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Effects of partially defatted larvae meal of Black Soldier Fly (*Hermetia illucens*) on caecal microbiota and volatile compounds of Muscovy ducks (*Cairina moschata domestica*)

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ABSTRACT

The present study explored the effects of substituting maize gluten meal with increasing levels of partially defatted black soldier fly larvae meal (BSFLM) in Muscovy ducks' diets on their caecal microbiota and organic volatile compounds. The ducks were divided into four groups, each receiving a diet containing 0%, 3%, 6%, or 9% BSFLM (HI0, HI3, HI6, and HI9, respectively). At slaughter (50 days of age), caecal samples were collected and analysed. The alpha diversity indexes were lower in the HI9 than in the HI0 and HI3 treatments that did not differ between them. Bacteroidetes in the HI0 and Firmicutes in the HI6 treatment showed a higher abundance than in the HI9 treatment. *Faecalibacterium* and *Megamonas* were more abundant in the HI6 than in the HI9 treatment. Abundance of *Clostridium* and unclassified Coriobacteriaceae were higher and lower, respectively, in the HI9 than in the HI0 treatment. Canonical discriminant analysis revealed that *Faecalibacterium*, unclassified Victivallaceae and *Megamonas* in relation to *Ruminococcus* would separate the HI6 and HI9 treatments, while unclassified Coriobacteriaceae in relation to *Streptococcus* and *Faecalibacterium* would distinguish the HI0 and HI3 from the HI6 and HI9 treatments. Eleven volatile compounds were more abundant HI9 than in the HI6 treatment. Five of them were negatively correlated with *Faecalibacterium* and two with *Megamonas*. These findings indicate that diets with 6% and 9% BSFLM alter the caecal microbiota in Muscovy ducks, while a diet with 3% BSFLM has no effect. The distinct abundance of several volatile compounds in the 6% and 9% BSFLM treatments suggests a relationship between their characteristic microbiota profile and those compounds that warrants further investigation.

HIGHLIGHTS

- 3% black soldier fly larvae meal in duck feed does not affect caecal microbiota profile.
- Diets with 6% and 9% black soldier fly larvae meal trigger distinct caecal microbiota and volatile compound shifts.

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Poultry; insects; caecum; microbiota; volatile compounds

Introduction

A sustained increase in poultry meat demand for human consumption is expected to occur in the next decades (Henchion et al. 2021). Traditionally, soybean meal and fishmeal have been the primary protein sources in poultry diets, which might be considered undesirable from a sustainability point of view (Mottet and Tempio 2017). However, feed formulation taking into account environmental constraints considerably increases the cost of formulas based on traditional ingredients (Tallentire et al. 2017). Furthermore, the rising costs and fluctuating availability of conventional protein sources have led to a search for alternative options (Iji et al. 2017). Insects, particularly black soldier fly larvae, have attracted considerable attention in recent years due to their high nutritional value, minimal environmental footprint, and potential to reduce waste through bioconversion (Dörper et al. 2021).

Gut microbiota plays a crucial role in the health and performance of poultry (Yadav and Jha 2019). Research has shown that gut microbe profile has a

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profound impact on production performance and nutrient metabolism (Singh et al. 2012; Moula et al. 2018; Yang et al. 2022). The composition and diversity of the gut microbiota are influenced by several factors, including age, genetics, and environmental conditions, but among all factors the diet plays a leading role (Kogut 2022). In this regard, few papers have dealt with the effects of black soldier fly larvae meal (BSFLM) on gut microbiota in poultry (Moniello et al. 2019; Biasato et al. 2023). The inclusion of insect-based meals in the diet has a noteworthy impact on gut health, attributable not only to their outstanding nutritional composition but also to their intriguing nutraceutical elements. These elements, including chitin, antimicrobial peptides, and lauric acid, have recently been proposed as having a substantial influence on the gut microbiota of animals (Addeo et al. 2022, Atallah et al. 2023; Biasato et al. 2023). The incorporation of BSFLM in poultry diets has been shown to modulate the gut microbiota and the production of short-chain fatty acids in the caecum (Borrelli et al. 2017; Biasato et al. 2020; He et al. 2021). Furthermore, changes in gut microbiota in response to BSFLM seem to be related to the inclusion level in the diet (Biasato et al. 2020; He et al. 2021).

Gut bacteria release a diverse range of volatile organic compounds, including hydrocarbons, ketones, alcohols, acids and others, in their headspace that are potentially involved in their coexistence in dynamic communities and the adaptation to environmental changes (Audrain et al. 2015). Those volatile compounds are the resultant effect of substrate fermentation and might be potential markers for intestinal dysbiosis (Celi et al. 2019). However, information on volatile compounds other than short-chain fatty acids in the lower gut of poultry is scant (Yang et al. 2020; Hartinger et al. 2022). Thus far, the majority of research on the relationship between insects and the animal gut has primarily centred on analysing the bacterial composition of the intestinal microbiota. Only a limited number of studies have ventured into exploring the potential of the volatilome (Biasato et al. 2023).

The objective of the present study was to explore the effects of increasing levels of partially defatted BSFLM in the diet of Muscovy ducks (*Cairina moschata domestica*) on their caecal microbiota and volatile compounds.

Materials and methods

The experimental protocol used in the present study was carried out in compliance with the guidelines of the European and Italian laws on the protection of animals used for experimental and other scientific purposes and was approved by the Bioethical Committee of the University of Turin (Italy; protocol number 380576).

Animals and husbandry

A total of 3-day-old 192 female Muscovy ducklings (Canedins R71 L White, Grimaud Freres Selection, France) were housed at the poultry facility of the Department of Agricultural, Forest and Food Science of the University of Turin (Italy). The poultry house was 7 m wide × 50 m long × 7 m high, with an automatic ventilation system and equipped with a waterproof floor and walls. Each pen was 1.20 m wide × 2.20 m long and rice hulls were used as bedding. The poultry house was automatically heated to maintain an environmental temperature ranging from 25 °C to 18 °C, depending on the age of the ducklings. Additionally, during the first three weeks of their life, each pen was equipped with infrared infra-red lamps (Philips® 150 W) to ensure the appropriate floor-level temperature. Initially, the lamps were positioned approximately 40 cm above the floor. Subsequently, the lamps were gradually raised. During the initial week of their growth, the temperature at floor level was kept within the range of 29–32 °C. Following this, the temperature was gradually lowered by 3–5 °C each week until the birds had fully developed their feathers, maintaining a consistent range of 18–21 °C. Until day 3 of the trial (6 days old of birds) the daily lighting schedule was 23 L:1D and thereafter the dark period was progressively increased to 6 h and maintained constant until slaughtering. The relative humidity ranged between 60% and 70%. The environmental parameters (light, temperature an relative humidity) were monitored daily.

Experimental design

The birds were distributed in 24 pens of similar average initial body weight (71.3 ± 3.0 g). The pens were randomly assigned to four dietary treatments. Therefore, there were six pens (replicates) per treatment with eight birds per pen. The treatments consisted of four experimental diets with 0, 3, 6 and 9% as fed of partially defatted BSFLM (hereafter HI0, HI3, HI6 and HI9 treatments, respectively) which was substituted for maize gluten meal. The detailed composition of the experimental diets has been reported elsewhere (Gariglio et al. 2019). Briefly, in order to meet the ducks' nutritional requirements, a 3-phase

feeding program was applied: starter diet (from three to 17 days old), grower diet (from 18 to 38 days old), and finisher diet (from 39 to 51 days old). The diets were based on maize and soybean meal (> 75%, as fed). Calculated apparent metabolisable energy corrected to zero nitrogen balance and measured crude protein values were (as fed): 2890 kcal/kg and 22.4%, 2990 kcal/kg and 20.0%, and 3050 kcal/kg and 17.9%, in the starter, grower and finisher diets, respectively. Determined BSFLM composition was (% dry matter): crude protein, 56.71; ether extract, 10.70; ash, 16.38; chitin, 6.43. Feed and water were provided ad libitum during the experimental period.

Caecal sampling, microbial enumeration and volatile compound profile determination

At 50 days of age, two birds from each replicate (12 birds per treatment) with the closest body weight to the mean weight for their pen were selected and identified with a shank ring. Subsequently, after a feed withdrawal of 12 h were transferred to a commercial processing plant and slaughtered by electrical stunning and bleeding, according to the standard EU regulations.

Caecal content of the slaughtered birds was collected into sterile plastic tubes that were previously refrigerated (for a maximum of 2 h) and frozen at −80 °C until DNA extraction and sequencing. Analysis was carried out as described by Biasato et al. (2020). In brief, total DNA from the samples was extracted using the RNeasy Power Microbiome KIT (Qiagen, Milan, Italy). The DNA was then quantified using the NanoDrop and standardised at 5 ng/μL. The DNA was used to assess the microbiota diversity by the amplification of the V3-V4 region of the 16S rRNA gene. Sequencing was performed with a MiSeq Illumina instrument with V3 chemistry and generated 250 bp paired end reads. FLASH software (https://ccb.jhu.edu/software/FLASH/) was used to join the reads while QIIME 1.9.0 software (http://qiime.org/) was used for the other steps. Operational Taxonomic Units (OTUs) were picked at 97% of similarity and taxonomy was assessed by Greengenes16S rRNA gene database v. 2013. The OTU table was rarefied at the lowest number of sequences and display the higher taxonomy resolution.

Caecal volatile was analysed in five out of 12 samples because only samples with a similar amount of caecal content were retained. Analysis was carried out as described by Battelli et al. (2019). Briefly, volatile compounds in caecal contents were determined in the headspace of samples using a Head-Space Solid Phase Micro Extraction module (Combi-Pal automated sampler CTC Analytics, Zwingen, Switzerland) equipped with DVB/CAR/PDMS 50/30 μm fibre (Supelco, Bellefonte, USA) and coupled to a gas chromatograph-mass spectrometer (6890 N/5973N Agilent Technologies, Inc., Wilmington, DE). The volatile compounds were identified using the Wiley 7n-1 MS library on Agilent MSD ChemStation® software (Agilent Technologies Inc.). Confirmation of the identity of the volatile compounds was achieved by comparing the GC retention indexes and mass spectra of individual components with those of authentic reference compounds injected under the same operating conditions. The results were expressed as log10 of the peak area of the corresponding compound.

Statistical analyses

The present study includes data on growth performance to support the findings, but these data have already been published in Gariglio et al. (2019, 2021), where the associated statistics were previously described. SAS OnDemand for Academics (SAS Institute, Cary, NC, USA) was used for statistical analyses. Means in the text were presented as means ± standard deviation. Statistical significance was declared at $p < 0.05$. Differences between treatments in the alpha diversity measures (Chao1, Shannon and observed species indexes), the relative abundance of phyla, families and genera were assessed by the multiple comparison method of Dwass-Steel-Critchlow-Fligner within the non-parametric analysis of variance (Kruskall-Wallis' test) carried out with the NPAR1WAY procedure. The CANDISC procedure was used to perform a canonical discriminant analysis. The variables that would be included as predictors in the canonical discriminant analysis were selected with the STEPDISC procedure (P to enter, 0.15; P to stay, 0.15). Canonical discriminant analysis is a useful multivariate statistical technique to determine if hypothesised groups are distinguishable on the basis of a set of predictor variables (Paliy and Shankar 2016). In the current analysis, the feeding treatments were the hypothetical grouping variable, and the quantitative variables used as predictors were the bacterial genera identified in the caecal contents. Only genera with relative abundance higher than 1% were used in the analysis. Zero values were replaced by 0.55 times the lowest measured value in each variable prior to the application of the centred log-ratio transformation recommended for multivariate analysis of compositional data (Aitchison

1982; Sandford et al. 1993), then multivariate normality was assessed by Mardia's test. Pearson's correlation analysis was used when appropriate.

Results and discussion

To the best of our knowledge this is the first time that the effects of feeding BSFLM to ducks on caecal microbiota and volatile compounds is reported. For this reason, the discussion of the observed effects is mostly referred to other poultry species fed BSFLM.

Caecal microbiota

The growth performance results from the same experiment published elsewhere (Table 1) (Gariglio et al. 2019; 2021) indicated that any differences in the microbiota profile found in the present study did not have any effects on average final body weight (2516 ± 70 g), average daily gain (52 ± 2 g), daily feed intake (120 ± 4 g), and feed conversion ratio (2.3 ± 0.1 g/g). In this regard, it has been shown that up to 15% BSFLM in chicken diets is not expected to have any negative effects on growth performance (Martínez Marín et al. 2023).

Table 1. Growth performance of Muscovy ducks fed diets containing 0% (HI0), 3% (HI3), 6% (HI6) or 9% (HI9) of partially defatted black soldier larvae meal substituted for maize gluten meal (from Gariglio et al. 2021).

Item	HI0	HI3	HI6	HI9	SEM
Initial live weight (g)	71	70	73	72	0.60
Final live weight (g)	2,541	2,511	2,456	2,555	20.13
Average daily gain (ADG) (g/d)	52	52	52	53	0.43
Daily feed intake (DFI) (g/d)	121	121	118	121	1.20
Feed conversion ratio (FCR) (g/g)	2.29	2.34	2.32	2.30	0.019

None of the alpha diversity measures (Figure 1) differ between the H0 and HI3 groups ($p > 0.05$) and they were always lower in the HI9 treatment as compared with those treatments ($p < 0.05$), with the HI6 treatment in an intermediate position between the H0 and the HI9 treatments for Shannon and observed species indexes. Biasato et al. (2020) also found a lower Shannon index in the caecal contents of chickens fed the diet with the highest level of partially defatted BSFLM (15%) in their study but they did not detect differences in Chao 1 and observed species indexes in comparison with the control diet and the diets that included 5 and 10% BSFLM. On the contrary, He et al. (2021) found a higher Chao 1 index in chickens, whose feed included 5% full fat BSFLM as compared with the controls and those fed the diet with 1% BSFLM, but observed no differences in the Shannon index. In hens that were fed a diet with 10% full fat BSFLM, Kawasaki et al. (2019) reported a higher Chao 1 index and no differences in the Shannon index with the control diet. Moreover, Borrelli et al. (2017) described higher observed species and Shannon indexes in hens fed a diet with 17% defatted BSFLM than in those that received a control diet. Such discrepancies between studies on the effects of BSFLM

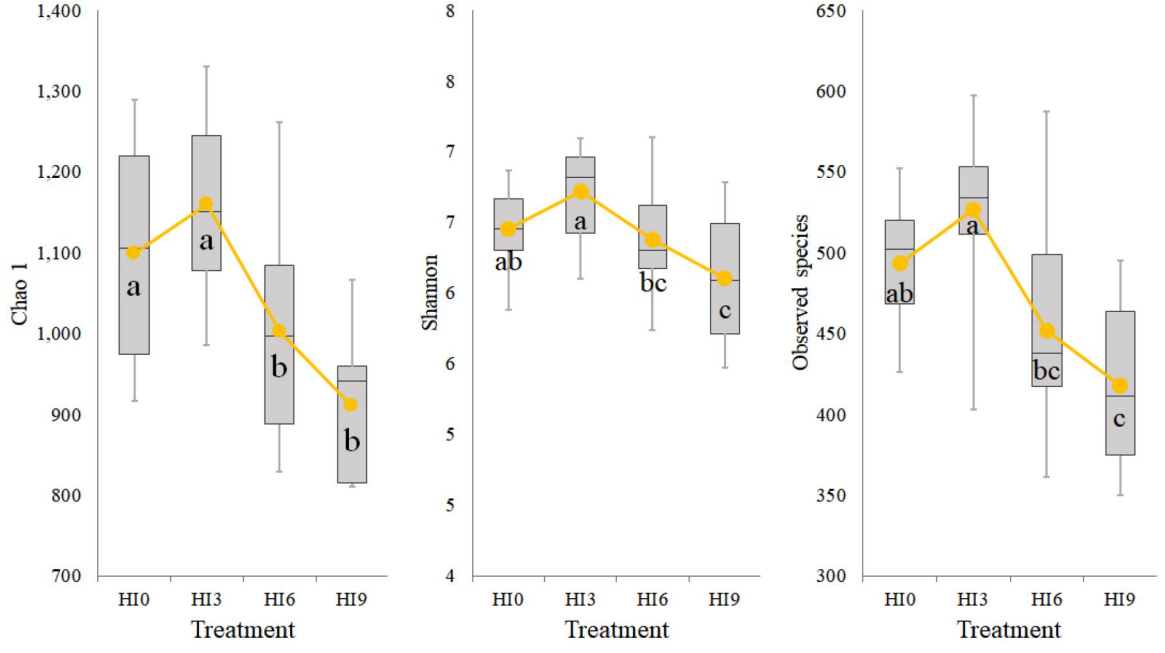

Figure 1. Box plots of the alpha diversity indexes in caecal samples of Muscovy ducks fed diets containing 0% (HI0), 3% (HI3), 6% (HI6) or 9% (HI9) of partially defatted black soldier larvae meal substituted for maize gluten meal. Treatments without a common letter are statistically different by the Dwass-Steel-Critchlow-Fligner test for multiple comparisons at $p < 0.05$.

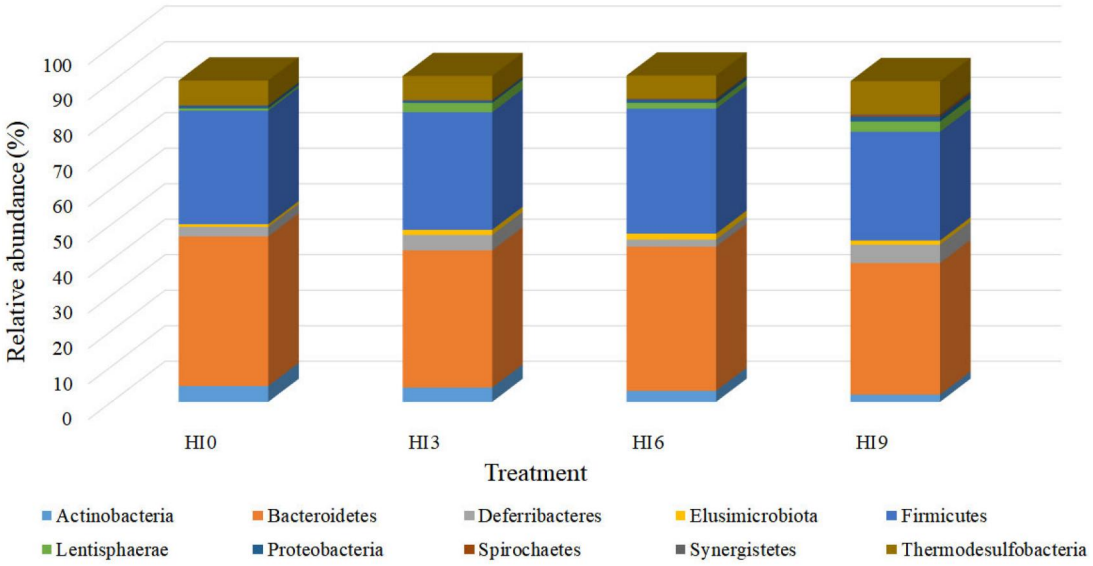

Figure 2. Relative abundance of bacterial phyla in caecal samples of Muscovy ducks fed diets containing 0% (HI0), 3% (HI3), 6% (HI6) or 9% (HI9) of partially defatted black soldier larvae meal substituted for maize gluten meal.

on the richness and evenness of caecal microbiota are difficult to explain. Higher Chao1 and Shannon's indexes represent a more rich and diverse microbial population, respectively, which in turn should be beneficial for the intestinal ecosystem and its performance (Torok et al. 2011; Stanley et al. 2012). However, as observed in the present study, lower alpha diversity indexes of caecal microbiota have not always translated into poorer performance despite it might be detrimental to the host's immunity and gut health in the long term (Siegerstetter et al. 2017; Diaz Carrasco et al. 2019).

A total of 38 genera belonging to 10 phyla were identified. Thirty-three families were represented by those genera and up to 12 families out of the 36 identified included unclassified members. Bacteroidetes closely followed by Firmicutes were the predominant phyla in all treatments (Figure 2). This agreed with the fact that, contrary to what was reported in chickens and turkeys (Wei et al. 2013), Bacteroidetes are equivalent to or more prevalent than Firmicutes in duck caecal microbiota (Vasaï et al. 2014; Wang et al. 2018; Yang et al. 2020; Zhu et al. 2020). The relative abundance of Bacteroidetes was lower in the HI9 treatment than in the HI0 treatment ($p < 0.05$), whereas the relative abundance of Firmicutes was higher in the HI6 treatment than in the HI9 treatment ($p < 0.05$). On the contrary, neither Borreli et al. (2017), Biasato et al. (2020) nor He et al. (2021) reported changes in the relative abundance of those phyla. The quantitatively minor phylum Lentisphaerae was more abundant in the HI3 treatment than in the HI0 treatment ($p < 0.05$). This phylum was found positively related to BSFLM consumption in the study of Borrelli et al. (2017). The ratio Firmicutes to Bacteroidetes did not differ between treatments (0.84 ± 0.14; $p > 0.05$), which would support that the utilisation efficiency of feed energy and fat metabolism were not affected by BSFLM in the diet (Huang et al. 2021; Yang et al. 2022). Furthermore, the absence of differences in the performance results would be also supported by the fact that the relative abundances of Lachnospiraceae ($1.7 \pm 0.8\%$), Lactobacillaceae ($0.7 \pm 2.0\%$), Rikenellaceae ($2.1 \pm 1.2\%$), Synergistaceae ($0.16 \pm 0.14\%$) and Prevotellaceae ($1.7 \pm 1.9\%$) families, which are positively correlated with average daily weight gain or negatively correlated with feed conversion ratio in chickens (Singh et al. 2012; Moula et al. 2018), did not differ between treatments ($p > 0.05$).

In coincidence with previous studies in ducks (Yang et al. 2020; Zhu et al. 2020), the most abundant genus was *Bacteroides*, followed by *Desulfovibrio*, *Clostridium*, and *Faecalibacterium* (Figure 3). Significant differences ($p < 0.05$) were found for *Faecalibacterium* (HI6 > HI0 and HI9), *Megamonas* (HI6 > HI9) and *Suterella* (HI6 > HI3), as well as unclassified Victivallaceae (HI3 > HI0). *Faecalibacterium* and *Sutterella* are negatively related to fat deposition in ducks, whereas *Megamonas* shows a positive relationship (Yang et al. 2022). However, no significant correlations between abdominal fat deposition ($2.08 \pm 0.29\%$ slaughter weight; Gariglio et al. 2021) and those genera were found in the present study ($r = -0.14$, -0.09 and -0.02 in *Faecalibacterium*, *Sutterella* and *Megamonas*, respectively; $p > 0.05$). There was a higher relative

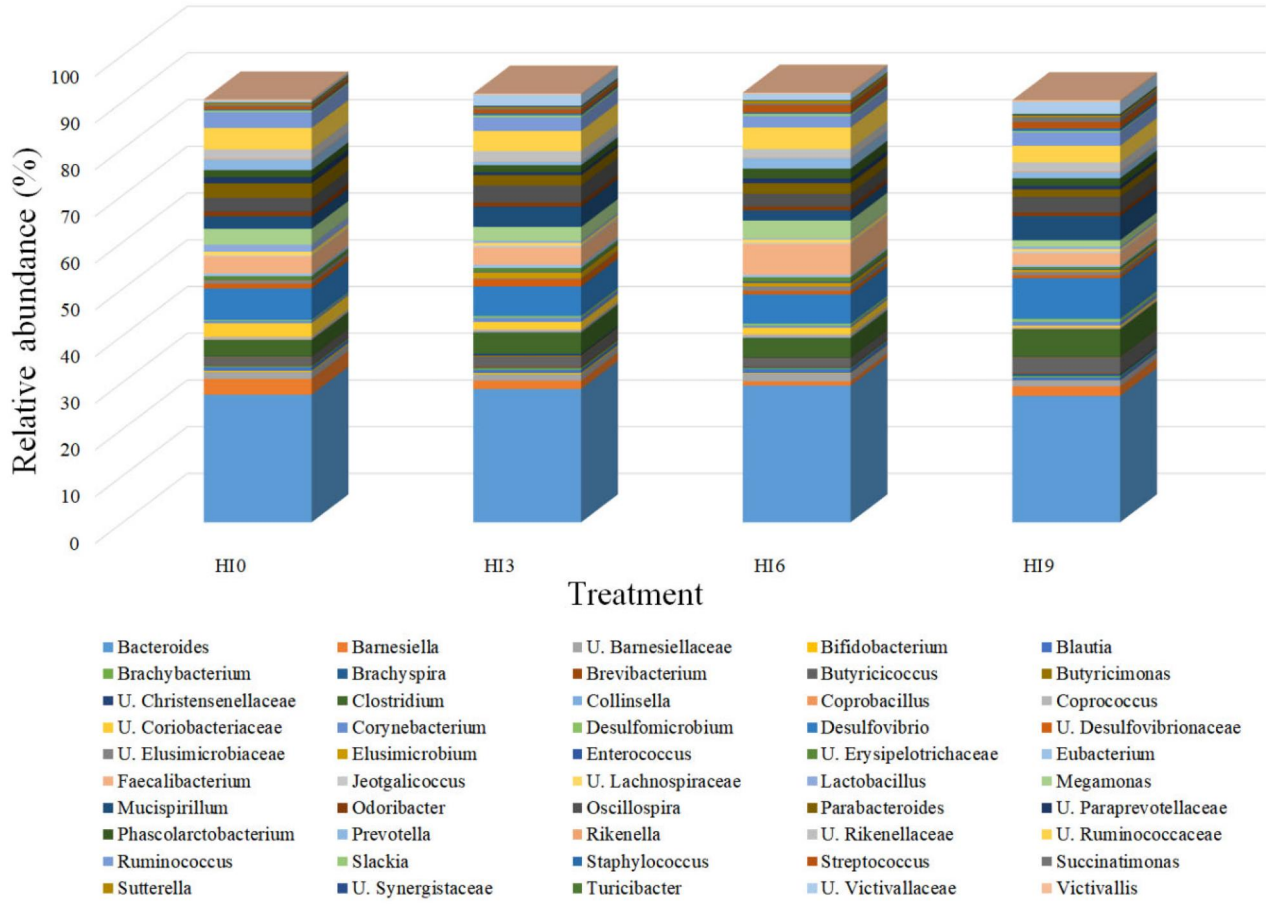

Figure 3. Relative abundance of bacterial genera in caecal samples of Muscovy ducks fed diets containing 0% (HI0), 3% (HI3), 6% (HI6) or 9% (HI9) of partially defatted black soldier larvae meal substituted for maize gluten meal. U.: unclassified.

abundance of *Clostridium* and a lower relative abundance of *Bifidobacterium*, *Coprococcus*, and unclassified Coriobacteriaceae in the HI9 treatment than in the HI0 treatment ($p < 0.05$). The latter genera were not described by Borreli et al. (2017) and He et al. (2021) as affected by their BSFLM treatments. The abundance of *Clostridium* was found by Biasato et al. (2020) to be linked to their 5% BSFLM treatment but not to the 10 and 15% BSFLM treatments, while Kawasaki et al. (2019) also found a lower abundance of *Bifidobacterium* due to BSFLM consumption. The reason why *Bifidobacterium*, which is recognised to have positive effects on gut health (Ali et al. 2022), might show a negative response to certain levels of BSFLM in the diet warrants future research. Noticeably, none of the genera with species related to chitin degradation according to the results of Borrelli et al. (2017) were identified in the HI9 treatment despite the higher expected chitin consumption (~ 0.70 g/d vs 0.46 and 0.23 g/d in the HI6 and HI3 treatments).

Six out of the 20 genera with relative abundance higher than 1% were selected as predictors in the canonical discriminant analysis (Table 2 and Figure 4). Wilks' lambda test, which checks the linear relationship between the predictors and the grouping variable (i.e. the validity of the analysis) was highly significant ($p < 0.001$). The first two discriminant functions reached significance and explained more than 88% of the total variance between groups. Discriminant function 1 mainly separated the HI6 and HI9 treatments (Squared Mahalanobis distance = 9.0; $p < 0.05$), whereas discriminant function 2 differentiate the HI0 and HI3 treatments from the HI6 and HI9 treatments (Squared Mahalanobis distance HI0 to HI6 = 6.1, HI0 to HI9 = 4.9, HI3 to HI6 = 3.6, and HI3 to HI9 = 5.1; $p < 0.05$). The HI0 and HI3 treatments were virtually indistinguishable between them (Squared Mahalanobis distance = 2.4; $p > 0.05$). The class means for each group's canonical observation scores in discriminant function 1 indicated that a high relative abundance of *Faecalibacterium*, unclassified Victivallaceae and *Megamonas* in relation to *Ruminococcus* would separate the HI6 and HI9 treatments. Conversely, class means for each group's canonical observation scores in discriminant function 2 showed that an elevated relative abundance of unclassified Coriobacteriaceae in

Table 2. Canonical discriminant analysis results.

	Pooled within standardised canonical coefficients		Total canonical structure	
	DF1[a]	DF2	DF1	DF2
Variables[b]				
U. m. Coriobacteriaceae	0.35	0.79	0.42	0.80
Faecalibacterium	0.79	−0.41	0.73	−0.03
Megamonas	0.57	−0.03	0.59	0.31
Ruminococcus	−0.60	0.09	−0.34	0.29
Streptococcus	−0.03	−0.50	−0.00	−0.58
U. m. Victivallaceae	0.61	−0.21	0.04	−0.53
Canonical details				
Canonical correlation	0.75	0.61		
Likelihood ratio test	$p < 0.001$	$p < 0.01$		
Variance explained (%)	59.77	28.39		
Class means[c]				
HI0	−0.31	1.05		
HI3	0.28	0.35		
HI6	1.51	−0.62		
HI9	−1.49	−0.78		

[a]DF: discriminant function.
[b]U.: unclassified.
[c]Diets for Muscovy ducks containing 0% (HI0), 3% (HI3), 6% (HI6) or 9% (HI9) of partially defatted black soldier larvae meal substituted for maize gluten meal. The pooled within standardised canonical coefficients indicate the relative contribution of each variable to the separation of the groups in the DF. The total canonical structure indicates the correlations between the continuous variables and the DF.

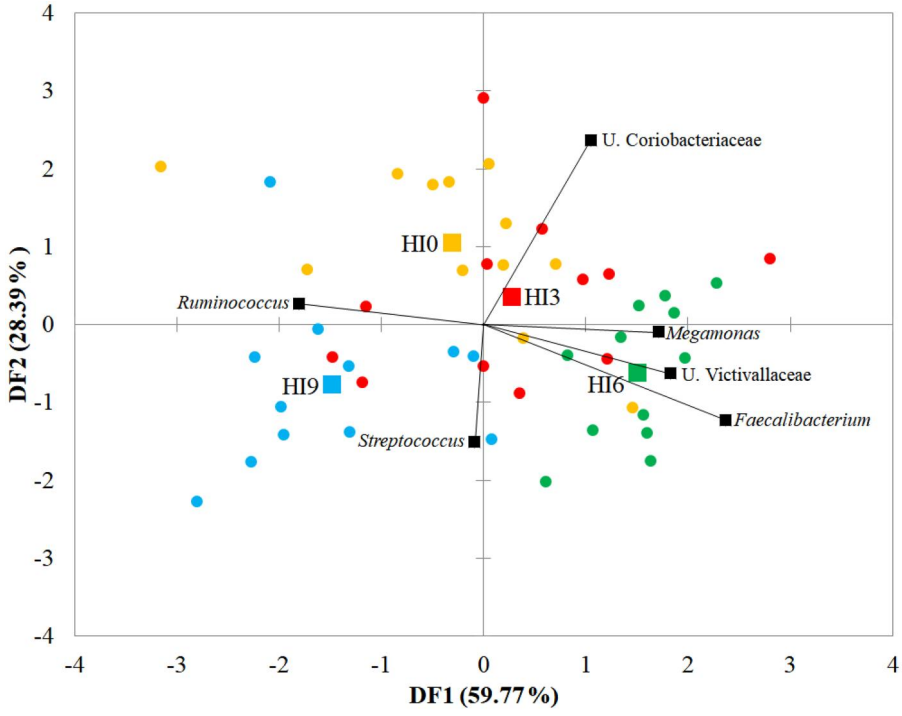

Figure 4. Canonical discriminant plot. Observations correspond to caecal samples of Muscovy ducks fed diets containing 0% (HI0; orange dots), 3% (HI3; red dots), 6% (HI6; green dots) or 9% (HI9; blue dots) of partially defatted black soldier larvae meal substituted for maize gluten meal. Class means of each treatment are indicated by squares of the corresponding colour. The black lines indicate the contribution of each predictor to the separation of the groups in the discriminant functions (×3 rescaling for the sake of readability). DF: discriminant function (within parentheses proportion of total variance explained). U.: unclassified.

relation to *Streptococcus* and *Faecalibacterium* would distinguish the HI0 and HI3 treatments from the HI6 and HI9 treatments.

Altogether the obtained results indicated that the diets with 6 and 9% BSFLM modified the caecal microbiota profile in different manners between them and with respect to the control and 3% BSFLM diets. In the experimental diets, maize gluten meal was substituted with BSFLM on a weight basis, assuming comparable nutritional values. However, the content of true protein in the crude protein is expected to be higher in maize gluten meal (90.1%; Mahesh et al.

2017) than in BSFLM (70.9%; Smets et al. 2021). Therefore, it might be hypothesised that the observed differences in the microbiota profile between treatments could be caused by a higher proportion of dietary crude protein in the 6 and 9% BSFLM diets (i.e. the fraction comprised by chitin and other non-protein nitrogen compounds not digestible in the upper gut) being fermented in the lower gut rather than being digested practically. This point would be supported by the higher relative abundance of *Clostridium* observed in the HI9 treatment in comparison with the HI0 treatment and the contribution of *Streptococcus* to discriminate the HI6 and HI9 treatments from the HI0 and HI3 treatments since both genera are regarded as proteolytic (Rinttilä and Apajalahti 2013). Again, fermentation of protein in the lower gut would not be reflected in the faecal apparent digestibility and thus in the apparent efficiency usage of total dietary nitrogen (Gariglio et al. 2019).

Volatile compounds

A total of 47 volatile organic compounds belonging to five chemical families (nine aldehydes, five ketones, eight alcohols, nine organic acids, and six esters) and a heterogenous group of other compounds (10) were identified in caecal samples (Table 3). Distinctly, eleven volatile compounds were more abundant in the HI9 treatment than in the HI6 treatment ($p < 0.05$). This suggested that the differences in the microbiota profile between the HI6 and HI9 treatments (e.g. the higher relative abundance of *Faecalibacterium*, unclassified Victivallaceae and *Megamonas* in relation to *Ruminococcus*) were responsible for the observed differences in the volatile compounds between those treatments. In this regard, Pearson's analysis of the treatment means of the bacteria genera that contributed to the separation of the groups in the canonical discriminant analysis and the volatile compounds that exhibited significant differences between treatments showed that *Faecalibacterium* was negatively correlated with pentanoic acid, 4-methylphenol, ethyl butanoate, 3,5-dimethyl-2-furyl methyl ketone and 1,3-bis(1,1-dimethyl-ethyl)-benzene ($r = -0.99$, -0.97, -0.96, -0.96 and -0.97, respectively; $p < 0.05$), while *Megamonas* was negatively correlated with hexanal and ethyl propanoate ($r = -0.97$ and -0.98; $p < 0.05$).

Published literature on the relationship between the microbiota and the volatile compounds in the caecal contents of poultry is scarce and makes it difficult to discuss the above results. Chang and Yu (2022) did not observe any correlation between *Faecalibacterium* and pentanoic acid in the caecal contents of chickens. However, Liu et al. (2023) found a negative relationship between the relative abundance of *Faecalibacterium* and pentanoic acid concentration in an *in vitro* study of colonic fermentation using pig faecal microbiota. The phenolic compound 4-methylphenol has been related to the *in vitro* fermentation of glucose and ethanol as carbon sources by *Clostridium*

Table 3. Effects of diets containing 0% (HI0), 3% (HI3), 6% (HI6) or 9% (HI9) of partially defatted black soldier larvae meal substituted for maize gluten meal on the caecal volatile compounds of Muscovy ducks.

Volatile compounds	HI0	HI3	HI6	HI9	SEM
Aldehydes					
Benzaldehyde	5.74	5.60	5.77	6.04	0.072
Benzeneacetaldehyde	4.52	4.53	4.52	4.83	0.062
Butanal	4.37	4.81	4.15	5.12	0.149
Hexanal	5.43^{ab}	5.95^{ab}	5.06^{b}	6.44^{a}	0.16
Propanal	4.35	4.49	4.39	4.88	0.136
2-Methylbutanal	4.52	5.05	3.80	5.45	0.206
2-Methylpropanal	5.72	5.66	5.84	5.79	0.061
2-Phenyl-2-butenal	4.09^{ab}	4.40^{ab}	3.65^{b}	4.68^{a}	0.143
3-Methylbutanal	5.30	5.32	5.44	5.39	0.064
Ketones					
Acetone	4.82	4.65	4.71	4.83	0.074
2-Butanone	2.63	3.26	2.35	3.75	0.233
2,3-Butanedione	3.90	3.78	3.55	4.20	0.137
3-Hydroxy-2-butanone	5.67	5.89	5.48	6.07	0.082
3-Octanone	3.66	3.91	2.97	4.61	0.229
Alcohols					
Ethanol	5.10	5.00	4.83	5.04	0.053
Isopropanol	4.31	4.52	4.08	4.66	0.105
Phenol	6.74^{ab}	7.06^{ab}	6.50^{b}	7.10^{a}	0.076
1-Hexanol	6.10	6.19	5.73	6.33	0.088
1-Propanol	5.25	5.35	5.48	5.42	0.039
3-Methyl-1-butanol	4.43	4.83	4.05	4.50	0.149
4-Ethylphenol	4.66	4.89	4.76	4.77	0.067
4-Methylphenol	5.79^{ab}	6.04^{ab}	5.15^{b}	6.16^{a}	0.113
Acids					
Acetic acid	5.63	5.52	5.27	5.78	0.083
Butanoic acid	6.29	6.37	6.45	6.34	0.035
Propanoic acid	4.36	4.90	4.50	4.99	0.108
Pentanoic acid	6.11^{ab}	6.14^{ab}	5.70^{b}	6.28^{a}	0.077
Hexanoic acid	6.37^{ab}	6.51^{ab}	5.94^{b}	6.46^{a}	0.073
2-Methylbutanoic acid	5.04	5.44	4.73	5.79	0.168
2-Methylpropanoic acid	5.87	5.76	5.50	6.03	0.086
3-Methylbutanoic acid	5.98	5.79	5.60	5.96	0.075
4-Methylpentanoic acid	5.20	5.25	5.73	5.60	0.093
Esters					
Ethyl acetate	2.85	4.04	2.32	4.41	0.249
Ethyl anteisovalerate	6.08	6.43	5.71	6.57	0.117
Ethyl butanoate	6.07^{ab}	6.39^{a}	5.51^{b}	6.49^{a}	0.108
Ethyl isovalerate	5.30	5.17	5.31	5.25	0.031
Ethyl propanoate	3.62^{ab}	4.11^{ab}	3.21^{b}	4.78^{a}	0.222
Methyl 4,6-dimethyloctanoate	4.67	4.72	4.83	4.87	0.066
Others					
Indole	5.18	5.03	5.16	5.06	0.075
1,3-bis(1,1-dimethylethyl)-benzene	4.51^{ab}	4.76^{ab}	3.65^{b}	5.33^{a}	0.194
2,4,6-Trimethylpyridine	4.35	4.92	3.60	5.19	0.227
2,4-Dimethylbenzaldehyde	5.19	5.42	5.28	5.54	0.050
2,5-Dimethylbenzaldehyde	5.96	6.23	6.02	6.25	0.088
2-Pentylfuran	5.56	5.47	5.50	5.45	0.023
3,4-Dimethylbenzyl alcohol	6.62^{ab}	7.15^{ab}	6.26^{b}	7.38^{a}	0.137
3,5-Dimethyl-2-furyl methyl ketone	6.30^{ab}	6.62^{a}	5.66^{b}	6.73^{a}	0.114
4,5-Dimethyl-2-isopropyloxazole	5.83	6.31	5.35	6.62	0.179
4-Methoxyphenol	4.56^{ab}	4.76^{a}	4.58^{b}	5.02^{a}	0.105

Treatment means without a common superscript letter are statistically different by the Dwass-Steel-Critchlow-Fligner test for multiple comparisons at $p < 0.05$. SEM: standard error of the mean.

and *Ruminococcus*, respectively, isolated from pit mud (Ji et al. 2020). Allowing for the differences, in the present study neither *Ruminococcus* nor *Clostridium*, which defined the HI9 treatment in contrast to the HI6 treatment, showed a significant correlation with 4-methylphenol ($r = 0.52$ and 0.54; $p > 0.05$). Ethyl propanoate and ethyl butanoate synthesis by *Lactobacillus* strains has been demonstrated during cheese ripening (Mukdsi et al. 2018), but no correlation between them was observed in the present study ($r = -0.12$ and 0.12; $p > 0.05$).

Conclusions

The results of the present study indicated that the inclusion of 3% BSFLM in the diet of fattening female Muscovy ducks in replacement of maize gluten meal does not elicit significant changes in the caecal microbiota as compared to a control diet without BSFLM. Conversely, the inclusion of 6% and 9% BSFLM in the diet alters the microbiota profile in a distinctively manner, although it would not be reflected in production performance. The differences in the abundance of several caecal volatile compounds between the diets with 6% and 9% BSFLM suggest a close relationship between their distinguishing microbiota and those compounds that deserve further research.

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Ethical approval

The experimental protocol was approved by the Bioethical Committee of the University of Turin (Italy) (protocol number: 380576, 04/12/2017).

Authors' contributions

MG, AS, and IB conceived and designed the experiment. MTC, IB, and MG performed the trial and collected the experimental data. SP performed the volatile compounds analysis. IF performed the microbiota analysis. ALMM and IF performed the statistical analysis. ALMM, MG, and AS wrote the first draft of the manuscript. All the authors critically reviewed the intellectual content of the manuscript and gave their approval for the final version to be published.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author, [M. G.], upon reasonable request.

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