.1545
Isthmus length	0.76	0.72	0.71	0.08	0.4894
Uterus length	0.53 ^b	0.54 ^b	0.64 ^a	0.06	0.0174

Values are means. Within a row, means with different superscript letters differ significantly (a–b: $P < 0.05$; A–B: $P < 0.01$). RMSE, root mean square error. GIT, gastrointestinal tract. Organ weights are expressed as percentage of live weight. Organ weights are expressed as a percentage of live weight, while organ lengths are expressed as cm/100 g of live weight.**Table 5**

Effect of chitin source on short-chain fatty acid concentration in caecal content of the hens.

	Control	Extracted chitin	Exuviae	RMSE	P-value
Total SCFA	mmol/L 108.5 C	158.3B	187.4A	12.81	< 0.0001
% of total SCFA					
Acetate	47.13B	48.13AB	51.77A	1.84	< 0.0001
Propionate	20.54A	18.45B	19.48AB	1.31	0.0068
Isobutyrate	4.67A	3.05B	2.95B	0.38	< 0.0001
Butyrate	15.93 C	18.45B	20.17A	1.36	< 0.0001
Isovaleric acid	7.78A	4.91B	5.45B	0.71	< 0.0001
Valeric acid	3.97 ^a	3.37 ^b	3.82 ^{ab}	0.42	0.0112

Values are means. Within a row, means with different superscript letters differ significantly (a–b: $P < 0.05$; A–C: $P < 0.01$). RMSE, root mean square error. SCFA, short-chain fatty acids.

chitin per hen per day Therefore, the observed differences between EXU and CHIT may be related to the presence of additional components in the whole exuviae matrix rather than to chitin alone. However, differences in actual chitin intake cannot be entirely excluded. . The improved FCR observed in the EXU group suggests that crude chitin, supplied within the natural exuvia matrix, may have promoted more efficient feed utilization compared to the control group. Since intake and oviposition were not significantly different, the effect may reflect mechanisms beyond production intensity alone, potentially mediated by interactions between the exuvia matrix, intestinal function, and the microbiota. This interpretation is consistent with evidence that insect-based diets modulate

Table 6

Physical characteristics of eggs on day 70 of the experiment.

	Control	Extracted chitin	Exuviae	RMSE	P-value
Whole egg Weight (g)	61.21	56.84	57.68	6.15	0.471
Egg Length (mm)	55.83	55.33	54.28	0.22	0.493
Egg Width (mm)	43.72	42.50	42.72	0.13	0.254
Egg Circumference (mm)	139.17	137.83	136.17	0.51	0.601
Form Index (%)	80.04	79.25	79.39	3.02	0.854
Shell Weight (g)	8.23	7.34	7.70	1.05	0.394
Shell (%)	12.75	13.30	13.23	1.45	0.708
Albumen Weight (g)	36.75	36.68	35.56	4.50	0.841
Albumen (%)	62.48	62.18	61.04	2.79	0.560
Yolk Weight (g)	14.28	13.66	14.05	1.24	0.617
Yolk (%)	24.28	23.32	24.28	1.70	0.439
Shell Index (g/cm ²)	0.31	0.33	0.33	0.03	0.673
Shell Breaking Strength (N)	40.24	54.09	46.99	9.84	0.105
Shell Deformation (mm)	0.63 ^b	0.77 ^{ab}	0.81 ^a	0.10	0.032
Shell Thickness (mm)	0.38	0.37	0.39	0.05	0.731
Albumen Height (mm)	10.28	8.01	13.20	3.90	0.148
Haugh Unit (HU)	99.38	88.35	109.05	14.42	0.114
Vitelline Membrane Strength (N)	0.17B	0.18A	0.17B	0.002	0.0001
Yolk Height (mm)	14.94 ^a	14.62 ^{ab}	13.33 ^b	1.03	0.038
Yolk Width (mm)	40.12	40.10	39.47	1.38	0.657
Yolk Index	0.37	0.36	0.34	0.02	0.084
Yolk Colour (Roche scale)	10.83	10.33	10.67	1.01	0.693

Values are means. Within a row, means with different superscript letters differ significantly (a–b: $P < 0.05$; A–B: $P < 0.01$). HU, Haugh Unit.

Table 7

Chemical characteristics of whole eggs on day 70 of the experiment.

	Control	Extracted chitin	Exuviae	RMSE	P-value
Moisture (%)	75.20	75.64	74.53	1.69	0.536
Ash (%)	0.99	1.04	1.13	0.11	0.141
Crude Protein (%)	12.84	12.27	12.63	0.51	0.183

Values are means. No significant differences were detected among treatments ($P > 0.05$)

Table 8

Chemical characteristics of albumen on day 70 of the experiment.

	Control	Extracted chitin	Exuviae	RMSE	P-value
Moisture (%)	87.82	87.53	87.34	0.79	0.630
Ash (%)	0.74	0.74	0.73	0.03	0.712
Crude Protein (%)	10.57	11.36	11.06	1.13	0.393

Values are means. No significant differences were detected among treatments ($P > 0.05$)

the intestinal microbiota and metabolites, so the response may also depend on structural complexity and co-components rather than isolated chitin (Borrelli et al., 2017; Moniello et al., 2019; Złotko et al., 2021; Falgayrac et al., 2024). Apparent ileal digestibility data indicated that dietary chitin primarily influenced crude protein utilization. Both the extracted chitin and EXU groups showed lower protein digestibility compared with the control ($P < 0.01$), with no differences between the two chitin sources. This result is consistent with the nature of chitin as a largely indigestible polysaccharide, which shares functional properties with dietary fibres that cannot be hydrolysed by endogenous digestive enzymes (Šimůnek and Bartoňová, 2005). However, this reduction in crude protein digestibility should be interpreted in the context of a functional trade-off, where reduced enzymatic digestion is accompanied by enhanced microbial fermentation, as evidenced by the increased SCFA production. This shift may contribute to improved gut functionality and metabolic modulation rather than representing a purely negative nutritional outcome. Consistent with this mechanism, the presence of chitin in insect-derived feed ingredients has been reported as a factor that can impair nutrient utilization, particularly protein digestibility, in monogastric animals, including poultry (Józefiak et al., 2018; Gasco et al., 2019). The similar response observed for the EXU and CHIT groups suggests that the native structure of pupal exuviae did not exert additional detrimental effects compared to purified chitin, indicating that the reduction in protein digestibility is likely related to chitin itself rather than other components of the exuviae matrix. More broadly, dietary manipulations in laying hens can affect nutrient digestibility and egg traits, supporting the view that relatively subtle changes in diet formulation may translate into measurable differences in nutrient utilization and production responses (Attia et al., 2025b). Interestingly, the inclusion of chitin, either extracted or crude, exerted a similar effect on the weight of the glandular stomach compared with the control group. Poultry are able to partially digest chitin in the proventriculus due to the presence of an enzyme called acidic chitinase (Chia) and the presence of chitin in the diet can reduce proventriculus pH enhancing the

Table 9

Chemical characteristics of Yolk at day 70 of the experiment.

	Control	Extracted chitin	Exuviae	RMSE	P-Value
Moisture (%)	44.99	46.66	47.92	1.91	0.072
Ash (%)	2.45	2.76	2.48	0.22	0.069
Crude Protein (%)	16.97	15.98	16.02	1.12	0.297
Lipid (%)	30.79	32.73	32.61	2.50	0.392
Fatty acids profile, g/100g of total identified FAMES					
C12:0	0.01	0.02	0.02	0.00	0.051
C14:0	0.386	0.35	0.37	0.04	0.361
C14:1	0.07 ^a	0.04 ^b	0.05 ^{ab}	0.01	0.016
C16:0	28.70	29.04	27.72	1.19	0.174
C16:1	2.93 ^a	1.94 ^b	2.27 ^b	0.40	0.004
C17:0	0.19 ^b	0.27 ^a	0.24 ^{ab}	0.04	0.014
C17:1	0.13	0.13	0.13	0.02	0.674
C18:0	10.60	11.71	10.42	1.15	0.149
C18:1n9	37.20	35.12	36.74	1.57	0.099
C18:1n7	1.66	1.49	1.59	0.18	0.339
C18:2n6	15.13	16.59	16.98	1.66	0.194
C18:3n3	0.44	0.46	0.50	0.09	0.526
C20:0	0.04	0.05	0.04	0.00	0.538
C20:1n9	0.21	0.19	0.21	0.03	0.742
C20:2	0.28	0.32	0.30	0.04	0.283
C20:3n6	0.14	0.13	0.13	0.03	0.841
C20:3n3	1.37	1.46	1.59	0.39	0.646
C20:5n3	0.07	0.08	0.06	0.02	0.224
C22:0	0.05	0.06	0.05	0.00	0.121
C22:1n9	0.04	0.05	0.04	0.01	0.557
C22:6n3	0.36	0.47	0.53	0.13	0.113
SFA	39.00	41.51	38.86	1.80	0.068
MUFA	42.23 ^a	38.98 ^b	41.05 ^{ab}	1.93	0.041
PUFA	17.77	19.51	20.09	2.08	0.202
UFA	60.00	58.49	61.14	1.80	0.068
UFA/SFA	1.50	1.41	1.58	0.11	0.071
PUFA/SFA	0.45	0.47	0.52	0.06	0.203
n3	2.23	2.48	2.68	0.56	0.432
n6	15.26	16.72	17.11	1.67	0.202
n3/n6	0.15	0.15	0.16	0.02	0.709
AI	0.51	0.52	0.48	0.03	0.128
TI	0.82	0.84	0.76	0.07	0.192
H/H	0.61	0.65	0.70	0.08	0.181
PI	20.86	23.29	24.19	2.48	0.112
n6/n3	6.90	7.01	6.62	1.19	0.842
Cholesterol mg/100 g	1096.97	1170.54	1189.67	119.55	0.432

Values are means. Within a row, means with different superscript letters differ significantly (a–b: $P < 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; UFA/SFA, ratio of unsaturated to saturated fatty acids; PUFA/SFA, ratio of polyunsaturated to saturated fatty acids; n3, omega-3 fatty acids; n6, omega-6 fatty acids; n3/n6, omega-3 to omega-6 ratio; n6/n3, omega-6 to omega-3 ratio; AI, atherogenic index; TI, thrombogenic index; H/H, hypocholesterolemic/hypercholesterolemic ratio; PI, peroxidation index.

activity of Chia (Tabata et al., 2017). Not many studies are available in the literature in which the effect of the inclusion of insect meals or chitin in the diets on proventriculus of poultry was investigated. Lokman et al. (2019) including 0.5 g/kg of chitin from cricket and shrimps in broiler diets observed no significant effects on proventriculus weight, even if its % on live weight was 0.8 in the control group and an average 0.65 in the other groups. In our study, the effect of chitin on proventriculus weight is evident considering that the main factors affecting proventriculus weight such as particle size of feed and feed intake are the same or not statistically different, respectively, among the groups. The significant reduction in jejunal weight observed in the CHIT and EXU groups suggests a morpho-functional adaptation of the small intestine to the presence of chitin in the diet. A study conducted with *Tenebrio molitor* meal showed that high inclusion levels can induce morphological changes in the small intestine, particularly the jejunum, such as reduced villous height, increased crypt depth, and a decreased villus/crypt ratio, without, however, causing histopathological lesions of the mucosa or systemic alterations (Biasato et al., 2018). These changes indicate an adaptive response of the intestinal tissue rather than a degenerative process. A possible modulatory mechanism of mucosal homeostasis is the action of butyrate, a short-chain fatty acid capable of stimulating the expression of antimicrobial peptides and supporting innate intestinal defences in chickens (Sunkara et al., 2011). Although the direct link between chitin fermentation and butyrate production has not yet been conclusively demonstrated, this evidence supports the potentially beneficial role of microbial metabolites in maintaining intestinal homeostasis. Available evidence indicates that the observed jejunal changes likely reflect a trophic adaptation of the mucosa to the chitinous matrix rather than a direct atrophying effect. It is known that chitin, although partially hydrolysed by the acidic chitinase enzyme expressed in the proventriculus (Tabata et al., 2017), largely reaches the distal intestine where it can function as a functional fibre. The presence of fibrous matrices can

reduce the immediate availability of protein nutrients, as confirmed by the decrease in ileal digestibility of crude protein observed in the present study, with possible repercussions on the trophic development of the ileum. Modulating effects on the digestive system and nutrient utilization efficiency in the presence of chitin- or chit oligosaccharide rich sources have been described in poultry (Lokman et al., 2019) and other avian species (Tufan et al., 2015). The different response between extracted chitin and raw exuviae also suggests that the complex matrix of the exuviae may exert a different mechanical and fermentative effect than the purified polymer. The increase in total SCFAs at the caecal level, particularly evident in the EXU group, indicates a greater fermentative activity that may contribute to the functional remodelling of the distal segments of the intestine (Bovera et al., 2018a), also through immunometabolism mechanisms mediated by short-chain fatty acids (Sunkara et al., 2011). In particular, the increase in butyrate is of special biological relevance, as this SCFA represents the primary energy source for enterocytes and plays a key role in maintaining intestinal barrier integrity, modulating immune responses, and promoting gut homeostasis. Therefore, the higher butyrate production observed in the EXU group suggests a potential functional benefit extending beyond nutrient utilization. This finding reinforces the hypothesis that insect-derived substrates can act as modulators of the gut microbiota, with downstream effects on host physiology. Similar increases in caecal fermentation end-products, including butyrate, have been reported in avian models fed insect-based ingredients, supporting the hypothesis of a microbiota-mediated effect on gut functionality (Addeo et al., 2022). Dietary inclusion of ground exuviae significantly increased abdominal fat deposition in laying hens. In contrast with some research showing that chitin and its derivatives such as chitosan, have an indirect effect on abdominal fat, reducing the intestinal fat absorption and thus its deposition (Kobayashi et al., 2002; Huang et al., 2020). A possible explanation for this discrepancy could lie in differences in the structure and processing of chitin, which can determine different metabolic responses, including through modulation of the microbiota and microbial metabolites. Several dietary factors have been reported to influence abdominal fat deposition in poultry (Fouad and El-Senousey, 2014). Moreover, caecal production of SCFAs by the intestinal microbiota is increasingly recognized as a metabolic signal potentially affecting host lipid metabolism and body fat accretion (Sun et al., 2021). In the present study, the greater abdominal fat observed in the EXU group may therefore be associated with changes in energy partitioning linked to microbial fermentation and SCFA production. However, since no direct measurements of lipid metabolism or gene expression were performed, the underlying mechanisms cannot be determined and warrant further investigation. Previous studies in poultry reported that the inclusion of *T. molitor* larvae in broiler diets (Biasato et al., 2018), chito-oligosaccharides in growing Japanese quail (Tufan et al., 2015), and low dietary inclusion levels of *T. molitor* larva meal in Japanese quail (Secchi et al., 2022) can modulate gut function and nutrient utilization, although their effects on abdominal fat deposition are not always consistent across studies. In a previous trial on laying hens feeding diets with *H. illucens* larvae at 25 and 50 % of replacing protein level (Bovera et al., 2018a) despite the significant decrease of lipids blood levels in both groups in comparison to the control, the group feeding the highest level of larvae with an estimated intake of chitin of 0.99 g/day showed a percentage of abdominal fat similar to that of the control group. To our knowledge, no studies are available on the effect of chitin on oviduct morphology in laying hens, so it is impossible to compare our data with the literature. However, effects of black soldier fly meals on the oviduct were recently investigated by Tajudeen et al. (2025). The authors, using an expanded (heated under pressure) full fat BSF meal at 3, 6, 9 and 12 % of the diet, observed an increase of gap-junction connexins in the infundibulum, uterus and vagina of laying hens. In the present study, both chitin-supplemented diets were associated with increased infundibulum length compared with the control diet, whereas uterus length was significantly greater only in the EXU group. Further investigations can be useful to better understand possible relationships between chitin and oviduct changes in hens. In any case, the most important changes due to the administration of chitin are in the fatty acid production and profile of the hen's caecal content, confirming our previous findings (Bovera et al., 2018a) in which the insect meals are able to modify the activity of caecal microbiota of poultry and that these changes could be mainly due to the chitin. It should be noted that the EXU diet provided not only chitin but also other structural components naturally present in the exuviae matrix, even if not measured in this trial. Therefore, the observed responses in the EXU group likely reflect the combined effect of the whole exuviae complex rather than chitin alone. This interpretation is consistent with evidence that BSF-derived materials can vary in their compositional profile depending on processing and origin, potentially contributing to different physiological responses compared with purified polymers (Addeo et al., 2024a). These differences are mainly in the total SCFA production, higher for EXU group, reflecting the increase of all the single SCFA measured. This suggests a general increase of microbiota activity. However, extracted chitin is able to increase the microbiota activity in laying hens caecal content in comparison to the control group. The result is in line with other findings in literature (Deng et al., 2022; Józefiak et al., 2018). When the SCFAs were expressed as percentage of the total SCFA production, the differences between the two chitin sources are strongly reduced: in fact, only the butyrate was higher in EXU than in CHIT group. The last result is interesting because the butyrate, coming from the fermentation of structural carbohydrates of the diet, plays a key role in the health of digestive system of poultry: it is considered, in general, the main enterocytes' energy source (Bovera et al., 2018a) and is required for a proper development of the Gut-Associated Lymphoid Tissue (Mroz, 2005). Therefore, ground exuviae appear more effective than extracted chitin in improving health conditions of hen's digestive system, but both chitin sources improve butyrate ratio in comparison to the control group. Regarding the other SCFA expressed in percentage of total produced SCFAs, only in the EXU group we have a relative increase of acetate; extracted chitin decreases the relative percentages of propionate and valeric acid while both chitin sources reduce the relative percentages of isobutyrate and isovaleric acid. Looking at the last point, it is well known that branched chain fatty acids (BCFA: isobutyric, isovaleric and valeric acids) come from the fermentation of some aminoacids such as valine, leucine and isoleucine. Dietary inclusion of chitin did not affect most external and internal egg quality parameters, in agreement with previous studies in laying hens fed *Hermetia illucens* meals at moderate inclusion levels (Secchi et al., 2018; Cutrignelli et al., 2018). Although shell weight and thickness were unchanged, the higher deformation observed in EXU eggs may indicate alterations in the organic matrix of the shell rather than in mineral deposition per se. Roberts (2004) highlighted that shell mechanical properties are not solely dependent on thickness, but also on matrix protein organization and calcium metabolism. Chitin, as an insoluble polysaccharide with fiber-like behavior, has been described to bind divalent minerals and to modify their

intestinal availability (Khoushab and Yamabhai, 2010), which could contribute to subtle changes in shell biomechanics. The most evident effect concerned vitelline membrane strength, a parameter known to be sensitive to dietary and metabolic factors (Kirunda and McKee, 2000). The improvement observed with purified chitin may be related to microbiota-mediated modulation of lipid metabolism and oxidative status, mechanisms previously associated with chitinous substrates (Deng et al., 2022). Conversely, the lower membrane resistance and yolk height in EXU eggs might reflect enhanced fermentative activity and SCFA production, which can influence yolk water balance and lipid deposition (Dai et al., 2022). Analysis of the yolk lipid profile revealed statistically significant differences limited to some minor monounsaturated and saturated fatty acids, while most nutritional parameters remained unchanged between treatments. This reduction in the treated groups can be interpreted in light of the modulatory effect of chitin on intestinal lipid metabolism, previously described as capable of interfering with lipid absorption and enteric lipase activity (Kobayashi et al., 2002). Similar effects of reduction in specific lipid fractions have been observed in poultry models fed chitin derivatives or chito-oligosaccharides (Świątkiewicz et al., 2015). The reduction in C16:1 acid was even more marked in the CHIT and EXU groups compared to the control. This finding suggests a possible inhibition of palmitate desaturation mediated by $\Delta 9$ -desaturase, a mechanism that may be influenced by microbial metabolites produced by caecal chitin fermentation. The increased production of SCFAs observed in the same groups, may be associated with metabolic changes that could influence lipid deposition and, consequently, the fatty acid composition transferred to the yolk (Sunkara et al., 2011). Regarding C17:0, the higher value recorded in the CHIT group compared to the control group could reflect a different contribution of microbial lipids. Odd-chain fatty acids, such as C17:0, are known to be associated with the fermentative activity of the gut microbiota and de novo bacterial synthesis (Józefiak et al., 2018). The increase in this fraction in the purified chitin group therefore suggests a direct interaction between the fibrous matrix and the caecal microbial community. At the lipid class level, the total MUFA content and their reduction in the presence of extracted chitin are consistent with studies indicating that non-digestible polysaccharides can limit the absorption of monounsaturated fatty acids or alter their hepatic transfer to the yolk (Razdan and Pettersson, 1994). Interestingly, this effect is less evident in the exuviae group, likely due to the presence of a complex matrix capable of differently modulating lipid bioavailability (Biasato et al., 2018).

5. Conclusion

Dietary supplementation with purified chitin and ground *H. illucens* pupal exuviae elicited different physiological responses in laying hens. Although both chitin sources reduced crude protein digestibility, exuviae supplementation enhanced caecal fermentative activity and butyrate production, suggesting a stronger interaction with the gut microbiota. The observed differences appear to be associated with the combined effect of exuviae matrix complexity and chitin supply level, rather than with purified chitin alone. Overall, pupal exuviae represent a promising functional feed ingredient for poultry, warranting further investigation into their long-term metabolic and productive implications.

Limitations of the study

Although the present study provides novel insights into the use of *Hermetia illucens* pupal exuviae as a functional feed additive, some limitations should be acknowledged. First, while both supplemented diets were formulated to provide a similar intake of chitin (~1 g/hen/day), the experimental design cannot fully decouple the effects of the isolated chitin polymer from the structural complexity of the whole exuviae matrix. Consequently, the enhanced fermentative responses observed in the EXU group must be interpreted as the combined contribution of the whole exuviae complex—including potential residual proteins, minerals, and other bioactives—rather than chitin alone. Second, although dietary treatments significantly modulated abdominal fat deposition and caecal short-chain fatty acid (SCFA) profiles, no direct measurements of host lipid metabolism pathways, gene expression (such as $\Delta 9$ -desaturase activity), or high-throughput caecal microbiota sequencing were conducted. Thus, the definitive microbiological and biochemical mechanisms driving these shifts remain partly speculative. Finally, this study evaluated a specific production phase (28–39 weeks of age) over an 11-week trial; therefore, longer-term investigations extending across the entire laying cycle are required to verify the prolonged physiological sustainability and economic viability of these insect-derived additives.

Institutional review board statement

The animal study protocol was approved by the Ethical Animal Care and Use Committee of the Department of Veterinary Medicine and Animal Production of the University of Napoli Federico II, Italy (prot. n. 2017/0017676).

Author contributions

Conceptualization, NFA, FB; methodology, ML, NM, AG, RS, CS, ADB, PF; investigation, ML, VP, LB, FA, RA, AG, RS, CS, ADB; data curation, ML, NM; formal analysis, NFA; writing—original draft preparation, ML; writing—review and editing, NFA, ARDR, AG, RS, CS, ADB, PF, FB; supervision, FB; project administration, FB; funding acquisition, NM, CS, NFA, FB. All authors have read and agreed to the published version of the manuscript.

CRedit authorship contribution statement

Lanzieri M.: Methodology, Investigation, Data curation, Writing – original draft. **Musco N.:** Methodology, Data curation, Funding

acquisition. **Accetta F.:** Investigation. **Addeo N.F.:** Conceptualization, Formal analysis, Writing – review & editing, Funding acquisition. **Panettieri V.:** Investigation. **Borrelli L.:** Investigation. **Guarnieri A.:** Methodology, Investigation, Writing – review & editing. **Salvia R.:** Methodology, Investigation, Writing – review & editing. **Scieuzo C.:** Methodology, Investigation, Writing – review & editing, Funding acquisition. **De Bonis A.:** Methodology, Investigation, Writing – review & editing. **Falabella P.:** Methodology, Writing – review & editing. **Armone R.:** Investigation. **Di Rosa A.R.:** Writing – review & editing. **Bovera F.:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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Not applicable.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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