

Alternative nutritional evaluation of black soldier fly with various substrates in cat diets and its effects on fecal microbiota

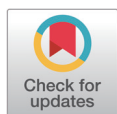
Kyeongho Jeon^{1#}, Jihwan Lee^{2#}, Minhong Song^{3#}, Kwanho Park⁴,
Seyeon Chang¹, Dongcheol Song¹, Hyuck Kim¹, Jinmo Yang¹, Jinho Cho^{1*}

¹Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea

²Swine Science Division, National Institute of Animal Science, Rural Development Administration, Cheonan 31000, Korea

³Division of Animal and Dairy Science, Chungnam National University, Daejeon 34134, Korea

⁴Industrial Insect and Sericulture Division, Department of Agricultural Biology, National Institute of Agricultural Sciences, Rural Development Administration, Wanju 55365, Korea



Received: May 12, 2025

Revised: Jun 19, 2025

Accepted: Jun 19, 2025

#These authors contributed equally to this work.

*Corresponding author

Jinho Cho

Department of Animal Science,
Chungbuk National University,
Cheongju 28644, Korea
Tel: +82-43-261-2544

E-mail: jinhcho@chungbuk.ac.kr

Copyright © 2025 Korean Society of Animal Science and Technology. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID

Kyeongho Jeon

<https://orcid.org/0000-0003-2321-3319>

Jihwan Lee

<https://orcid.org/0000-0001-8161-4853>

Minho Song

<https://orcid.org/0000-0002-4515-5212>

Kwanho Park

<https://orcid.org/0000-0002-3147-8651>

Seyeon Chang

<https://orcid.org/0000-0002-5238-2982>

Abstract

This study aimed to examine the effects of proper substitution ratios and organic substrates of black soldier fly larvae (*Hermetia illucens* larvae; HIL) on cats. A total of 30 mixed-sex domestic cats (*Felis domestica*) with an initial body weight of 5.06 ± 0.89 kg were used in this experiment, which used an incomplete 5×5 replicated Latin square design. The experimental period was 10 d, with 7 d of diet adaptation followed by 3 d of dietary treatment. The dietary treatments were provided: 1) CON (basal diet), 2) HA3 (replacing 3% of the poultry meal (PM) in the basal diet with HIL fed on animal-based substrate), 3) HA6 (replacing 6% of the PM in the basal diet with HIL fed on animal-based substrate), 4) HP3 (replacing 3% of the PM in the basal diet with HIL fed on plant-based substrate) 5) HP6 (replacing 6% of the PM in the basal diet with HIL fed on plant-based substrate). The HA3 group showed significantly higher ($p < 0.05$) crude protein digestibility than the CON group. The groups replaced by HIL had significantly higher ($p < 0.05$) ether extract digestibility than the CON group. The leucine, tryptophan, serine, and aspartic acid digestibility were significantly increased ($p < 0.05$) in the groups replaced by HIL compared to the CON group. The HP6 group showed distinct ($p < 0.05$) unweighted UniFrac distances from all other groups and weighted UniFrac distances from the CON and HP3 groups. In addition, the CON group exhibited significant differences ($p < 0.05$) in weighted UniFrac distances compared to all treatment groups. At the genus level, the HP6 group showed increased ($p < 0.05$) abundance of Bacteroidota compared with the CON group, while the CON exhibited higher ($p < 0.05$) levels of Firmicutes than the HP6 group. In conclusion, substituting PM with HIL in cat diets improved nutrient digestibility and had no negative impact on the fecal microbiota. Therefore, it is considered safe and effective to substitute up to 6% of the protein source with HIL in cat diets.

Keywords: Fecal microbiota, Feline, *Hermetia illucens* larvae, Nutrient digestibility, Organic substrates

Dongcheol Song
<https://orcid.org/0000-0002-5704-603X>
 Hyuck Kim
<https://orcid.org/0000-0002-5280-0734>
 Jinmo Yang
<https://orcid.org/0009-0007-4272-3441>
 Jinho Cho
<https://orcid.org/0000-0001-7151-0778>

Competing interests

No potential conflict of interest relevant to this article was reported.

Funding sources

This work was carried out with the support of 'Cooperative Research Program for Agriculture Science and Technology Development (Project No. RS-2024-00398491)' Rural Development Administration, Korea.

Acknowledgements

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Authors' contributions

Conceptualization: Jeon K, Lee J, Song M, Cho J.
 Data curation: Jeon K, Chang S, Yang J.
 Formal analysis: Song D, Kim H.
 Methodology: Chang S, Yang J.
 Software: Jeon K, Chang S.
 Validation: Kim H, Yang J.
 Investigation: Park K, Cho J.
 Writing - original draft: Jeon K, Lee J, Song M.
 Writing - review & editing: Jeon K, Lee J, Song M, Park K, Chang S, Song D, Kim H, Yang J, Cho J.

Ethics approval and consent to participate

This experiment was examined and approved (approval # CBNUA-24-0039-01) by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea.

INTRODUCTION

Cats and dogs foster social interactions and entertainment [1,2]. Although both dogs and cats are classified as carnivores nutritionally, dogs are omnivorous, whereas cats are strictly carnivorous [3]. Therefore, protein and fat constitute the most essential components of cat diets [4]. According to Green et al. [5], cats require 16% of their metabolizable energy to be protein to maintain themselves, significantly higher than rats, which need just 1.5%–3.5%. Research into alternative protein sources for cat diets has been conducted to optimize nutrient digestibility and improve overall fecal consistency due to fundamental dietary disparities [6,7].

As part of the companion animal industry, animal-sourced foodstuffs, such as feather meal and poultry by-product meal, are excellent sources of functional amino acids (AAs), which are essential for maintaining the structure, function, and health of the specialized connective tissues in dogs and cats [8]. However, FAO projections estimate a strong demand growth for poultry meat (PM) through 2050, creating an increasingly real challenge to sustainable PM production [9]. Insects have potential to be utilized as nutrient replacements for companion animal diets [10]. Black soldier fly larvae (*Hermetia illucens* larvae [HIL]), mealworm larvae (*Tenebrio molitor* larvae), and house cricket (*Acheta domesticus*) are among insects that have been authorized for use in companion animal diets [11]. Insects are similar in protein content to PM and abundant in fat, proteins, high-quality AAs (e.g., cysteine, lysine, methionine, and threonine), minerals, and essential vitamins (e.g., vitamin A, B complex, and vitamin C) [12]. Insects exhibit a favorable saturated-to-unsaturated fatty acid ratio and serve as a sustainable protein source due to their high feed efficiency compared to livestock animals [13]. Insect exoskeletons contain chitin, a naturally occurring carbohydrate that may have immune-modulatory properties and function akin to dietary fibers in promoting the formation of fecal metabolites [14,15]. In addition, insects are recognized as sustainable raw materials due to their high production rates, industrial output, and ability to consume diverse food sources [16].

Nutrient profiles of ingredients derived from insects can differ greatly based on factors such as rearing substrates, type of insects, developmental stage, and how they are raised [17]. Prior studies have demonstrated that HIL can consume a wide range of substrates with varying properties, such as mushrooms, food waste from restaurants, and animal manure [18–20]. Among them, vegetal and seaweed substrates are mainly utilized in HIL farming due to European legislation [21]. However, there is a notable lack of research on nutrient compositions of HIL reared on animal-based substrates. In addition, few studies have evaluated the impact of substrate variation by substituting conventional protein sources in cat diets with HIL raised on various substrates. Therefore, this study investigated the effects of replacing PM, a protein source in cat diets, with HIL reared on animal-based or plant-based substrates at two ratios (3% and 6%) on nutrient digestibility, gas emissions, and fecal microbiota.

MATERIALS AND METHODS

Animal ethics

This experiment was examined and approved (approval # CBNUA-24-0039-01) by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea.

Preparations for *Hermetia illucens* larvae and experimental diet

The HIL was reared on animal-based (milk sludge) and plant-based (citrus pulp) substrates. The HIL reared on an animal-based substrate was provided by Chungbuk Agricultural Research and

Extension Services (Cheongju, Korea), and the HIL reared on a plant-based substrate was obtained from Inseong Industry (Jeju, Korea). The HIL raised on the animal-based substrate were fed a mix of milk sludge and feed waste in a 7:3 ratio, maintained at $28 \pm 2^\circ\text{C}$ and $60 \pm 10\%$ humidity. The HIL reared on the plant-based substrate were provided with citrus pulp and soybean meal in an 8:2 ratio, at $25 \pm 3^\circ\text{C}$ and $70 \pm 5\%$ humidity. All HIL used in the experiment were 3rd instar larvae that had been reared for 10 days. All samples were then analyzed for dry matter ([DM] method 930.15), crude protein ([CP] method 990.03), and ether extract ([EE] method 920.29) following the procedures by the AOAC [22]. The gross energy (GE) of diets and feces was analyzed using an adiabatic oxygen bomb calorimeter (6400 Automatic Isoperibol calorimeter, Parr, Moline, IL, USA). The chemical composition of the experimental diets and the ingredient profiles are presented in Tables 1 and 2, respectively. The AAs were analyzed using high-performance liquid chromatography ([HPLC] Shimadzu model LC-10AT, Shimadzu, Kyoto, Japan).

The experimental treatments were as follows: CON, basal diet; HA3, replacing 3% of the PM in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the PM in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the PM in the basal diet with

Table 1. Nutrient components of HIL

Items	HIL fed with animal-based substrates	HIL fed with plant-based substrates
Dry matter (%)	96.68	93.46
Gross energy (kcal/g)	5,753.30	5,703.73
Crude protein (%)	48.37	38.51
Ether extract (%)	23.66	35.65
Crude fiber (%)	8.11	7.78
Crude ash (%)	10.06	10.49
Indispensable amino acids (%)		
Thr	1.57	1.56
Val	3.01	2.31
Ile	2.31	1.65
Leu	3.76	2.65
Phe	2.07	1.76
Lys	3.56	2.82
His	1.93	1.95
Arg	1.64	1.61
Trp	0.23	0.24
Met	0.65	0.71
Dispensable amino acids (%)		
Asp	4.22	3.40
Ser	2.97	1.62
Glu	6.42	4.16
Gly	0.45	0.77
Ala	4.19	3.09
Tyr	3.53	1.49
Cys	0.48	0.49
Pro	2.72	2.28

HIL, *Hermetia illucens* larvae; Thr, threonine; Val, valine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; Lys, lysine; His, histidine; Arg, arginine; Trp, tryptophane; Met, methionine; Asp, aspartic acid; Ser, serine; Glu, glutamic acid; Gly, glycine; Ala, alanine; Tyr, tyrosine; Cys, cysteine; Pro, proline.

Table 2. Ingredient composition of experimental diets

Items	CON ¹⁾	HA3	HA6	HP3	HP6
Ingredient (%)	100.00	100.00	100.00	100.00	100.00
Poultry meal	35.00	32.00	29.00	32.00	29.00
HIL fed on animal-based substrate	-	3.00	6.00	-	-
HIL fed on plant-based substrate	-	-	-	3.00	6.00
Rice	21.36	21.36	21.36	21.36	21.36
Wheat flour	14.74	14.74	14.74	14.74	14.74
Wheat bran	10.00	10.00	10.00	10.00	10.00
Soybean meal	5.00	5.20	5.50	5.70	6.40
DDGS	5.00	5.00	5.00	5.00	5.00
Poultry fat	5.00	4.80	4.50	4.30	3.60
Beet pulp	2.00	2.00	2.00	2.00	2.00
Salt	0.70	0.70	0.70	0.70	0.70
Taurine	0.20	0.20	0.20	0.20	0.20
Vitamin-mineral premix ²⁾	1.00	1.00	1.00	1.00	1.00
Chemical composition					
ME (kcal/kg)	3,633	3,635	3,632	3,633	3,633
CP (%)	32.01	31.61	31.25	31.55	31.09
EE (%)	10.56	10.74	10.82	10.66	10.77
CF (%)	3.64	3.83	4.03	3.86	4.07
Ash (%)	2.33	2.56	2.80	2.59	2.85
Ca (%)	1.11	1.18	1.26	1.19	1.28
P (%)	0.69	0.68	0.68	0.69	0.68

¹⁾CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate.

²⁾Provided per kg diet: 15.00 mg copper (CuSO₄), 0.30 mg selenium (Na₂SeO₃), 75.20 mg zinc (ZnSO₄, ZnO), 7.80 mg manganese (MnSO₄), 80.00 mg iron (FeSO₄), 1.80 mg iodine (KI), 22,600.00 IU vitamin A, 3,500.00 IU vitamin D, 54.00 mg vitamin E, 0.10 mg vitamin K3, 16.80 mg vitamin B1, 7.40 mg vitamin B2, 8.40 mg vitamin B6, 0.03 mg vitamin B12, 98.00 mg nicotinic acid, 9.48 mg calcium pantothenate, 0.11 mg D-biotin, 0.90 mg folic acid, 2641.80 mg choline chloride.

HIL, *Hermetia illucens* larvae; DDGS, dried distiller's grains with solubles; ME, metabolic energy; CP, crude protein; EE, ether extract; CF, crude fiber.

HIL fed on plant-based substrate; HP6, replacing 6% of the PM in the basal diet with HIL fed on plant-based substrate

Animal and experiment design

A total of 30 mixed-sex cats (*Felis domestica*), involving 12 males and 18 females, participated in a triplicated 5 × 5 Latin square design study. The cats had an average body weight of 5.06 ± 0.89 kg, and their diet was carefully regulated to meet or exceed the nutrient guidelines for adult cats by the Association of American Feed Control Officials (AAFCO) [23]. The experimental period lasted 10 d, including 7 d for diet adaptation and 3 d for sampling (starting from 0 d). Each cat was kept in a cage measuring 0.9 m × 0.9 m × 0.9 m, except during the feeding and sampling phases. The cats were randomly assigned to one of five experimental diets and were fed to maintain their body weight, with water available *ad libitum*. Cats were individually housed during feeding (twice daily from 08:00 h to 10:00 h and 15:00 h to 17:00 h) and fecal collection but were grouped together outside of the experimental periods. The cats were kept on a 12 h light cycle, with lights turned off

from 19:00 h to 07:00 h.

Nutrient digestibility

Apparent total tract digestibility (ATTD) of DM, CP, GE, and AAs was determined using 1% celite as an inert indicator by Scott and Boldaji [24] method. Cats were fed diets mixed with celite from 1 to 3 d and diet samples were also collected. Fresh fecal samples were collected from 2 to 4 d. Fresh fecal and diet samples were stored in a freezer at -20°C immediately after collection. At the end of the experiment, fecal samples were dried in a drying oven (OF-02GW, Jeio Tech, Daejeon, Korea) at 70°C for 72 h. Cat hairs and litter granules were manually removed from the dried feces and then ground finely using a laboratory mill (Mill CM 290 CemotecTM, FOSS, Hoganas, Sweden) with a 1 mm screen. All diet and fecal samples were then analyzed for DM (method 930.15), CP (method 990.03), and EE (method 920.29) following the procedures by the AOAC [22]. The GE of diets and feces was analyzed using an adiabatic oxygen bomb calorimeter (6400 Automatic Isoperibol calorimeter, Parr). The AAs were analyzed using HPLC (Shimadzu model LC-10AT, Shimadzu, Kyoto, Japan). For calculating the ATTD digestibility of nutrients used the following formula: $\text{Digestibility} = 1 - [(\text{Nf} \times \text{Cd}) / (\text{Nd} \times \text{Cf})] \times 100$, where Nf = concentration of nutrient in fecal, Nd = concentration of nutrient in the diet, Cd = concentration of celite in the diet, and Cf = concentration of celite in the fecal.

Fecal gas measurements

The fecal samples were collected and weighed at 100 g from each cage during the adaptation period (-7 d) and the sampling period (3 d). Samples were stored in 4.2 L plastic boxes in duplicate. The samples were allowed to ferment at room temperature (26°C) for 5 d. The plastic boxes with small holes sealed with adhesive plaster were used to analyze the fecal ammonia (NH_3), acetic acid (CH_3COOH), and hydrogen sulfide (H_2S) emissions of the samples. Before the measurement, the samples in plastic boxes are shaken for 20 s to break up clumps. The different kinds of detection tubes NH_3 (5.0–100.0 ppm; No.3La, detection tube, Gastec, Kanagawa, Japan), CH_3COOH (2.5–10.0 ppm; No.81L, detection tube, Gastec), and H_2S (2.0–20.0 ppm; No.4LK, detection tube, Gastec) were detected using the GV-100 (gas sampling pump, Gastec).

Fecal 16S metagenomic sequencing

Fecal samples were analyzed in 3 replicates per treatment. Fecal 16S rRNA sequencing data were analyzed using the QIIME2 next-generation microbiome bioinformatics pipeline. On -7 and 3 d, the fresh feces were collected in a sample bag from each pen and stored at -20°C until analysis. Samples were sent to Sanigen (Anyang, Korea) for microbial sequencing using the 16S rRNA technique. All raw data were converted into QIIME2 artifacts, encompassing information about data types and sources for subsequent analysis. Amplicon sequence variants (ASVs) were identified from the raw sequence data using the Divisive Amplicon Denoising Algorithm 2, a QIIME2 plugin. This algorithm corrects amplicon errors and filters out potential base errors and chimeric sequences [25]. The Relative Classification Frequency Table, which shows differential abundance tests at specific taxonomic levels, was generated using collapse and feature functions within the QIIME2 plugins. Alpha-diversity measurements were estimated and plotted using the QIIME2 'diversity' plugin in conjunction with R bioinformatics packages. This microbial diversity analysis pipeline used the ASV table (a more refined alternative to the traditional operational taxonomic unit table) as input data. Species richness and evenness scores, accounting for sampling depth, were measured using Chao1, Shannon, and Simpson indices. Each index evaluates the V3-V4 hypervariable region of the bacterial 16S rRNA gene. Relative abundance differences were analyzed

by comparing average bacterial proportions and compositions at each taxonomic level. Furthermore, bacterial classification accuracy across different amplicon regions was verified by comparing the taxonomy matching rate of each ASV's taxonomy with the National Center for Biotechnology Information (NCBI) bacterial reference genome database at the phylum and genus levels.

Statistical analysis

Statistical analyses and visualized graphs were performed using JMP Pro 16 (SAS Institute, Cary, NC, USA) and GraphPad Prism (Version 9.1.0; GraphPad Software, San Diego, CA, USA), respectively. All data, except fecal 16S metagenome data, were analyzed using one-way ANOVA with the Standard Least Squares model, treating each cat as an experimental unit. Alpha diversity was calculated from raw counts using Shannon estimators. For quantitative beta diversity analysis, each treatment group was considered the control group, and comparisons were made using the PROC MIXED procedure followed by Dunnett's post-hoc test. Results were considered statistically significant at $p < 0.05$.

RESULTS

Nutrient digestibility and fecal gas measurements

The CP digestibility was significantly higher ($p < 0.05$) in the HA3 group than in the CON group (Table 3). The groups replaced by HIL had significantly higher ($p < 0.05$) EE digestibility than the CON group. However, the DM and GE digestibility showed no significant differences among the groups. In the AA digestibility, the Lys and Cys digestibility were significantly higher ($p < 0.05$) in the HA3 group than in the CON group (Table 4). The Leu and Trp digestibility were significantly increased ($p < 0.05$) in the groups replaced by HIL compared to the CON group. The HA3 and HA6 groups had significantly higher ($p < 0.05$) Ser and Asp digestibility than the CON group. However, there was no significant difference in other AA digestibility parameters among the groups. In fecal gas measurement parameters, there was no significant difference among the groups (Table 5).

Diversity of the fecal microbiome

There was no significant difference in alpha-diversity parameters among the groups (Table 6). On 3 d, the HP6 group showed differences ($p < 0.05$) in unweighted unifracs distance from the other groups and in weighted unifracs distance from the CON and HP3 groups (Figs. 1, 2, and 3). In addition, the CON group showed differences ($p < 0.05$) in weighted unifracs distance from the other groups. However, there was no significant difference in weighted and unweighted unifracs distance

Table 3. Effects of HIL substitute ratio based on substrate differences on nutrient digestibility in cats

Item (%)	CON ¹⁾	HA3	HA6	HP3	HP6	SE	p-value
DM	83.29	87.74	86.89	86.00	85.70	1.260	0.167
CP	87.11 ^b	90.82 ^a	89.68 ^{ab}	89.02 ^{ab}	89.05 ^{ab}	0.781	0.038
EE	93.31 ^b	95.40 ^a	94.87 ^a	94.92 ^a	94.63 ^a	0.307	0.001
GE	81.19	83.88	83.70	81.01	80.04	1.262	0.148

¹⁾CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate.

^{a,b}Different letters within the same row reflect significant differences ($p < 0.05$).

HIL, *Hermetia illucens* larvae; DM, dry matter; CP, crude protein; EE, ether extract; GE, gross energy.

Table 4. Effects of HIL substitute ratio based on substrate differences on amino acid digestibility in cats

Item (%)	CON ¹⁾	HA3	HA6	HP3	HP6	SE	p-value
Indispensable							
His	93.86	94.96	94.93	94.47	94.36	0.357	0.204
Arg	92.85	94.20	93.06	93.09	93.36	0.461	0.295
Thr	90.03	91.02	90.12	90.43	90.24	0.508	0.666
Lys	91.06 ^b	92.84 ^a	92.54 ^{ab}	92.29 ^{ab}	92.09 ^{ab}	0.394	0.040
Met	92.37	93.36	92.07	92.81	92.23	0.482	0.350
Val	82.44	83.40	83.69	81.85	81.59	0.932	0.432
Ile	91.25	92.01	91.59	91.87	91.21	0.676	0.886
Leu	91.62 ^b	93.71 ^a	93.41 ^a	93.43 ^a	93.24 ^a	0.368	0.004
Phe	91.08	91.52	91.09	90.73	90.49	0.523	0.696
Trp	93.74 ^b	95.35 ^a	94.99 ^a	95.30 ^a	95.41 ^a	0.259	0.001
Dispensable							
Ser	86.74 ^b	90.93 ^a	90.49 ^a	88.88 ^{ab}	88.50 ^{ab}	0.846	0.020
Gly	92.38	93.52	93.46	92.36	92.12	0.476	0.130
Asp	91.57 ^b	94.13 ^a	94.18 ^a	92.50 ^{ab}	92.27 ^{ab}	0.536	0.005
Glu	93.49	94.43	94.62	94.14	93.99	0.381	0.287
Ala	93.45	94.69	94.53	93.58	93.45	0.484	0.202
Pro	93.74	94.56	94.43	93.85	93.57	0.354	0.223
Cys	90.52 ^b	93.43 ^a	92.87 ^{ab}	91.46 ^{ab}	91.42 ^{ab}	0.578	0.010
Tyr	93.63	94.49	94.70	94.32	93.94	0.376	0.296

¹⁾CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate.

^{ab}Different letters within the same row reflect significant differences ($p < 0.05$).

HIL, *Hermetia illucens* larvae; His, histidine; Arg, arginine; Thr, threonine; Lys, lysine; Met, methionine; Val, valine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; Trp, tryptophane; Ser, serine; Gly, glycine; Asp, aspartic; Glu, glutamic acid; Ala, alanine; Pro, proline; Cys, cysteine; Tyr, tyrosine.

Table 5. Effects of HIL substitute ratio based on substrate differences on fecal gas measurement in cats

Items (ppm)	CON ¹⁾	HA3	HA6	HP3	HP6	SE	p-value
-7 d							
NH ₃	19.75	18.94	19.12	18.98	22.01	1.989	0.789
CH ₃ COOH	12.57	11.82	12.52	12.82	12.86	0.450	0.495
H ₂ S	5.89	3.83	4.91	4.08	4.77	0.849	0.469
3 d							
NH ₃	20.45	17.21	17.74	17.38	18.37	1.784	0.703
CH ₃ COOH	12.39	12.33	12.13	12.6	11.86	0.459	0.826
H ₂ S	5.73	4.59	6.65	4.11	4.26	0.833	0.165

¹⁾CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate.

HIL, *Hermetia illucens* larvae; NH₃, ammonia; CH₃COOH, acetic acid; H₂S, hydrogen sulfide.

Table 6. Effects of HIL substitute ratio based on substrate differences on alpha-diversity in cats

Items	CON ¹⁾	HA3	HA6	HP3	HP6	SE	p-value
-7 d							
Chao1	356.77	454.74	367.78	307.33	461.84	46.426	0.161
Shannon	6.13	5.59	5.38	5.97	5.57	0.420	0.709
Simpson	0.97	0.92	0.94	0.96	0.92	0.026	0.629
3 d							
Chao1	272.71	327.71	312.14	277.71	336.84	38.196	0.686
Shannon	4.59	6.09	5.79	5.03	6.05	0.492	0.195
Simpson	0.90	0.97	0.95	0.91	0.97	0.024	0.173

¹⁾CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate.

HIL, *Hermetia illucens* larvae.

among the other groups.

Relative abundance

At the phylum level, there was no significant difference in the abundance of microbiota among the groups on -7 d (Table 7) (Fig. 4). On 3 d, the CON group showed a significantly higher abundance of Firmicutes ($p < 0.05$) compared to the HP6 group. The abundance of Bacteroidota was significantly increased ($p < 0.05$) in the HP6 group compared to the CON and HP3 groups.

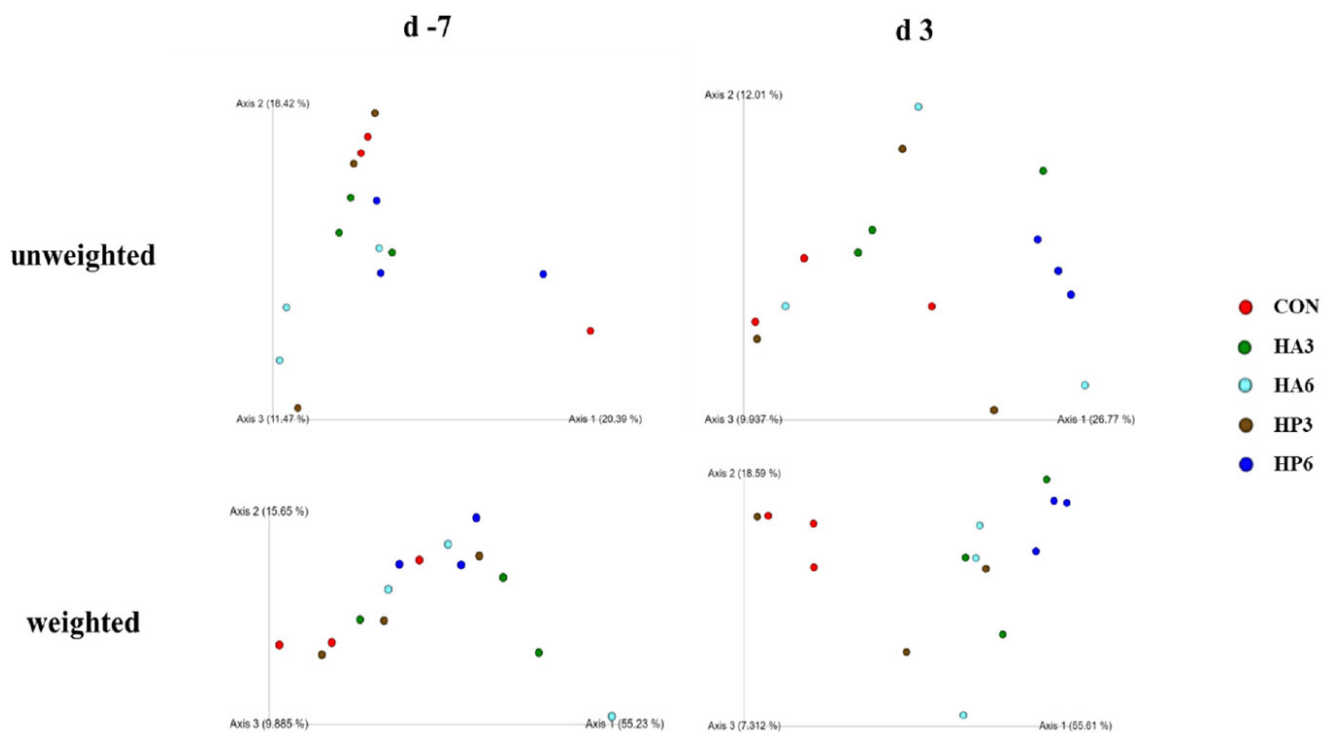


Fig. 1. Visualized beta-diversity indices including unweighted and weighted emperor in HIL substitute ratio based on substrate differences. CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate. HIL, *Hermetia illucens* larvae.

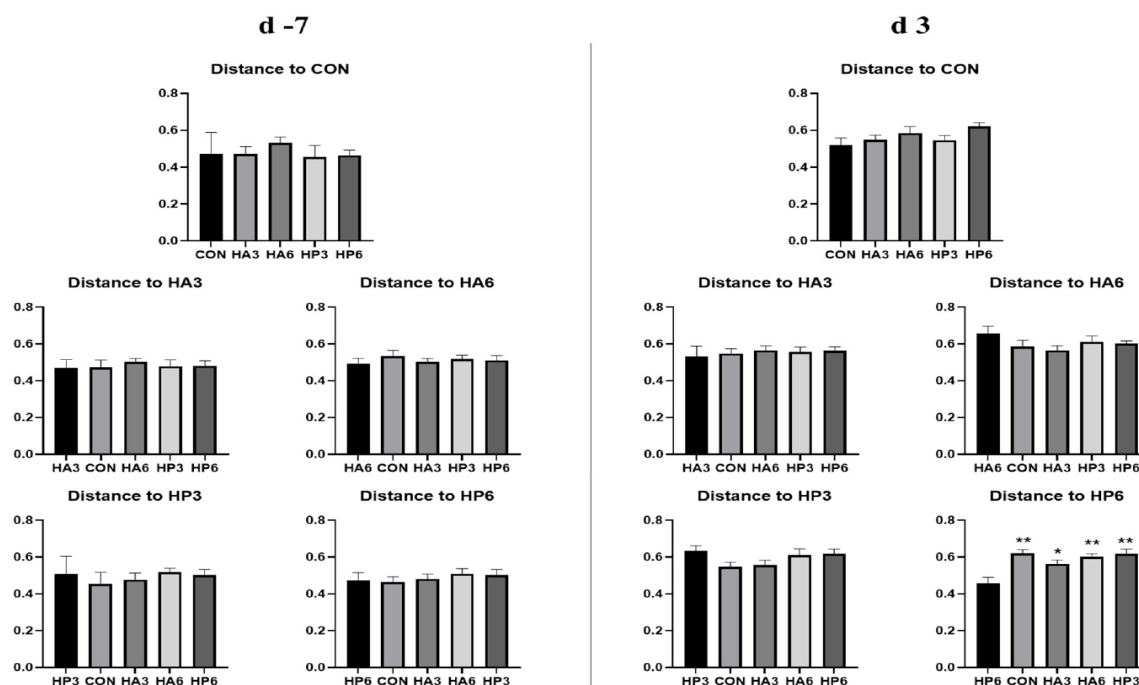


Fig. 2. Unweighted Unifrac measurement in HIL substitute ratio based on substrate differences. CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate. Each treatment group was placed as the control group, and treatment groups were compared by using one-way PROC MIXED with Dunnett's post-hoc test. * $p < 0.05$, ** $p < 0.01$. HIL, *Hermetia illucens* larvae.

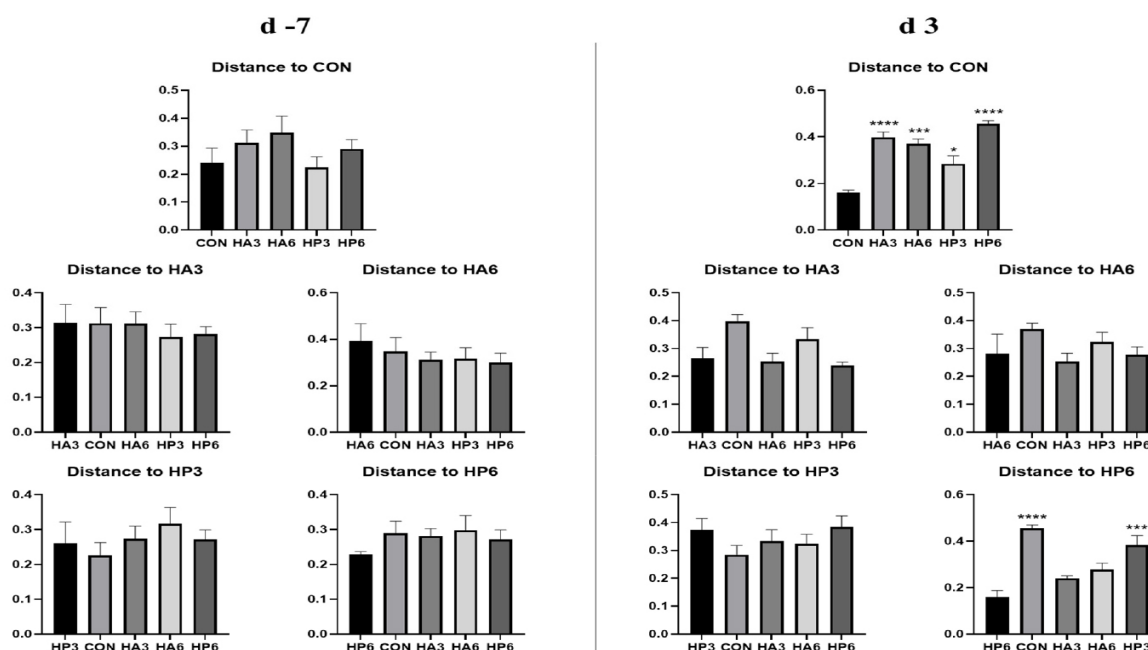


Fig. 3. Weighted Unifrac measurement in HIL substitute ratio based on substrate differences. CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate. Each treatment group was placed as the control group, and treatment groups were compared by using one-way PROC MIXED with Dunnett's post-hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. HIL, *Hermetia illucens* larvae.

In addition, the CON group had a significantly higher ($p < 0.05$) abundance of Deinococcota than the HA3 and HP6 groups and significantly higher ($p < 0.05$) abundance of Verrucomicrobiota than the other groups. However, there were no significant differences in the abundance of the other microbiota among the groups.

At the family level, there was no significant difference in the abundance of microbiota among the groups on -7 d (Table 8) (Fig. 5). On 3 d, the HP6 group showed significantly higher ($p < 0.05$) abundance of Sutterellaceae compared to the other groups (Table 9). The abundance of Lactobacillaceae and Streptococcaceae was significantly increased ($p < 0.05$) in the CON group compared to the other groups, except for the HP3 group. In addition, the abundance of Prevotellaceae was significantly higher ($p < 0.05$) in the HP6 group compared to the other groups, except for the HA6 group. The HP6 group significantly increased ($p < 0.05$) the abundance of Acidaminococcaceae compared to the CON and HP3 groups. The HA6 group showed significantly higher ($p < 0.05$) abundance of Veillonellaceae compared to the other groups, except for the HA3 group. However, there were no significant differences in the abundance of the other microbiotas at the family level among the groups.

At the genus level, there was no significant difference in the abundance of microbiota among the groups on -7 d (Table 10) (Fig. 6). On 3 d, the CON group showed significantly higher ($p < 0.05$) abundance of *Muribaculaceae* and *Akkermansia* compared to the other groups (Table 11).

Table 7. Effects of HIL substitute ratio based on substrate differences on fecal microbiota at the phylum level in cats

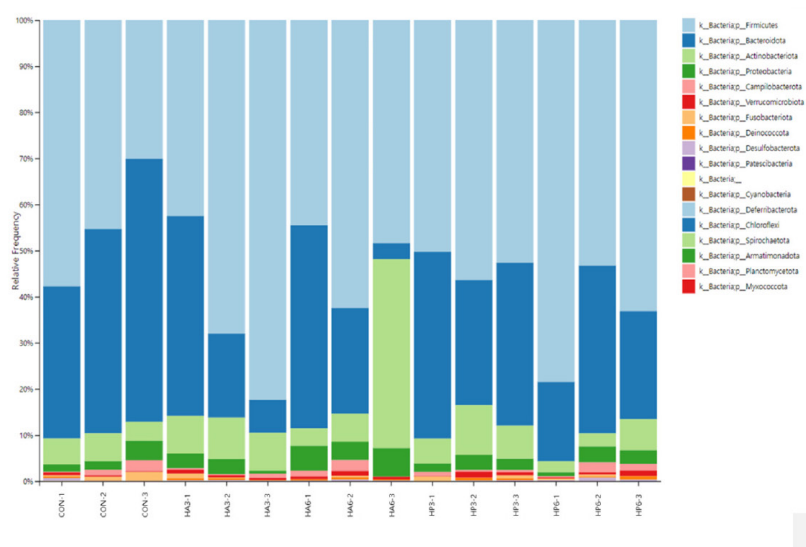
Items (%)	CON ¹⁾	HA3	HA6	HP3	HP6	SE	p-value
-7 d							
Firmicutes	44.39	64.31	52.04	53.11	64.96	7.550	0.318
Bacteroidota	44.78	22.87	23.72	34.30	25.67	8.399	0.360
Actinobacteriota	5.29	8.48	16.98	7.80	3.99	5.460	0.518
Campilobacterota	1.26	0.49	1.24	0.61	1.30	0.494	0.641
Proteobacteria	2.50	2.36	4.63	2.47	2.41	0.759	0.230
Deinococcota	0.16	0.31	0.35	0.34	0.33	0.125	0.793
Desulfobacterota	0.24	0.16	0.17	0.18	0.40	0.120	0.495
Verrucomicrobiota	0.34	0.58	0.72	0.70	0.65	0.233	0.788
Others	1.04	0.44	0.14	0.58	0.28	0.287	0.286
3 d							
Firmicutes	76.59 ^a	48.74 ^{ab}	60.03 ^{ab}	68.60 ^{ab}	41.12 ^b	6.128	0.013
Bacteroidota	7.19 ^b	31.52 ^{ab}	24.90 ^{ab}	10.55 ^b	43.72 ^a	5.710	0.006
Actinobacteriota	15.04	10.88	13.64	18.45	3.95	3.738	0.152
Campilobacterota	0.06	0.46	0.41	1.29	0.34	0.460	0.449
Proteobacteria	0.57	7.74	0.70	0.75	5.39	1.858	0.061
Deinococcota	0.12 ^a	0.01 ^b	0.02 ^{ab}	0.03 ^{ab}	0.01 ^b	0.022	0.027
Desulfobacterota	0.02	0.20	0.10	0.08	0.25	0.118	0.655
Verrucomicrobiota	0.15 ^a	0.01 ^b	0.01 ^b	0.03 ^b	0.01 ^b	0.014	<0.001
Others	0.25	0.44	0.18	0.21	5.22	1.720	0.236

¹⁾CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate.

^{a,b} Different letters within the same row reflect significant differences ($p < 0.05$).

HIL, *Hermetia illucens* larvae.

d -7



d 3

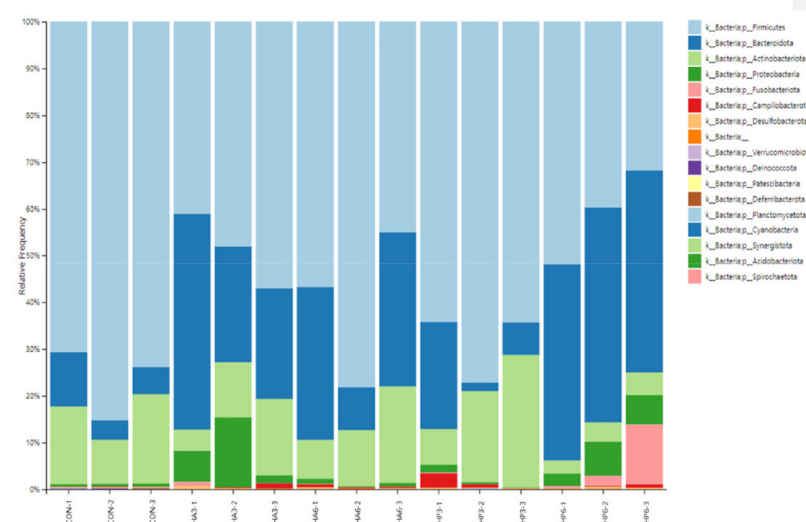


Fig. 4. 16S rRNA gene analysis revealed the relative abundance of fecal bacterial community at the phylum level in HIL substitute ratio based on substrate differences. CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate. HIL, *Hermetia illucens* larvae.

In addition, the abundance of *Lactobacillus* was significantly increased ($p < 0.05$) in the CON group compared to the other groups, except for the HP3 group. However, the HP6 group showed a significantly higher ($p < 0.05$) abundance of *Colidextribacter* and *Alloprevotella* than the other groups, except for the HA3 group, and significantly higher ($p < 0.05$) abundance of *Prevotella* than the CON group. Additionally, the abundance of *Oscillibacter* was significantly increased ($p < 0.05$) in the HP6 group compared to the other groups. The abundance of *Deinococcus* was significantly increased ($p < 0.05$) in the CON group compared to the HA3 and HP6 groups. However, there were no significant differences in the abundance of the other microbiotas at the genus level among the groups.

DISCUSSION

The HIL has the potential to be an alternative ingredient in diet due to its high protein and

Table 8. Effects of HIL substitute ratio based on substrate differences on fecal microbiota at the family level in cats at -7 d

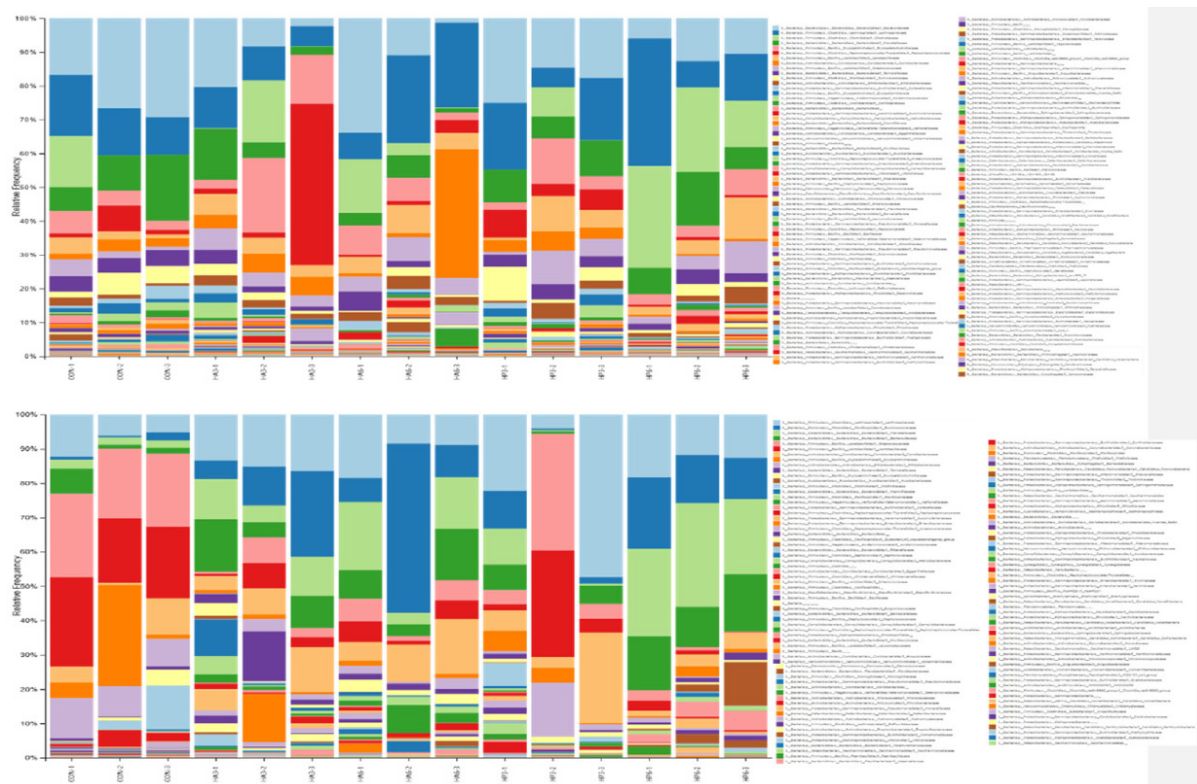
Items (%)	CON ¹⁾	HA3	HA6	HP3	HP6	SE	p-value
Streptococcaceae	2.66	4.63	5.40	5.34	5.45	1.696	0.743
Lachnospiraceae	14.40	9.48	19.65	14.69	21.93	5.986	0.631
Oscillospiraceae	1.15	0.54	0.58	1.13	0.82	0.305	0.497
Flavobacteriaceae	0.08	0.24	0.19	0.17	0.20	0.064	0.524
Helicobacteraceae	0.75	0.24	0.67	0.40	1.30	0.412	0.463
Sutterellaceae	1.99	0.98	1.22	1.45	0.39	0.632	0.512
Coriobacteriaceae	2.55	5.00	13.44	4.15	0.67	5.539	0.559
Erysipelatoclostridiaceae	4.58	13.11	5.95	6.58	4.96	3.190	0.372
Lactobacillaceae	2.78	4.78	5.99	5.47	6.43	1.988	0.725
Ruminococcaceae	4.48	2.17	1.24	5.09	2.11	1.483	0.346
Deinococcaceae	0.16	0.31	0.35	0.34	0.35	0.123	0.777
Tannerellaceae	4.52	2.37	2.54	3.74	3.16	0.954	0.519
Butyricicoccaceae	0.07	0.04	0.07	0.10	0.04	0.023	0.402
Prevotellaceae	12.33	7.44	3.89	8.87	10.98	2.978	0.363
Enterobacteriaceae	0.03	0.11	1.85	0.15	0.11	0.769	0.436
Bacillaceae	0.05	0.14	0.15	0.09	0.16	0.059	0.648
Bacteroidaceae	24.21	10.29	15.39	19.10	10.47	5.467	0.379
Peptostreptococcaceae	3.39	16.88	1.85	5.93	0.83	7.001	0.519
Bifidobacteriaceae	1.44	2.25	2.94	2.51	2.75	0.821	0.735
Staphylococcaceae	0.16	0.35	0.39	0.40	0.38	0.122	0.601
Rikenellaceae	0.51	0.32	0.14	0.28	0.45	0.110	0.212
Enterococcaceae	0.07	0.13	0.28	0.12	0.27	0.079	0.301
Marinifilaceae	1.22	0.75	0.28	0.63	0.68	0.223	0.136
Veillonellaceae	0.19	1.33	1.21	0.09	0.03	0.752	0.591
Desulfovibrionaceae	0.24	0.15	0.17	0.10	0.44	0.126	0.428
Campylobacteraceae	0.51	0.25	0.54	0.19	0.09	0.234	0.596
Clostridiaceae	5.04	6.11	6.55	2.90	16.97	4.383	0.259
Leuconostocaceae	0.04	0.15	0.15	0.10	0.32	0.097	0.381
Acidaminococcaceae	0.34	0.58	0.72	0.70	0.68	0.231	0.772
Others	7.99	6.28	4.38	6.49	5.36	1.682	0.644

¹⁾CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate.

HIL, *Hermetia illucens* larvae.

lipid content and low environmental footprint [10]. Previous studies have suggested that HIL adapts well to environments with poor or unbalanced diets, but the quality of the substrate has a significant impact on their nutrient profile [26]. In this experiment, we raised and processed the HIL on different organic substrates and analyzed their nutrient compositions. Spranghers et al. [18] reported that variations in rearing substrate could influence the nutrient profile in the HIL. In the present study, the HIL fed with animal-based substrates had numerically higher CP than the HIL fed with plant-based substrates. Dietary protein is an essential macronutrient for the development of the HIL and is necessary to support adequate protein and lipid accumulation in the fat cells of the HIL [27]. The current study showed similar values for AA contents of the HIL with different rearing substrates. In previous studies, various rearing substrates showed different AA contents in

d -7



d 3

Fig. 5. 16S rRNA gene analysis revealed the relative abundance of fecal bacterial community at the family level in HIL substitute ratio based on substrate differences. CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate. HIL, *Hermetia illucens* larvae.

the HIL; supplementation with seaweed had high amounts of threonine [28], while fed vegetable mixtures contained sufficient amounts of leucine [18], or aspartic acid, glutamate, and arginine [29]. Hopkins et al. [30] reported that the HIL nutrient composition was compounded by factors such as harvest stage and age. In this study, the HIL was reared in the same harvest environment and age, further studies considering other factors are needed.

This study evaluated the effects of replacing the PM with the HIL in the cat diets, examining how the rearing substrate and replacement ratio impacted nutrient digestibility. The monogastric animals cannot digest the chitin in insects due to their lack of endogenous chitinase enzymes [31]. However, insect-based meals lacked collagen compared with vertebrate protein meals, which could explain the improvement in protein digestibility in insect-based meals [32]. Collagen, a fibrous protein, tends to be tough, insoluble, and resistant to digestion compared to the globular proteins [33]. This study suggested that the collagen content was attributed to different nutrient digestibility among treatments. The current results indicated that the HIL could improve the utilization and absorption of specific AAs in cats. Lysine is important for the growth and function of the immune system, while arginine promotes hair growth and the health of the scalp [34]. According to Fuso et al. [35], lysine and leucine contents vary depending on substrate, whereas Spranghers et al. [18] found no significant differences. The results of this study showed that while the HIL improved the digestibility of some AAs, it did not have the same effect on all AAs. Regarding differences based on the rearing substrate, the HIL fed on an animal-based substrate exhibited better digestibility of certain nutrients than HIL fed on a plant-based substrate. This suggests that the substrate used for raising the HIL can influence the nutrient profile of the HIL, resulting in differences in

Table 9. Effects of HIL substitute ratio based on substrate differences on fecal microbiota at the family level in cats at 3 d

Items (%)	CON ¹⁾	HA3	HA6	HP3	HP6	SE	p-value
Streptococcaceae	21.97 ^a	3.56 ^b	4.59 ^b	14.13 ^{ab}	1.40 ^b	4.627	0.049
Lachnospiraceae	7.94	15.05	11.94	24.56	14.28	5.982	0.428
Oscillospiraceae	0.15	2.18	1.90	0.28	1.28	0.718	0.238
Flavobacteriaceae	0.08	0.01	0.01	0.05	0.01	0.023	0.172
Helicobacteraceae	0.04	0.40	0.32	1.17	0.29	0.479	0.557
Sutterellaceae	0.15 ^b	0.42 ^b	0.16 ^b	0.09 ^b	4.03 ^a	0.440	< 0.001
Coriobacteriaceae	5.21	8.24	10.69	11.92	3.24	3.976	0.531
Erysipelatoclostridiaceae	1.99	1.58	2.73	1.42	0.85	0.825	0.595
Lactobacillaceae	21.94 ^a	3.68 ^b	4.21 ^b	12.25 ^{ab}	1.42 ^b	4.485	0.047
Ruminococcaceae	1.24	13.89	21.93	4.46	15.56	6.916	0.276
Deinococcaceae	0.12 ^a	0.01 ^b	0.02 ^{ab}	0.03 ^{ab}	0.01 ^b	0.022	0.027
Tannerellaceae	0.65	6.21	1.60	0.72	1.35	1.889	0.269
Butyricicoccaceae	0.02	0.10	0.11	0.04	0.12	0.034	0.229
Prevotellaceae	2.25 ^b	9.33 ^b	12.71 ^{ab}	3.81 ^b	25.90 ^a	3.015	0.002
Enterobacteriaceae	0.16	4.72	0.14	0.13	0.02	2.096	0.468
Bacillaceae	0.36	0.05	0.03	0.25	0.03	0.096	0.105
Bacteroidaceae	3.35	9.66	8.94	4.47	14.68	2.496	0.058
Peptostreptococcaceae	2.75	1.36	1.46	0.60	0.38	0.608	0.126
Bifidobacteriaceae	9.49	1.77	2.05	6.01	0.60	2.292	0.101
Staphylococcaceae	0.23	0.03	0.02	0.13	0.01	0.060	0.111
Rikenellaceae	0.16	1.13	0.20	0.20	0.53	0.512	0.641
Enterococcaceae	0.04	0.01	0.11	0.09	0.59	0.220	0.373
Marinifilaceae	0.25	4.01	0.68	0.44	0.39	1.500	0.391
Veillonellaceae	0.03 ^b	1.35 ^{ab}	4.19 ^a	0.46 ^b	0.03 ^b	0.937	0.049
Desulfovibrionaceae	0.02	0.20	0.10	0.08	0.25	0.118	0.655
Campylobacteraceae	0.01	0.05	0.09	0.12	0.05	0.061	0.785
Clostridiaceae	5.09	0.22	0.01	2.71	0.15	1.968	0.339
Leuconostocaceae	0.07	0.06	0.03	0.03	0.04	0.020	0.449
Acidaminococcaceae	0.00 ^b	0.35 ^{ab}	0.63 ^{ab}	0.11 ^b	1.86 ^a	0.366	0.030
Others	14.22	10.38	8.35	9.26	10.67	5.180	0.940

¹⁾CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate.

^{a,b}Different letters within the same row reflect significant differences ($p < 0.05$).

HIL, *Hermetia illucens* larvae.

digestibility. Previous studies have reported that animal-based substrates can improve fatty acid and AA profiles of the HIL, thus enhancing nutrient digestibility [36,37]. In this study, as the proportion of the HIL in the diet 3%, there was a tendency towards improved nutrient digestibility, suggesting that a proper the HIL content might enhance nutrient absorption in cats. However, it is essential to note that excessive substitution may lead to digestive issues or nutritional imbalances, underscoring the need to determine the optimal substitution level.

It has been shown that fecal odor can directly affect owner satisfaction with diet selection, particularly in the case of indoor cats [38]. The results of this study indicated no significant

Table 10. Effects of HIL substitute ratio based on substrate differences on fecal microbiota at the genus level in cats at -7 d

Items (%)	CON	HA3	HA6	HP3	HP6	SE	p-value
<i>Lactococcus</i>	2.68	4.63	5.37	5.47	5.20	1.733	0.773
<i>Helicobacter</i>	0.77	0.24	0.67	0.42	1.22	0.402	0.521
<i>Muribaculaceae</i>	0.27	0.53	0.65	0.57	0.57	0.210	0.743
<i>Collinsella</i>	2.60	5.02	13.44	4.31	0.62	5.539	0.560
<i>Catenibacterium</i>	4.64	13.07	5.18	6.44	4.65	3.255	0.365
<i>Lactobacillus</i>	2.81	4.80	5.99	5.62	6.17	2.036	0.767
<i>Colidextribacter</i>	0.25	0.17	0.17	0.38	0.15	0.072	0.210
<i>Deinococcus</i>	0.16	0.31	0.35	0.35	0.33	0.126	0.797
<i>Parabacteroides</i>	4.58	2.38	2.54	3.90	2.92	0.937	0.444
<i>Alloprevotella</i>	5.44	3.17	0.74	3.19	2.43	1.616	0.411
<i>Prevotella</i>	5.86	3.51	3.00	5.18	7.01	1.558	0.394
<i>Bacteroides</i>	24.63	10.34	15.39	20.04	9.74	5.551	0.328
<i>Bifidobacterium</i>	1.46	2.26	2.94	2.58	2.63	0.841	0.772
<i>Ruminococcus torques</i> group	0.39	0.66	0.17	0.62	0.20	0.242	0.503
<i>Macrococcus</i>	0.16	0.35	0.39	0.41	0.35	0.125	0.647
<i>Enterococcus</i>	0.07	0.13	0.28	0.13	0.24	0.077	0.358
<i>Oscillibacter</i>	0.42	0.15	0.12	0.27	0.15	0.102	0.274
<i>Megasphaera</i>	0.19	1.33	1.21	0.09	0.02	0.755	0.592
<i>Desulfovibrio</i>	0.24	0.15	0.17	0.11	0.40	0.120	0.497
<i>Campylobacter</i>	0.51	0.25	0.54	0.20	0.08	0.234	0.588
<i>Holdemanella</i>	0.19	0.23	0.04	0.26	0.06	0.077	0.237
<i>Peptoclostridium</i>	1.15	1.10	1.08	1.38	0.68	0.193	0.219
<i>Roseburia</i>	0.67	0.49	1.92	0.68	0.08	0.476	0.157
<i>Tyzzarella</i>	1.75	1.43	0.37	0.22	14.91	4.371	0.161
<i>Clostridium sensu stricto</i> 1	3.53	3.40	4.97	2.73	13.60	3.802	0.299
<i>Ruminococcus gnavus</i> group	0.23	0.49	2.24	0.54	0.09	0.971	0.551
<i>Faecalibacterium</i>	3.16	1.32	0.76	4.11	1.29	1.364	0.406
<i>Akkermansia</i>	0.35	0.59	0.72	0.72	0.65	0.235	0.791
Others	29.19	35.83	27.95	27.26	23.45	6.868	0.784

¹CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate.

HIL, *Hermetia illucens* larvae.

difference in odor when the PM was replaced with the HIL. It would seem that the HIL undergoes a similar metabolic pathway in the digestion of cats to that of existing protein sources, resulting in no significant difference in the production of odor-causing substances. Several studies have demonstrated the nutritional and environmental benefits of the HIL [39,40]. Based on rearing substrate and substitution levels, there was no significant difference in fecal odor depending on the HIL cultivation conditions. Results of this study suggested that differences in nutritional components of the HIL according to rearing substrate may not directly affect odor occurrence. The finding that the increase in substitution level did not affect odor indicated that the HIL could be metabolized consistently during the digestion process in cats.

This study evaluated differences in fecal microbiome abundance between the PM and the HIL

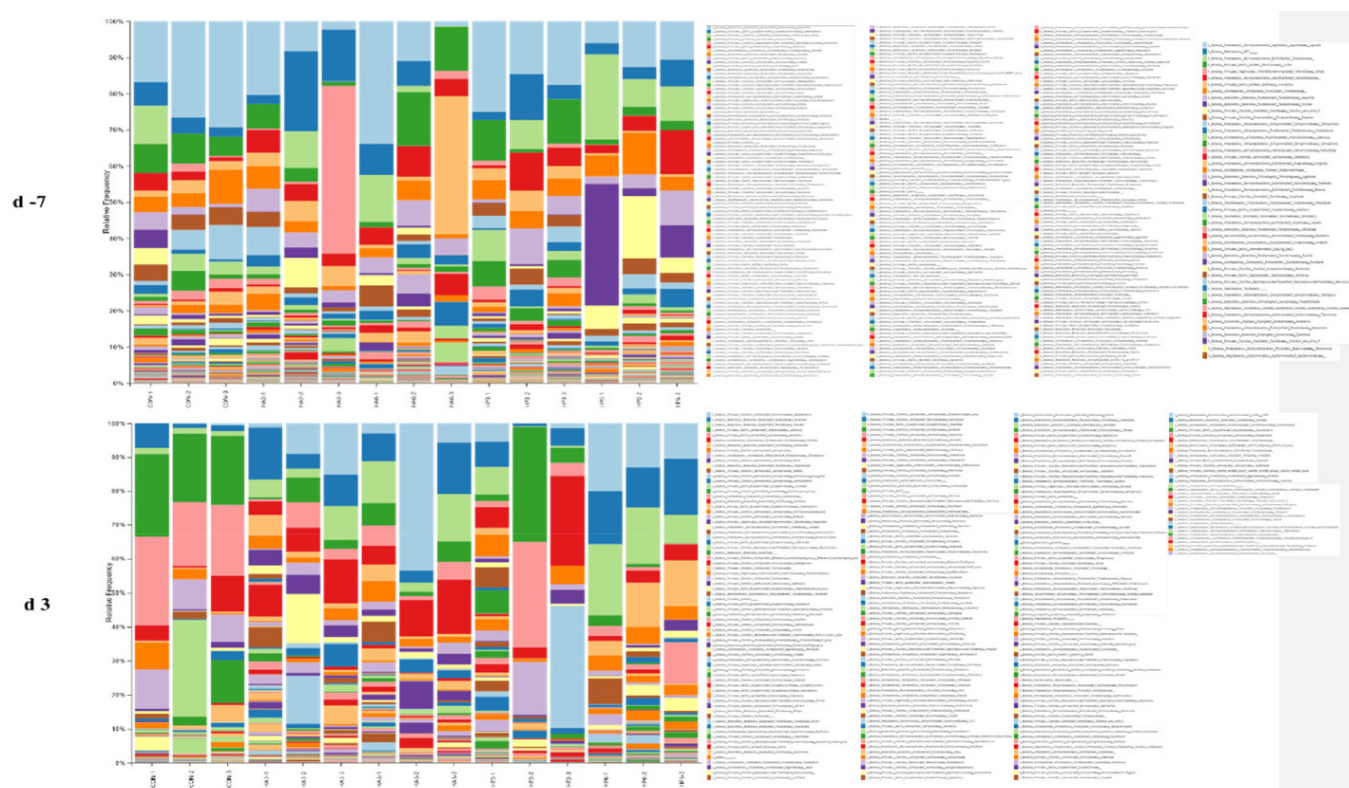


Fig. 6. 16S rRNA gene analysis revealed the relative abundance of fecal bacterial community at the genus level in HIL substitute ratio based on substrate differences. CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate.

substitution groups. Although many unknowns remain regarding microbial genes and/or bacterial species in the gastrointestinal (GI) tract, many of the predominant taxa in the GI tract have been identified through decades of traditional culture methods and recent microbiome research. The predominant phyla in companion animals such as dogs and cats are Firmicutes, Bacteroidota, and Fusobacteria [41]. Firmicutes is especially important for energy extraction and maintenance of gut barrier [42]. Xie et al. [43] reported that decreasing Firmicutes might negatively impact metabolic processes. In contrast, Bacteroidota increases proportionately to carbohydrate metabolism and short-chain fatty acids (SCFAs) production [44], positively affecting gut health. According to Miller et al. [45], SCFAs play a crucial role in regulating immunity and protecting the intestinal system. The comparatively low abundance bacteriome, such as Deinococcota and Verrucomicrobiota, has been shown to influence stress resistance, gut resilience, and inflammatory conditions in the gut, respectively [46,47]. Dietary components might influence intestinal microbial composition based on the difference in phylum relative abundance among treatments.

The abundances of Lactobacillaceae, Prevotellaceae, and Veillonellaceae could lead to a more equilibrated gut microbiome in cats, potentially enhancing digestive health, immune system function, and promoting the overall health of the GI [48,49]. Among them, Prevotellaceae produce propionate by digesting glucose or lactate [50]. In addition, the abundance of Acidaminococcaceae in cats has been closely associated with the synthesis of propionate that might help maintain intestinal homeostasis [51]. Marsilio et al. [52] have reported that facultative anaerobes such as Streptococcaceae tend to be reduced in healthy cats. The present study results indicated that the reduction in Streptococcaceae abundance due to the HIL substitution is thought to affect

Table 11. Effects of HIL substitute ratio based on substrate differences on fecal microbiota at the genus level in cats at 3 d

Items (%)	CON ¹⁾	HA3	HA6	HP3	HP6	SE	p-value
<i>Lactococcus</i>	21.67	3.55	4.59	13.39	1.40	4.799	0.066
<i>Helicobacter</i>	0.04	0.40	0.32	1.17	0.29	0.479	0.557
<i>Muribaculaceae</i>	0.18 ^a	0.01 ^b	0.02 ^a	0.04 ^a	0.01 ^a	0.022	0.001
<i>Collinsella</i>	5.21	8.07	10.68	11.92	3.24	3.980	0.533
<i>Catenibacterium</i>	1.96	0.64	2.57	0.92	0.43	0.723	0.246
<i>Lactobacillus</i>	21.94 ^a	3.68 ^a	4.21 ^a	12.25 ^{ab}	1.42 ^a	4.485	0.047
<i>Colidextribacter</i>	0.03 ^b	0.15 ^{ab}	0.10 ^b	0.08 ^b	0.30 ^a	0.042	0.008
<i>Deinococcus</i>	0.12 ^a	0.01 ^b	0.02 ^{ab}	0.03 ^{ab}	0.01 ^b	0.022	0.027
<i>Parabacteroides</i>	0.65	6.21	1.60	0.72	1.35	1.890	0.269
<i>Alloprevotella</i>	0.45 ^b	3.03 ^{ab}	2.16 ^b	0.69 ^b	10.19 ^a	1.647	0.011
<i>Prevotella</i>	1.69 ^b	6.00 ^{ab}	9.68 ^{ab}	3.02 ^{ab}	15.34 ^a	2.749	0.034
<i>Bacteroides</i>	3.35	9.66	8.94	4.47	14.68	2.496	0.058
<i>Bifidobacterium</i>	9.49	1.77	2.05	6.01	0.60	2.292	0.101
<i>Ruminococcus torques</i> group	0.59	0.75	0.41	1.20	2.10	0.546	0.265
<i>Macrococcus</i>	0.17	0.03	0.02	0.12	0.01	0.053	0.189
<i>Enterococcus</i>	0.04	0.01	0.11	0.09	0.59	0.220	0.371
<i>Oscillibacter</i>	0.01 ^c	0.07 ^{bc}	0.09 ^b	0.02 ^{bc}	0.18 ^a	0.016	< 0.001
<i>Megasphaera</i>	0.02	0.56	3.79	0.43	0.02	1.033	0.120
<i>Desulfovibrio</i>	0.02	0.20	0.10	0.08	0.25	0.118	0.655
<i>Campylobacter</i>	0.01	0.05	0.09	0.12	0.05	0.061	0.785
<i>Holdemanella</i>	0.65	0.75	0.47	1.06	0.99	0.473	0.894
<i>Peptoclostridium</i>	0.81	1.22	1.36	0.40	0.26	0.301	0.099
<i>Roseburia</i>	0.06	1.47	1.06	1.08	1.48	0.821	0.737
<i>Tyzzereella</i>	0.03	0.30	0.17	0.45	0.27	0.233	0.782
<i>Clostridium sensu stricto</i> 1	5.08	0.22	0.01	2.71	0.15	1.967	0.340
<i>Ruminococcus gnavus</i> group	0.03	0.63	0.31	11.97	0.14	5.346	0.471
<i>Faecalibacterium</i>	0.26	8.47	17.44	2.33	14.52	6.297	0.302
<i>Akkermansia</i>	0.14 ^a	0.01 ^b	0.01 ^b	0.03 ^b	0.01 ^b	0.013	< 0.001
Others	23.10	34.27	23.36	20.09	27.24	8.384	0.787

¹⁾CON, basal diet; HA3, replacing 3% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HA6, replacing 6% of the poultry meal in the basal diet with HIL fed on animal-based substrate; HP3, replacing 3% of the poultry meal in the basal diet with HIL fed on plant-based substrate; HP6, replacing 6% of the poultry meal in the basal diet with HIL fed on plant-based substrate.

^{a-c}Different letters within the same row reflect significant differences ($p < 0.05$).

HIL, *Hermetia illucens* larvae.

inflammation positively. Sutterellaceae abundance is a pathogenic microorganism that impacts dogs with acute diarrhea [53]. In this study, the HP6 group increased the abundance of Sutterellaceae, which we may expect to harm the substitution of the HIL of plant-based substrates. There is a possibility that host health is impacted by the abundance of these genera and their end products.

Muribaculaceae is a beneficial bacterium associated with promoting the production of SCFAs [54]. In addition, *Alloprevotella* and *Prevotella* improve gut barrier function and reduce inflammation [50]. The current results suggested that substituting HIL may numerically increase the number of these bacteria by enhancing the availability of fermentable fibers or chitin and creating a conducive environment due to bioactive compounds such as antimicrobial peptides and lauric acid. A decrease in *Oscillibacter* indicates that the HIL inclusion might alter protein fermentation dynamics in the

gut, possibly reducing the production of undesirable metabolites and enhancing overall gut health [55]. These variations in bacterial populations were influenced by different substrates and inclusion levels of the HIL used in different treatments. A difference in nutrient composition of the HIL might affect gut microbiota among those raised on other substrates. The HIL substitution may be beneficial in the cat diet because the HIL substitution selectively affects key bacterial taxa associated with gut health.

Consequently, substituting the PM with the HIL improved fecal microbiota and nutrient digestibility, which suggests that the HIL could serve as a viable alternative protein source in cat diets. Especially, replacing animal-based substrates with the HIL at a level of 3% might increase nutrient digestibility and confirm that replacing animal-based substrates with the HIL did not negatively affect fecal microbiota. However, precise mechanisms underlying fecal microbial diversity and SCFAs production remain unclear; more research is needed to clarify these correlations. Based on the above results, it is suggested that the HIL reared on animal substrates is a potential alternative source of protein.

CONCLUSION

In this study, substituting PM, a protein source in cat diet, with HIL reared on animal-based or plant-based substrates at replacement ratios of 3% and 6% resulted in a positive effect on nutrient digestibility compared to the CON group. Both the 3% and 6% HIL substitutions had no adverse effects on gas emissions and positively modulated the fecal microbiota. In specific nutrient digestibility, although the HA3 group showed more significant differences compared to the CON group, there were no significant differences observed among the HIL substitution groups. Therefore, substituting up to 6% of the protein source with HIL in cat diets is considered safe and effective.

References

1. Legrand-Defretin V. Differences between cats and dogs: a nutritional view. *Proc Nutr Soc.* 1994;53:15-24. <https://doi.org/10.1079/PNS19940004>
2. Menchetti L, Calipari S, Mariti C, Gazzano A, Diverio S. Cats and dogs: best friends or deadly enemies? What the owners of cats and dogs living in the same household think about their relationship with people and other pets. *PLOS ONE.* 2020;15:e0237822. <https://doi.org/10.1371/journal.pone.0237822>
3. Tazerji SS, Elahinia A, Akhtardanesh B, Kabir F, Vazir B, Duarte PM, et al. Nutritional risks and consequences of meat-only diets for dogs and cats. *Ger J Vet Res.* 2024;4:62-76. <https://doi.org/10.51585/gjvr.2024.1.0076>
4. Hamper BA, Kirk CA, Bartges JW. Apparent nutrient digestibility of two raw diets in domestic kittens. *J Feline Med Surg.* 2016;18:991-6. <https://doi.org/10.1177/1098612X15605535>
5. Green AS, Ramsey JJ, Villaverde C, Asami DK, Wei A, Fascetti AJ. Cats are able to adapt protein oxidation to protein intake provided their requirement for dietary protein is met. *J Nutr.* 2008;138:1053-60. <https://doi.org/10.1093/jn/138.6.1053>
6. Beynen AC. Insect-based petfood. *Creature Companion;* 2018;40-1:1-6.
7. Yamka RM, Koutsos EA, McComb A. Evaluation of black soldier fly larvae as a protein and fat source in pet foods. Presented at: Petfood Forum 2019; 2019 April 29-May 1; Kansas City, MI.
8. Li P, Wu G. Functional molecules of intestinal mucosal products and peptones in animal

- nutrition and health. In: Wu G, editor. Recent advances in animal nutrition and metabolism. Cham: Springer; 2022. p. 263-77.
9. Macelline SP, Chrystal PV, Liu SY, Selle PH. The dynamic conversion of dietary protein and amino acids into chicken-meat protein. *Animals*. 2021;11:2288. <https://doi.org/10.3390/ani11082288>
 10. Do S, Koutsos EA, McComb A, Phungviwatnikul T, de Godoy MRC, Swanson KS. Palatability and apparent total tract macronutrient digestibility of retorted black soldier fly larvae-containing diets and their effects on the fecal characteristics of cats consuming them. *J Anim Sci*. 2022;100:skac068. <https://doi.org/10.1093/jas/skac068>
 11. Jacuńska W, Biel W, Zych K. Evaluation of the nutritional value of insect-based complete pet foods. *Appl Sci*. 2024;14:10258. <https://doi.org/10.3390/app142210258>
 12. Shah AA, Totakul P, Matra M, Cherdthong A, Hanboonsong Y, Wanapat M. Nutritional composition of various insects and potential uses as alternative protein sources in animal diets. *Anim Biosci*. 2022;35:317-31. <https://doi.org/10.5713/ab.21.0447>
 13. Reilly LM, Hu Y, von Schaumburg PC, de Oliveira MRD, He F, Rodriguez-Zas SL, et al. Chemical composition of selected insect meals and their effect on apparent total tract digestibility, fecal metabolites, and microbiota of adult cats fed insect-based retorted diets. *J Anim Sci*. 2022;100:skac024. <https://doi.org/10.1093/jas/skac024>
 14. Elieh Ali Komi D, Sharma L, Dela Cruz CS. Chitin and its effects on inflammatory and immune responses. *Clin Rev Allergy Immunol*. 2018;54:213-23. <https://doi.org/10.1007/s12016-017-8600-0>
 15. Lei XJ, Kim TH, Park JH, Kim IH. Evaluation of supplementation of defatted black soldier fly (*Hermetia illucens*) larvae meal in beagle dogs. *Ann Anim Sci*. 2019;19:767-77. <https://doi.org/10.2478/aoas-2019-0021>
 16. Liceaga AM, Aguilar-Toalá JE, Vallejo-Córdova B, González-Córdova AF, Hernández-Mendoza A. Insects as an alternative protein source. *Annu Rev Food Sci Technol*. 2022;13:19-34. <https://doi.org/10.1146/annurev-food-052720-112443>
 17. Pezzali JG, Shoveller AK. Short communication: the effects of a semi-synthetic diet with inclusion of black soldier fly larvae meal on health parameters of healthy adult cats. *J Anim Sci*. 2021;99:skab290. <https://doi.org/10.1093/jas/skab290>
 18. Sprangers T, Ottoboni M, Klootwijk C, Olyn A, Deboosere S, De Meulenaer B, et al. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *J Sci Food Agric*. 2017;97:2594-600. <https://doi.org/10.1002/jsfa.8081>
 19. Cai M, Zhang K, Zhong W, Liu N, Wu X, Li W, et al. Bioconversion-composting of golden needle mushroom (*Flammulina velutipes*) root waste by black soldier fly (*Hermetia illucens*, Diptera: Stratiomyidae) larvae, to obtain added-value biomass and fertilizer. *Waste Biomass Valorization*. 2019;10:265-73. <https://doi.org/10.1007/s12649-017-0063-2>
 20. Bortolini S, Macavei LI, Saadoun JH, Foca G, Ulrici A, Bernini F, et al. *Hermetia illucens* (L.) larvae as chicken manure management tool for circular economy. *J Clean Prod*. 2020;262:121289. <https://doi.org/10.1016/j.jclepro.2020.121289>
 21. European Union. Commission Regulation (EU) 2017/893 of 24 May 2017 amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards the provisions on processed animal protein. *Off J Eur Union L138*. 2017;60:92-116.
 22. AOAC (Association of Official Analytical Chemists) International. Official methods of analysis of the AOAC International. 18th ed. Gaithersburg, MD: AOAC International; 2007.

23. AAFCO (Association of American Feed Control Officials). Official publication. Champaign, IL: AAFCO; 2023.
24. Scott TA, Boldaji F. Comparison of inert markers [chromic oxide or insoluble ash (Celite)] for determining apparent metabolizable energy of wheat- or barley-based broiler diets with or without enzymes. *Poult Sci*. 1997;76:594-8. <https://doi.org/10.1093/ps/76.4.594>
25. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol*. 2019;37:852-7. <https://doi.org/10.1038/s41587-019-0209-9>
26. Fitriana EL, Laconi EB, Astuti DA, Jayanegara A. Effects of various organic substrates on growth performance and nutrient composition of black soldier fly larvae: a meta-analysis. *Bioresour Technol Rep*. 2022;18:101061. <https://doi.org/10.3390/insects12070608>
27. Pimentel AC, Montali A, Bruno D, Tettamanti G. Metabolic adjustment of the larval fat body in *Hermetia illucens* to dietary conditions. *J Asia Pac Entomol*. 2017;20:1307-13. <https://doi.org/10.1016/j.aspen.2017.09.017>
28. Liland NS, Biancarosa I, Araujo P, Biemans D, Bruckner CG, Waagbø R, et al. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. *PLOS ONE*. 2017;12:e0183188. <https://doi.org/10.1371/journal.pone.0183188>
29. Cappellozza S, Leonardi MG, Savoldelli S, Carminati D, Rizzolo A, Cortellino G, et al. A first attempt to produce proteins from insects by means of a circular economy. *Animals*. 2019;9:278. <https://doi.org/10.3390/ani9050278>
30. Hopkins I, Newman LP, Gill H, Danaher J. The influence of food waste rearing substrates on black soldier fly larvae protein composition: a systematic review. *Insects*. 2021;12:608. <https://doi.org/10.3390/insects12070608>
31. Bonomini MG, Prandi B, Caligiani A. Black soldier fly (*Hermetia illucens* L.) whole and fractionated larvae: in vitro protein digestibility and effect of lipid and chitin removal. *Food Res Int*. 2024;196:115102. <https://doi.org/10.1016/j.foodres.2024.115102>
32. Abd El-Wahab A, Meyer L, Kölln M, Chuppava B, Wilke V, Visscher C, et al. Insect larvae meal (*Hermetia illucens*) as a sustainable protein source of canine food and its impacts on nutrient digestibility and fecal quality. *Animals*. 2021;11:2525. <https://doi.org/10.3390/ani11092525>
33. Kies C. Bioavailability: a factor in protein quality. *J Agric Food Chem*. 1981;29:435-40. <https://doi.org/10.1021/jf00105a002>
34. Connolly ED, Wu G. Functions and metabolism of amino acids in the hair and skin of dogs and cats. In: Wu G, editor. *Nutrition and metabolism of dogs and cats*. Cham: Springer; 2024. p. 135-54.
35. Fuso A, Barbi S, Macavei LI, Luparelli AV, Maistrello L, Montorsi M, et al. Effect of the rearing substrate on total protein and amino acid composition in black soldier fly. *Foods*. 2021;10:1773. <https://doi.org/10.3390/foods10081773>
36. Lu S, Taethaisong N, Meethip W, Surakhunthod J, Sinpru B, Sroichak T, et al. Nutritional composition of black soldier fly larvae (*Hermetia illucens* L.) and its potential uses as alternative protein sources in animal diets: a review. *Insects*. 2022;13:831. <https://doi.org/10.3390/insects13090831>
37. Moula N, Scippo ML, Douny C, Degand G, Dawans E, Cabaraux JF, et al. Performances of local poultry breed fed black soldier fly larvae reared on horse manure. *Anim Nutr*. 2018;4:73-8. <https://doi.org/10.1016/j.aninu.2017.10.002>
38. Robins LI, Napier S, Seek CM, Gao X, Flegler C, Mackenzie CD. Control of felinine-

- derived malodor in cat litter. *J Feline Med Surg.* 2022;24:123-30. <https://doi.org/10.1177/1098612X211009136>
39. van Huis A. Potential of insects as food and feed in assuring food security. *Annu Rev Entomol.* 2013;58:563-83. <https://doi.org/10.1146/annurev-ento-120811-153704>
 40. Makkar HPS, Tran G, Heuzé V, Ankers P. State-of-the-art on use of insects as animal feed. *Anim Feed Sci Technol.* 2014;197:1-33. <https://doi.org/10.1016/j.anifeedsci.2014.07.008>
 41. Suchodolski JS, Markel ME, Garcia-Mazcorro JF, Unterer S, Heilmann RM, Dowd SE, et al. The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease. *PLOS ONE.* 2012;7:e51907. <https://doi.org/10.1371/journal.pone.0051907>
 42. Sutoyo DA, Atmaka DR, Sidabutar LMGB. Dietary factors affecting firmicutes and bacteroidetes ratio in solving obesity problem: a literature review. *Media Gizi Indones.* 2020;15:94-109. <https://doi.org/10.20473/mgi.v15i2.94-109>
 43. Xie Q, Zhang Y, Zhang Z, Gong S, Mo Q, Li J. Characteristics and dynamic changes of gut microbiota in cats with colitis. *Pak Vet J.* 2024;44:414-22. <https://doi.org/10.29261/pakvetj/2024.175>
 44. Wang Y, Sun P, Guo H, Zheng K, Zhu T, Liu Y. Performance and mechanism of sodium percarbonate (SPC) enhancing short-chain fatty acids production from anaerobic waste activated sludge fermentation. *J Environ Manage.* 2022;313:115025. <https://doi.org/10.1016/j.jenvman.2022.115025>
 45. Miller J, Żebrowska-Róžańska P, Czajkowska A, Szponar B, Kumala-Ćwikła A, Chmielarz M, et al. Faecal microbiota and fatty acids in feline chronic enteropathy. *BMC Vet Res.* 2023;19:281. <https://doi.org/10.1186/s12917-023-03824-9>
 46. Singhvi N, Talwar C, Nagar S, Verma H, Kaur J, Mahato NK, et al. Insights into the radiation and oxidative stress mechanisms in genus *Deinococcus*. *Comput Biol Chem.* 2024;112:108161. <https://doi.org/10.1016/j.compbiolchem.2024.108161>
 47. Wang Z, Guo M, Yang S, Chen Y, Cheng J, Huang Z, et al. Intestinal microflora and metabolites affect the progression of acute pancreatitis (AP). *Gut Pathog.* 2024;16:64. <https://doi.org/10.1186/s13099-024-00652-6>
 48. Bermingham EN, Young W, Kittelmann S, Kerr KR, Swanson KS, Roy NC, et al. Dietary format alters fecal bacterial populations in the domestic cat (*Felis catus*). *MicrobiologyOpen.* 2013;2:173-81. <https://doi.org/10.1002/mbo3.60>
 49. Zhang L, Wu W, Lee YK, Xie J, Zhang H. Spatial heterogeneity and co-occurrence of mucosal and luminal microbiome across swine intestinal tract. *Front Microbiol.* 2018;9:48. <https://doi.org/10.3389/fmicb.2018.00048>
 50. Butowski CF, Thomas DG, Young W, Cave NJ, McKenzie CM, Rosendale DI, et al. Addition of plant dietary fibre to a raw red meat high protein, high fat diet, alters the faecal bacteriome and organic acid profiles of the domestic cat (*Felis catus*). *PLOS ONE.* 2019;14:e0216072. <https://doi.org/10.1371/journal.pone.0216072>
 51. Díaz-Regañón D, García-Sancho M, Villaescusa A, Sainz Á, Agulla B, Reyes-Prieto M, et al. Characterization of the fecal and mucosa-associated microbiota in dogs with chronic inflammatory enteropathy. *Animals.* 2023;13:326. <https://doi.org/10.3390/ani13030326>
 52. Marsilio S, Pilla R, Sarawichitr B, Chow B, Hill SL, Ackermann MR, et al. Characterization of the fecal microbiome in cats with inflammatory bowel disease or alimentary small cell lymphoma. *Sci Rep.* 2019;9:19208.
 53. Zhang M, Mo R, Li M, Qu Y, Wang H, Liu T, et al. Comparison of the effects of enzymolysis seaweed powder and *Saccharomyces boulardii* on intestinal health and microbiota composition in kittens. *Metabolites.* 2023;13:637. <https://doi.org/10.3390/metabo13050637>

54. Pei L, Liu W, Liu L, Wang X, Jiang L, Chen Z, et al. Morel (*Morchella* spp.) intake alters gut microbial community and short-chain fatty acid profiles in mice. *Front Nutr.* 2023;10:1237237. <https://doi.org/10.3389/fnut.2023.1237237>
55. Rowe JC, Summers SC, Quimby JM, Winston JA. Fecal bile acid dysmetabolism and reduced ursodeoxycholic acid correlate with novel microbial signatures in feline chronic kidney disease. *Front Microbiol.* 2024;15:1458090. <https://doi.org/10.3389/fmicb.2024.1458090>