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Ethics approval and consent to participate	The experimental protocol was approved (CBNUA-25-0034-02) by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea.

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Abstract

This study evaluated the effects of two drying methods (microwave drying and hot-air drying) for black soldier fly larvae (*Hermetia illucens*; HI) on nutrient digestibility, palatability, and fecal microbiota composition in cats. Three experimental diets were formulated: CON, basal diet; MW, basal diet with 3% poultry meal (PM) replaced by microwave drying HI; HA, basal diet with 3% PM replaced by hot-air drying HI. In Experiment 1, *in vitro* digestibility was evaluated with 6 replicates per diet. In vitro organic matter (OM) digestibility was significantly higher ($p < 0.05$) in HA than CON, and crude protein (CP) digestibility was higher ($p < 0.05$) in HA compared with MW and CON. In Experiment 2, palatability was assessed using the two-bowl test with 18 cats, and no significant differences were observed in any pairwise comparison for feed intake, intake ratio, time to eat, first sniffing, or first eating. In Experiment 3, *in vivo* apparent total tract digestibility (ATTD) was determined using 12 cats in a replicated 3 × 3 Latin square design. The HA group showed significantly higher *in vivo* CP digestibility compared with both CON and MW, while ether extract (EE) digestibility tended to be higher in HA ($p = 0.063$). Fecal microbiota analysis revealed that the Chao1 index was significantly lower ($p < 0.05$) in MW at 10 d, and unweighted UniFrac analysis showed significant community separation. At the genus level, *Prevotella* significantly decreased ($p < 0.05$) with HI inclusion, while uncultured bacterial taxa were significantly lower ($p < 0.05$) in both HI groups. These results suggest that hot-air dried HI at 3% inclusion level is a viable alternative protein source for cat diets, offering improved CP digestibility without compromising palatability, while inducing selective shifts in fecal microbiota composition.

Keywords (3 to 6): Hermetia illucens, cat, nutrient digestibility, palatability, drying method, fecal microbiota

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Introduction

The rapid growth of the global companion animal industry has increased the demand for sustainable protein sources that fulfill pets' nutritional needs without competing with human food resources [1]. This challenge is especially significant for cats, which are obligate carnivores requiring much higher protein levels than omnivorous species and cannot sufficiently meet these needs through plant-based ingredients alone [2, 3]. As a result, identifying alternative protein sources that satisfy both feline nutritional requirements and environmental sustainability criteria is a crucial research goal.

Black soldier fly larvae (*Hermetia illucens*; HI) have emerged as a promising alternative protein source for pet food. These insects grow rapidly, have excellent feed conversion efficiency, and result in significantly lower greenhouse gas emissions compared to traditional animal protein sources [4, 5]. Additionally, HI are rich in crude protein (38.5–47.9%) and crude fat (14.6–39.2%) and possess a well-balanced profile of essential amino acids suitable for carnivorous species[6, 7]. Research using cecectomized rooster models has demonstrated that HI meal has high amino acid digestibility, highlighting its potential as a high-quality protein ingredient [8].

However, the nutritional value of insect-based ingredients is not solely based on their composition; it is also significantly influenced by the processing methods used during production [9, 10]. Different drying techniques can lead to distinct physicochemical changes, altering protein structure, amino acid availability, and nutrient accessibility. Comparative studies in monogastric animals, such as pigs and broilers, have shown that processing methods like hot-air drying and microwave drying can differentially impact nutrient digestibility and growth performance [11, 12]. Despite the increasing interest in HI as a pet food ingredient, systematic comparisons of processing effects on nutrient utilization in cats are notably lacking in the literature.

Processing methods can influence host health indirectly by altering gut microbiota, in addition to their direct effects on nutrient digestibility. The amount of dietary nutrients that escape digestion in the small intestine determines the substrate available for microbial fermentation in the colon [13]. Reductions in protein digestibility due to processing can increase the flow of protein to the colon, potentially favoring the growth of proteolytic bacteria and the production of harmful compounds like ammonia, phenols, and indoles, which are linked to negative health outcomes [14, 15]. Conversely, some processing methods may improve the fermentability of insect-derived chitin, promoting beneficial saccharolytic bacteria that produce short-chain fatty acids[16, 17]. Given the established links between gut microbiota composition and overall health outcomes in companion animals—such as immune function, metabolic regulation, and gastrointestinal homeostasis (Pilla & Suchodolski,

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54 2020)—it is crucial to understand how processing methods affect fecal microbiota for a thorough assessment of
55 ingredients.
56 High-throughput 16S rRNA gene sequencing has become the standard for characterizing gut microbial
57 communities in companion animal nutrition research, allowing for detailed taxonomic profiling that identifies
58 diet-induced changes in microbial ecology [18, 19]. Combining microbiota analysis with assessments of
59 digestibility and palatability creates a comprehensive evaluation framework that considers both nutritional
60 adequacy and potential health implications of new ingredients.
61 We hypothesized that hot-air drying, with its milder and more uniform thermal profile, would better preserve
62 protein quality and enhance digestibility than the rapid dielectric heating of microwave drying, which can produce
63 localized super-heating, advanced Maillard browning, and protein cross-linking. Accordingly, the objectives of
64 this study were to evaluate the effects of two high-heat processing methods (hot-air drying vs. microwave drying)
65 on (1) *in vitro* and *in vivo* nutrient digestibility, (2) diet palatability, and (3) fecal microbiota composition in cats.
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Materials and Methods

Preparations of HI and experiment diet

The HI was obtained from Inseong Industry (Jeju, Korea). The larvae were reared on a citrus pulp and soybean meal mixture (8:2, w/w) at 25 ± 3 °C and 70 ± 5% relative humidity, and all HI used in the experiment were 3rd-instar larvae reared for 10 d. After rearing, the larvae were starved for 24 h to allow gut clearance, washed with potable water to remove substrate residues, surface-dried at ambient temperature, and then subjected to one of two secondary drying programs: (1) microwave drying at 80 °C for 32 min using a microwave dryer (M-200, Entomo Co. Ltd., Cheongju, Republic of Korea), or (2) hot-air drying at 60 °C for 17 h using a forced-air drying oven (KAPD-195D; CNT Co. Ltd., Gwangju, Republic of Korea). Both drying programs were continued until the moisture content was below 1% to ensure microbial stability and storage longevity, after which the dried larvae were ground through a 1 mm screen for incorporation into the experimental diets. The chemical composition of the experimental diets and the ingredient profiles are presented in Tables 1 and 2. The experimental treatments were as follows: CON, basal diet; MW, basal diet with 3% poultry meal (PM) replaced by microwave-dried HI; HA, basal diet with 3% PM replaced by hot-air-dried HI.

Experiment 1

***In vitro* analysis**

The *in vitro* trial was slightly modified by the Soutar et al. [20] method. The trial was conducted with 6 replicates per diet. These samples were ground in the form of fine powder (< 1.00 mm). When simulating the stomach, weighing each sample (5.000 ± 0.005 g) then put in 250 mL Erlenmeyer flasks with adding 85 mL of ultrahigh-quality water (> 18 MΩ) Using 1M hydrochloric acid and 1M sodium bicarbonate solutions, the pH was gradually adjusted until 2.0 was reached. To simulate stomach digestion in the cat, 10 mL of pepsin solution was added to the flask to equilibrate for 15 minutes at 39°C (solid, P7000, porcine gastric mucosa pepsin, Sigma-Aldrich, St. Louis, MO, USA). To prevent bacterial fermentation, 5 mL of chloramphenicol solution (C0378, chloramphenicol, Sigma-Aldrich with 5 g/L ethanol) was added. After being covered with Parafilm M® film, the flasks were incubated in a shaking incubator (SWB-35, Hanyang Science Lab, Seoul, Korea) for 1.5 h at 39°C. Before the small intestine step of the simulation, the flask was cooled to room temperature, and then 1 M NaOH solution was added to adjust the pH to 6.8. To simulate small intestine digestion in the cat, 20 mL of an 80 mg/mL bile salts (B8756, Sigma Aldrich) solution and 20 mL of a pancreatin solution (1 mg/mL 8 × USP pancreatin composed of amylase [3,720 U/mg], protease [2880 U/mg], and lipase [100−650 U/mg]; P7545, Sigma-Aldrich) were added

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to the flask. After sealing the flasks with Parafilm M® film, the flasks were placed in a shaking incubator (SWB-35, Hanyang Science Lab) at 39°C for 3 h. Then, pre-weighed and pre-dried filter crucibles (Gooch Type Filter Crucibles, PYREX, Sunderland, UK) were used to filter the undigested samples. After the in vitro trial, the filter crucibles containing the undigested residues were dried at 70°C for 24 hours, and DM was calculated.

Chemical analysis and calculations

All diets and residues were crushed on a 1 mm screen and chemically analyzed in 6 replicates. The diets and residues of DM (method 930.15), and EE (method 920.39) were determined using the AOAC[21] method. The gross energy (GE) content was analyzed by bomb calorimeter (Parr 6400 Bomb Calorimeter, Parr Instrument, Moline, IL, USA). The CP content was determined using the dumas (Rapid MAX N-Exceed, Elementar, Langenselbold, Germany).

Calculating the *in vitro* digestibility of DM using the following formula:

“Digestibility (%) = 100 − [(residue weight / sample weight) × 100]

Calculating the in vitro digestibility of CP, EE, and GE used the following formula:

“Digestibility (%) = 100 − [Nr × (100 − IDDM) / Nd]”

Nr =nutrient concentration in residues (DM %), Nd = nutrient concentration in diet (DM %), IDDM =in vitro digestibility of DM (%).

Experiment 2

Palatability test

Animal and experimental procedures

18 healthy adult domestic cats of mixed sex (9 males and 9 females) aged 5–7 years with a body weight of 4.88 ± 1.90 kg, were used to determine palatability when substituting PM with HI using different process methods. The palatability test used the international palatability comparison test method, “Two-Bowl Test” among treatments. The palatability test used the two-bowl test method and estimated feed intake, intake ratio, first sniffing bout, first eating bout, and time to eat. Every experiment was implemented using a split-plate test, where cats were given 40 g of diet in each of two stainless steel bowls for 5 min. Each comparative experiment, including a total of 18 cats, was conducted in 2 continuous periods with 4 d per period, and the position of the bowl was changed every day. The detailed operation abided by the previous method with a slight change [22]. Each cat’s “first

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132 sniffing" and "first eating" were the first food bowls they touched and ate, respectively, and the relative ratio was
133 calculated. Feed intake was calculated by subtracting the remaining diet amount from the initially provided. The
134 intake ratio was determined by dividing feed intake by the amount provided.
135
136 **Experiment 3**
137
138 ***In vivo* analysis**
139
140 **Animal ethics**
141 The experimental protocol was approved (CBNUA-25-0034-02) by the Institutional Animal Care and Use
142 Committee of Chungbuk National University, Cheongju, Korea.
143
144 **Animals and experiment design**
145 Twelve healthy adult domestic cats of mixed sex (6 males and 6 females), aged 5–7 years and weighing 5.00 ±
146 1.64 kg, were assigned to four replicated 3 × 3 Latin squares, so that within each replicate every cat received each
147 of the three experimental diets in a different order across the three periods. Each period consisted of 7 d of dietary
148 adaptation followed by 3 d of total fecal collection, and consecutive periods were separated by a 5-d wash-out
149 period during which all cats received the basal (CON) diet. Their diet was controlled to meet or exceed the nutrient
150 profile for adult cats established by the AAFCO [23]. Each cat was housed in an individual cage (0.9 m × 0.9 m
151 × 0.9 m) outside of feeding and fecal-collection times, with water available *ad libitum* throughout. Cats were fed
152 twice daily (08:00–10:00 and 15:00–17:00) and were housed in groups outside the experimental periods. The cats
153 were maintained on a 12-h light cycle with lights off from 19:00 to 07:00.
154
155 **Nutrient digestibility**
156 Apparent total tract digestibility (ATTD) of DM, CP, and gross energy (GE) were determined using 1% celite
157 as an inert indicator by Scott and Boldaji [23] method. Cats were fed diets mixed with celite from 1 to 3 d and
158 diet samples were also collected. Fresh fecal samples were collected from 2 to 4 d. Fresh fecal and diet samples
159 were stored in a freezer at −20°C immediately after collection. At the end of the experiment, fecal samples were
160 dried at 70°C for 72 h and then crushed on a 1 mm screen. All diet and fecal samples were then analyzed for DM,
161 CP, and GE following the procedures by the AOAC [21]. The GE of diets and feces were analyzed using an
162 adiabatic oxygen bomb calorimeter (6400 Automatic Isoperibol calorimeter, Parr, USA). For calculating the
163 ATTD of the nutrients, we used the following equation: Digestibility = 1 − [(Nf × Cd)/ (Nd × Cf)] × 100, where

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164 Nf = concentration of nutrient in fecal, Nd = concentration of nutrient in the diet, Cd = concentration of celite in
165 the diet, and Cf = concentration of celite in the fecal.
166
167 **16S rRNA sequencing**
168 The 16S rRNA sequencing data were analyzed using QIIME 2, a next-generation microbiome bioinformatics
169 pipeline designed for metagenomic research. Fecal samples were submitted to Sanigen (Anyang, Korea) for
170 sequencing of the V3–V4 hypervariable region of the bacterial 16S rRNA gene on an Illumina MiSeq platform (2
171 × 300 bp paired-end). All raw paired-end reads were demultiplexed, primer-trimmed, and quality-filtered
172 (truncation at the position where the median Phred quality dropped below 25), and amplicon sequence variants
173 (ASVs) were inferred using the Divisive Amplicon Denoising Algorithm 2 (DADA2) implemented in QIIME 2,
174 which simultaneously corrects amplicon errors and removes potential base-calling errors and chimeric sequences.
175 Taxonomy was assigned against the SILVA 138 reference database using the QIIME 2 q2-feature-classifier plugin
176 with the naïve-Bayes classifier pre-trained for the V3–V4 region, and the assignments were cross-validated against
177 NCBI bacterial taxonomy references. To control differences in sequencing effort across samples, the ASV feature
178 table was rarefied to a uniform depth corresponding to the lowest-coverage sample prior to alpha-diversity
179 (observed OTUs, Chao1, Shannon, Simpson) and beta-diversity (unweighted and weighted UniFrac)
180 computations. Differential-abundance analyses at the phylum and genus levels were performed on per-sample
181 relative abundances (per-sample read counts divided by per-sample library size) without rarefaction, so as to retain
182 the full taxonomic information of rare taxa.
183
184 **Statistical analysis**
185 Palatability and *in vitro* digestibility data were analyzed by one-way ANOVA in JMP Pro (version 16.0.0, SAS
186 Institute, Cary, NC, USA), with diet as the sole fixed effect and the experimental replicate (or cat, in the case of
187 palatability) as the experimental unit. For the *in vivo* experiment, the apparent total tract digestibility (ATTD) data
188 were analyzed using a linear mixed-effects model with diet as the fixed effect and cat, sequence, and period as
189 random effects. Pairwise comparisons among diet least-squares means were performed using Tukey's HSD test.
190 A probability level of $p < 0.05$ was considered statistically significant, and $0.05 \leq p < 0.10$ was considered to
191 represent a tendency.
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193 **Results**

194 ***In vitro* Digestibility**

195 The effects of HI inclusion on *in vitro* nutrient digestibility are presented in Table 3. The *in vitro* digestibility
196 of OM was significantly affected by dietary treatment, with the HA group being significantly higher ($p < 0.05$)
197 than the CON group. The *in vitro* CP digestibility showed highly significant differences among treatments, with
198 HA being significantly higher ($p < 0.05$) than MW, which in turn was significantly higher than CON. No
199 significant differences were observed among treatments for DM, EE, and GE digestibility.

200

201 **Palatability**

202 Palatability results from the two-bowl test are shown in Table 4. None of the three pairwise comparisons
203 (CON vs. MW, CON vs. HA, and MW vs. HA) reached statistical significance for feed intake, intake ratio, time
204 to eat, first sniffing, or first eating.

205

206 ***In vivo* digestibility**

207 The ATTD of nutrients in cats fed the experimental diets is shown in Table 5. The *in vivo* CP digestibility was
208 significantly different among treatments, with the HA group being significantly higher ($p < 0.05$) than both the
209 CON and MW groups. The *in vivo* EE digestibility tended to differ among treatments ($p = 0.063$). No significant
210 differences were observed for DM and GE digestibility among the three dietary treatments.

211

212 **Diversity of the fecal microbiome**

213 Alpha diversity indices of the fecal microbiota are presented in Figure 1. At 0 d, no significant differences
214 were observed among treatments for any alpha diversity index, including the Chao1, Shannon, and Simpson
215 index. At 10 d, the Chao1 index was significantly lower in the MW group compared to the CON and HA
216 groups.

217 Beta diversity analysis based on UniFrac distances is shown in Figure 2. At 0 d, no significant separation
218 among treatment groups was observed in either unweighted and weighted UniFrac analyses. At 10 d, the
219 unweighted UniFrac analysis revealed a significant separation ($p < 0.05$) among treatment groups. However, the
220 weighted UniFrac analysis did not show significant differences among groups.

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221

222 **Relative abundance of the fecal microbiome**

223 The relative abundance of fecal microbiota at the phylum level is presented in Table 6 and Figure 3. At 0 d,
224 the fecal microbiota was with no significant differences observed among treatments for any phylum. At 10 d, the
225 overall phylum composition showed no significant differences among treatments. The relative abundance of
226 fecal microbiota at the genus level before and after dietary adaptation is presented in Tables 7, 8, and Figure 9
227 respectively. At 0 d, *Odoribacter* showed a significantly higher ($p < 0.05$) level than the HA group compared to
228 the CON group. At 10 d, significant changes were observed in the genus-level composition. The relative
229 abundance of *Prevotella* was significantly affected by dietary treatment, with a marked decrease from CON to
230 HA. The uncultured bacterial taxa also showed significant treatment effects, with CON being significantly
231 higher ($p < 0.05$) than both MW and HA. Other genera, including *Bacteroides*, *Collinsella*, *Megasphaera*,
232 *Alloprevotella*, and *Helicobacter*, showed no significant differences.

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233 **Discussion**

234 The CP and GE contents of hot-air dried HI were higher than those of microwave-dried HI, highlighting the
235 impact of the drying method on the proximate composition of insect meals [24]. The CP values were consistent
236 with the range reported for HI in various studies, confirming that HI retains a high protein density regardless of
237 the processing method. The increased ash content in microwave-dried HI may have diluted the CP fraction on a
238 dry matter basis [25]. Prolonged exposure during hot-air drying can denature proteins and promote non-enzymatic
239 browning, whereas microwave drying employs rapid dielectric heating that polymerizes protein particles and
240 alters their tertiary structure [26]. The amino acid composition of HI meals fell within previously reported ranges
241 [9, 27], although variations in certain amino acids across studies can be attributed to differences in drying
242 conditions and oil extraction methods [11].

243 The *in vitro* digestibility values were also within previously reported ranges [10], suggesting that a 3% HI
244 substitution did not compromise nutrient availability under simulated conditions. The lower *in vitro* digestibility
245 observed with microwave drying may be due to protein polymerization, which reduces the accessibility of peptide
246 bonds to digestive enzymes such as pepsin and pancreatin [26]. Chitin might overestimate CP content when
247 measured by the Kjeldahl method and reduce apparent digestibility by acting as a fiber source resistant to
248 mammalian digestive enzymes [28, 29]. However, comparable digestibility between HI-based and conventional
249 diets has been reported in cats [30], indicating that the high CP content of insect protein offsets the reductions
250 caused by chitin. Additionally, the inclusion of lipase in the *in vitro* method enhanced DM, OM, and EE
251 digestibility without impacting CP and GE digestibility. This suggests that standard two-step methods may
252 underestimate total nutrient availability due to inadequate fat hydrolysis [31].

253 The increased *in vivo* CP digestibility observed in the HA group (hot-air dried HI) supports the idea that hot-
254 air drying maintains protein structure in a form that is more conducive to enzymatic hydrolysis in the feline
255 gastrointestinal tract. Additionally, the lower ash concentration in hot-air dried HI enhances amino acid
256 bioavailability, as the essential amino acid content per unit of CP increases with reduced ash content [33]. The
257 improved digestibility of arginine and cystine is particularly significant for cats, considering their limited capacity
258 for *de novo* citrulline synthesis in the urea cycle and their reliance on cysteine for taurine biosynthesis [34]. This
259 enhancement in amino acid digestibility supports the potential of HI as an alternative protein source for obligate
260 carnivores [35]. The tendency toward higher EE digestibility in the HA group may suggest that hot-air drying
261 better preserves lipid structural integrity, thereby facilitating the favorable absorption kinetics of lauric acid
262 (C12:0), which makes up 40–60% of HI fatty acids and exhibits medium-chain triglyceride characteristics [28].

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263 The observed superiority of hot-air drying for *in vitro* and *in vivo* CP digestibility is consistent with our initial
264 hypothesis that the milder thermal profile of hot-air drying would better preserve protein structure than the rapid
265 dielectric heating of microwave drying. Several mechanistic considerations support this interpretation. First,
266 microwave drying generates internal heat through dielectric absorption, in which water molecules and polar
267 protein side chains absorb microwave energy directly and produce localized hot spots that can exceed the bulk
268 drying temperature, inducing thermal protein aggregation, peptide-bond cross-linking, and Maillard-type
269 browning between reducing sugars and the ε-amino groups of lysine [27, 38]. These reactions reduce the
270 accessibility of peptide bonds to pepsin and pancreatin and lower the apparent availability of reactive lysine,
271 which is consistent with the lower *in vitro* and *in vivo* CP digestibility observed for the MW diet. Second, the
272 higher residual ash fraction of microwave-dried HI dilutes the CP fraction on a dry-matter basis [25, 33], which
273 slightly reduces the absolute amount of digestible protein delivered per unit of insect meal. Third, hot-air drying
274 at 60 °C for 17 h subjects the larvae to a milder, more uniform thermal load than the 80 °C for 32 min microwave
275 program, allowing protein structures to denature in a controlled manner that opens the polypeptide backbone to
276 enzymatic attack without driving the more advanced Maillard products characteristic of rapid dielectric heating
277 [27]. Together, these mechanisms account for the superior protein preservation observed for hot-air drying in the
278 present study.

279 The lack of palatability differences across all pairwise comparisons indicates that substituting 3% HI for
280 chicken meal did not negatively impact diet acceptance, regardless of the processing method. Palatability in cats
281 involves a complex interplay of olfactory, gustatory, and textural signals, and cats tend to be more selective eaters
282 than dogs due to their narrower dietary niche as obligate carnivores [32]. Measures such as feed intake, intake
283 ratio, time to eat, initial sniffing, and first eating were all unaffected, suggesting that neither the olfactory profile
284 nor the post-oral sensory experience of HI-substituted diets differed from the control diet. Processing methods
285 can influence the sensory properties of insect meals by generating Maillard reaction products and volatile
286 compounds during thermal treatment, which may alter the aroma and flavor of the final diet [33]. Hot-air drying
287 at high temperatures produces melanoidins and heterocyclic compounds that contribute both desirable roasted
288 notes and potentially unappealing bitter flavors, while microwave drying creates a different volatile profile due to
289 its rapid internal heating mechanism [26, 34]. The absence of significant palatability differences between MW
290 and HA in this study indicates that the processing-induced changes in sensory properties were not enough to affect
291 diet acceptance at a 3% inclusion level. Previous research has shown varying palatability responses to insect-
292 based diets in cats, influenced by both inclusion levels and insect species. For instance, reduced acceptance was

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293 noted at inclusion levels exceeding 10% in some studies [35], while low to moderate levels did not elicit adverse
294 effects [36]. The chitin content in HI may also affect the textural properties of extruded diets, as chitin serves as
295 a structural polysaccharide that can influence kibble hardness and mouthfeel at higher inclusion rates [29]. The
296 3% inclusion level in this study appears to be below the threshold where sensory and textural changes become
297 noticeable to cats, supporting the commercial viability of HI as a partial protein substitute without the need for
298 palatability-enhancing additives like animal digest or flavor coatings [1].

299 The lower Chao1 index observed in the MW group on day 10, along with trends indicating decreased Shannon
300 and Simpson indices in both HI-supplemented groups, suggests that HI inclusion impacted fecal microbial
301 community structure, with a more pronounced effect seen in the microwave-dried HI. In animal nutrition, alpha
302 diversity is a practical indicator of gut microbial community stability [14]. The reduced species richness in the
303 MW group may be attributed to the lower CP digestibility of microwave-dried HI. Poorly digested protein
304 reaching the hindgut can lead to fermentation by-products such as ammonia, biogenic amines, and branched-chain
305 fatty acids, which inhibit commensal bacteria [37, 38]. The maintenance of alpha diversity in the HA group aligns
306 with the higher CP digestibility of hot-air dried HI, which limits the protein substrate available for fermentation
307 in the hindgut. The divergence in unweighted UniFrac on day 10, without a corresponding shift in weighted
308 UniFrac, supports the alpha diversity pattern, indicating that HI inclusion altered the presence of low-abundance
309 taxa while preserving the core community. Similar selective shifts in rare taxa have been reported in previous
310 studies on companion animal nutrition when novel protein sources were introduced [39, 40]

311 At the phylum level, Firmicutes and Bacteroidota dominated the fecal microbiota across all treatments, aligning
312 with the core composition found in healthy adult cats [41, 42]. Although the numerical increase in Firmicutes and
313 decrease in Bacteroidota in the HI-supplemented groups were not statistically significant, they may indicate the
314 effects of introducing chitin as a novel fiber substrate. Chitin has been shown to promote chitinolytic bacteria
315 within the Firmicutes phylum in monogastric species [17, 43]. This shift in the Firmicutes-to-Bacteroidota ratio
316 aligns with dietary substrate modulation observed in companion animals fed altered protein and fiber sources [44].
317 The lack of significant phylum-level differences at 3% inclusion supports previous findings regarding low to
318 moderate levels of insect meal [36].

319 The most notable genus-level change was the decrease in Prevotella abundance with HI substitution. Prevotella
320 is a saccharolytic genus that ferments non-starch polysaccharides and produces short-chain fatty acids [45, 46].
321 This decline can be attributed to the altered carbohydrate-to-protein substrate ratio; replacing wheat bran and
322 DDGS with HI reduced readily fermentable non-starch polysaccharides while increasing chitin content, which

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323 diminished Prevotella's competitive advantage in the hindgut. The more significant reduction in the HA group
324 suggests that hot-air drying preserved chitin in a more crystalline form, further limiting carbohydrate substrates
325 available to saccharolytic bacteria [47].

326 The higher abundance of *Odoribacter* in the HA group at day 0, observed prior to dietary intervention, is most
327 likely attributable to inter-individual variation in the baseline fecal microbiome [52]. At day 10, the divergent
328 trends for *Catenibacterium* and *Parabacteroides* between the MW and HA groups suggest processing-dependent
329 microbial responses. *Catenibacterium*, a proteolytic taxon known to expand under conditions of elevated colonic
330 protein availability [53], was numerically enriched in the MW group, which is consistent with the lower CP
331 digestibility of microwave-dried HI and the resulting increase in undigested protein reaching the hindgut.
332 *Parabacteroides*, which tended to be more abundant in the HA group, has been implicated in bile-acid
333 deconjugation and anti-inflammatory signaling [54]; the functional consequences of this shift warrant further
334 investigation through metagenomic and metabolomic approaches. The digestibility and microbiota findings can
335 be explained by a common underlying mechanism. The amount of undigested protein and fiber reaching the colon
336 largely determines the processing-dependent changes observed in the fecal microbiome. The reduced *in vitro* and
337 *in vivo* CP digestibility of microwave-dried HI indicates that a greater proportion of dietary protein bypassed
338 proximal enzymatic hydrolysis and entered the large intestine, where it became available for proteolytic
339 fermentation. This interpretation is supported by the concurrent elevation of *Catenibacterium* — a recognized
340 proteolytic taxon — and the reduction in Chao1 richness in the MW group at day 10, both of which constitute
341 established signatures of protein-driven dysbiosis in monogastric species [41, 42, 53]. Conversely, the higher CP
342 digestibility achieved with hot-air drying was accompanied by maintenance of alpha diversity at levels comparable
343 to the control, consistent with the dual effect of (i) enhanced small-intestinal protein bioavailability and (ii) a
344 concomitant reduction in colonic protein flux, the latter of which limits the substrate driving proteolytic
345 fermentation. The parallel decline of the saccharolytic genus *Prevotella* in both HI-supplemented groups, by
346 contrast, occurred independently of drying method and is attributable to the partial substitution of fermentable
347 non-starch polysaccharides with chitin upon HI inclusion. The microbiota response therefore reflects two distinct
348 substrate-level mechanisms: drying method governs protein-side fermentation in the hindgut, whereas HI
349 inclusion *per se* governs carbohydrate-side fermentation.
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352 **Conclusion**

353 The hot-air drying was superior to microwave drying for preserving nutrient digestibility of HI in cats, with
354 no adverse effects on palatability at a 3% inclusion level. HI inclusion selectively altered low-abundance fecal
355 microbial taxa without disrupting the core community structure, and hot-air-dried HI maintained alpha diversity
356 comparable to the control. These results support hot-air-dried HI at 3% inclusion as a nutritionally adequate and
357 palatable alternative protein source in feline diets, while indicating that the rapid microwave program tested here
358 is less suitable than conventional hot-air drying for preserving protein quality of HI.

359

360 **Disclosure statement**

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Table 1. Ingredient composition of experimental diets

Items, %	CON	MW	HA
Poultry meal	35.00	32.00	32.00
Hot-air dried HI	-	-	3.00
Microwave HI	-	3.00	-
Rice	21.36	21.36	21.36
Wheat flour	14.74	14.74	14.74
Wheat bran	10.00	9.38	9.90
Soybean meal	5.00	7.84	7.59
DDGS	5.00	3.35	3.00
Poultry oil	5.00	4.43	4.51
Beet pulp	2.00	2.00	2.00
Salt	0.70	0.70	0.70
Taurine	0.20	0.20	0.20
Mineral + Vitamin Premix[2)]	1.00	1.00	1.00
Total	100.00	100.00	100.00
Chemical composition			
ME, kcal/kg	3,633	3,633	3,633
CP, %	32.01	32.01	32.01
EE, %	10.56	10.54	10.57
CF, %	3.64	3.72	3.75
Ash, %	2.33	2.80	2.65
Ca, %	1.11	1.14	1.13
P, %	0.69	0.68	0.68

CON, basal diet; MW, basal diet with 3% poultry meal (PM) replaced by microwave drying HI; HA, basal diet with 3% PM replaced by hot-air drying HI.

[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.

HI, *Hermetia illucens* larvae; DDGS, Dried distiller's grains with solubles; ME, metabolic energy; CP, crude protein; EE, ether extract; CF, crude fiber; Ca, Calcium; P, Phosphorus.

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Table 2. Nutrient components of differently processed HI

Items, %	Microwave drying HI	Hot-air drying HI
Dry matter	96.61	95.43
Gross energy, kcal/kg	5,661.95	5,816.99
Crude protein	38.36	43.05
Ether extract	33.13	31.87
Crude fiber	6.75	7.41
Crude ash	16.32	11.54
Aspartic acid	3.38	3.84
Threonine	1.32	1.64
Serine	1.40	1.70
Glutamic acid	3.90	4.10
Glycine	2.28	2.39
Alanine	2.58	2.67
Valine	2.10	2.07
Isoleucine	1.53	1.49
Leucine	2.78	2.99
Tyrosine	2.87	3.22
Phenylalanine	1.72	1.38
Lysine	2.53	2.44
Histidine	1.35	1.47
Arginine	2.08	2.10
Cysteine	0.31	0.73
Methionine	0.76	0.86
Tryptophan	0.30	0.32
Proline	2.12	2.43

HI, *Hermetia illucens* larvae

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Table 3. Effects of replacing PM with differently processed HI on *in vitro* digestibility in cats

Item, %	CON	MW	HA	SE	*p*-value
DM	81.35	81.53	82.73	0.687	0.330
OM	80.03b	81.18ab	83.26a	0.666	0.012
CP	86.66c	87.30b	88.81a	0.146	<0.001
EE	80.47	80.19	81.27	0.797	0.619
GE	83.90	84.17	85.27	0.450	0.105

PM, poultry meal; HI, *Hermetia illucens* larvae; CON, basal diet; MW, basal diet with 3% PM replaced by microwave drying HI; HA, basal diet with 3% PM replaced by hot-air drying HI; DM, dry matter; CP, crude protein; EE, ether extract; GE, gross energy.

[a-c] Different letters within the same row indicate significant differences ($p < 0.05$).

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Table 4. Palatability of experimental diets replacing PM with differently processed HI in cats

Items			SE	*p*-value
	CON	MW		
Feed intake, g/5 min	4.29	4.35	0.241	0.851
intake ratio	51.04	48.96	4.257	0.730
Time to eat, s	53.13	46.88	5.150	0.393
First sniffing, %	149.40	176.31	12.175	0.121
First eating, %	10.73	10.88	0.601	0.851
	CON	HA		
Feed intake, g/5 min	3.87	3.60	0.253	0.452
intake ratio	52.86	47.14	0.436	0.355
Time to eat, s	49.48	47.14	4.724	0.727
First sniffing, %	147.92	160.40	11.517	0.446
First eating, %	9.67	9.00	0.633	0.452
	HA	MW		
Feed intake, g/5 min	3.79	3.90	0.257	0.759
intake ratio	52.60	47.40	4.823	0.447
Time to eat, s	49.22	50.78	4.743	0.816
First sniffing, %	149.31	172.94	12.168	0.173
First eating, %	9.48	9.76	0.641	0.759

PM, poultry meal; HI, *Hermetia illucens* larvae; CON, basal diet; MW, basal diet with 3% PM replaced by microwave drying HI; HA, basal diet with 3% PM replaced by hot-air drying HI.

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Table 5. Effects of replacing PM with differently processed HI on *in vivo* digestibility in cats

Item, %	CON	MW	HA	SE	*p*-value
DM	90.46	90.41	90.76	0.175	0.348
CP	83.38b	83.40b	84.77a	0.200	0.002
EE	82.21	82.58	83.43	0.313	0.063
GE	82.33	82.73	83.54	0.399	0.152

PM, poultry meal; HI, *Hermetia illucens* larvae; CON, basal diet; MW, basal diet with 3% PM replaced by microwave drying HI; HA, basal diet with 3% PM replaced by hot-air drying HI; DM, dry matter; CP, crude protein; EE, ether extract; GE, gross energy.
a, b Different letters within the same row indicate significant differences ($p < 0.05$).

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Table 6. Effects of replacing PM with differently processed HI on fecal microbiome relative abundance at Phylum in cats

Item, %	CON	MW	HA	SE	*p*-value
0 d					
Actinobacteriota	2.84	2.47	0.99	0.980	0.394
Bacteroidota	61.95	60.43	61.64	2.567	0.907
Campilobacterota	0.60	0.91	0.37	0.335	0.530
Firmicutes	32.13	33.49	32.66	2.957	0.948
Proteobacteria	2.21	2.44	3.99	0.578	0.098
Rest	0.28	0.26	0.35	0.103	0.840
10 d					
Actinobacteriota	11.01	13.88	5.74	3.445	0.269
Bacteroidota	50.07	36.84	36.21	4.978	0.118
Campilobacterota	3.03	4.79	3.16	1.891	0.768
Firmicutes	31.63	39.46	45.03	5.496	0.255
Proteobacteria	3.93	2.55	7.13	2.587	0.458
Rest	0.32	2.48	2.74	1.463	0.459

PM, poultry meal; HI, *Hermetia illucens* larvae; CON, basal diet; MW, basal diet with 3% PM replaced by microwave drying HI; HA, basal diet with 3% PM replaced by hot-air drying HI.

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Table 7. Effects of replacing PM with differently processed HI on fecal microbiome relative abundance at Genus in cats (0 d)

Item, %	CON	MW	HA	SE	*p*-value
Acidaminococcus	2.21	2.14	2.47	1.502	0.986
Allisonella	0.31	0.47	0.77	0.258	0.462
Alloprevotella	9.21	7.83	10.92	1.290	0.269
Anaerobiospirillum	0.34	0.32	1.28	0.393	0.177
Bacteroides	14.90	17.42	18.87	2.222	0.460
Blautia	0.97	0.86	1.00	0.400	0.969
Catenibacterium	3.76	1.69	1.52	0.963	0.219
Collinsella	2.30	2.01	0.67	0.874	0.395
Enterococcus	0.95	1.78	2.08	1.649	0.882
Megamonas	3.08	4.89	3.21	1.116	0.459
Megasphaera	7.28	12.80	13.18	2.107	0.120
Muribaculaceae	1.36	0.86	1.18	0.284	0.470
Odoribacter	1.35b	2.27ab	3.29a	0.459	0.030
Parabacteroides	2.57	3.22	4.06	0.455	0.099
Paraprevotella	3.33	3.02	2.95	1.527	0.983
Phascolarctobacterium	4.40	4.68	4.63	1.369	0.988
Prevotella	26.25	22.23	17.08	2.952	0.123
Solobacterium	1.60	0.08	0.16	0.557	0.129
Sutterella	1.83	1.72	2.38	0.481	0.588
uncultured	2.19	2.57	2.02	0.334	0.658
Rest	44.22	39.93	37.48	3.047	0.314

PM, poultry meal; HI, *Hermetia illucens* larvae; CON, basal diet; MW, basal diet with 3% PM replaced by microwave drying HI; HA, basal diet with 3% PM replaced by hot-air drying HI.

[a, b] Different letters within the same row indicate significant differences ($p < 0.05$).

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Table 8. Effects of replacing PM with differently processed HI on fecal microbiome relative abundance at Genus in cats (10 d)

Item, %	CON	MW	HA	SE	*p*-value
Allisonella	1.64	0.38	0.81	0.369	0.079
Alloprevotella	4.26	3.00	5.99	0.132	0.401
Anaerotruncus	0.01	0.01	0.02	0.005	0.674
Bacteroides	15.70	15.76	13.18	2.704	0.748
Blautia	1.25	0.81	0.99	0.216	0.371
Catenibacterium	0.92	6.26	0.82	1.688	0.061
Collinsella	10.35	12.38	5.25	3.257	0.309
Fusobacterium	0.14	0.24	2.26	1.417	0.467
Helicobacter	3.01	4.57	2.92	1.943	0.801
Megamonas	1.84	2.00	0.80	0.799	0.528
Megasphaera	7.60	10.89	23.79	6.677	0.227
Muribaculaceae	1.27	0.44	1.21	0.394	0.279
Odoribacter	2.01	1.47	1.19	0.533	0.553
Parabacteroides	4.08	2.26	6.12	1.081	0.070
Peptoclostridium	2.78	4.81	4.56	2.802	0.856
Phascolarctobacterium	3.67	2.12	3.04	0.758	0.361
Prevotella	18.26a	12.38ab	6.58b	2.153	0.006
Subdoligranulum	2.26	2.10	0.23	0.841	0.199
Sutterella	0.58	1.42	1.26	0.684	0.661
uncultured	3.80a	1.80b	1.43b	0.522	0.012
Rest	14.58	13.10	17.19	3.726	0.739

PM, poultry meal; HI, *Hermetia illucens* larvae; CON, basal diet; MW, basal diet with 3% PM replaced by microwave drying HI; HA, basal diet with 3% PM replaced by hot-air drying HI.
[a, b] Different letters within the same row indicate significant differences ($p < 0.05$).

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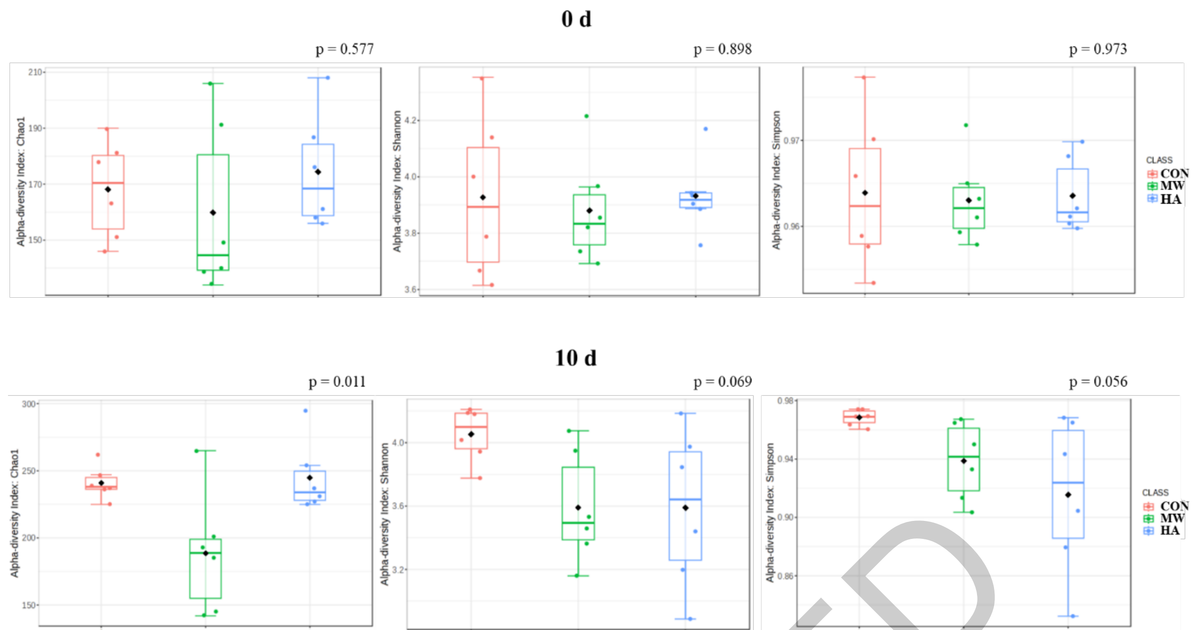

Figure 1. Effects of replacing PM with differently processed HI alpha diversity indices of fecal microbiota in cats fed experimental diets at day 0 and day 10. CON, basal diet; MW, basal diet with 3% PM replaced by microwave drying HI; HA, basal diet with 3% PM replaced by hot-air drying HI. Significant differences among treatments were determined at $p < 0.05$. PM, poultry meal; HI, *Hermitia illucens* larvae.

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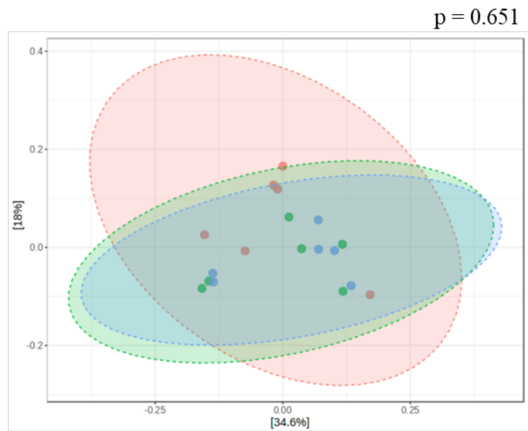
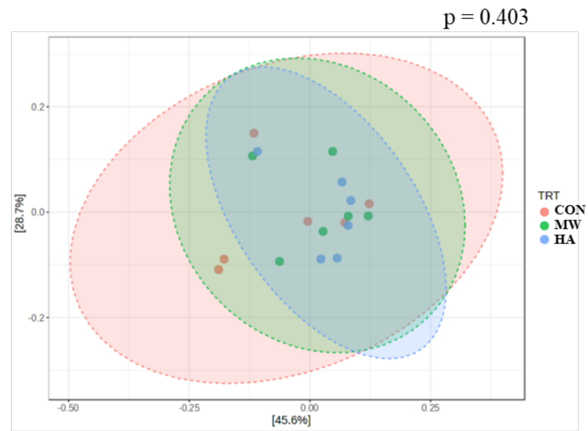
0 d

p = 0.651



Unweighted

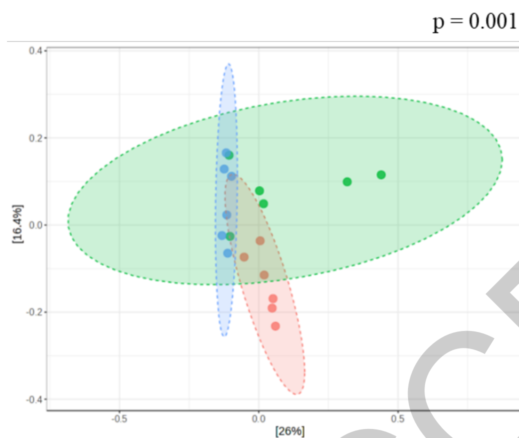
p = 0.403



Weighted

10 d

p = 0.001



Unweighted

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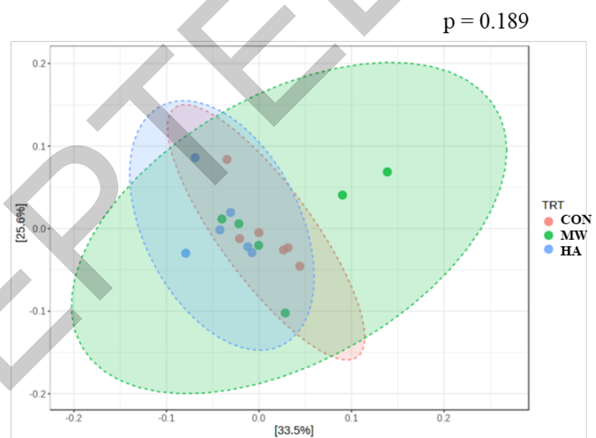

Weighted

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671 Figure 2. Effects of replacing PM with differently processed HI beta diversity analysis of fecal microbiota in
672 cats fed experimental diets at day 0 and day 10. CON, basal diet; MW, basal diet with 3% PM replaced by
673 microwave drying HI; HA, basal diet with 3% PM replaced by hot-air drying HI. Significant differences among
674 treatments were determined at $p < 0.05$. PM, poultry meal; HI, *Hermitia illucens* larvae.

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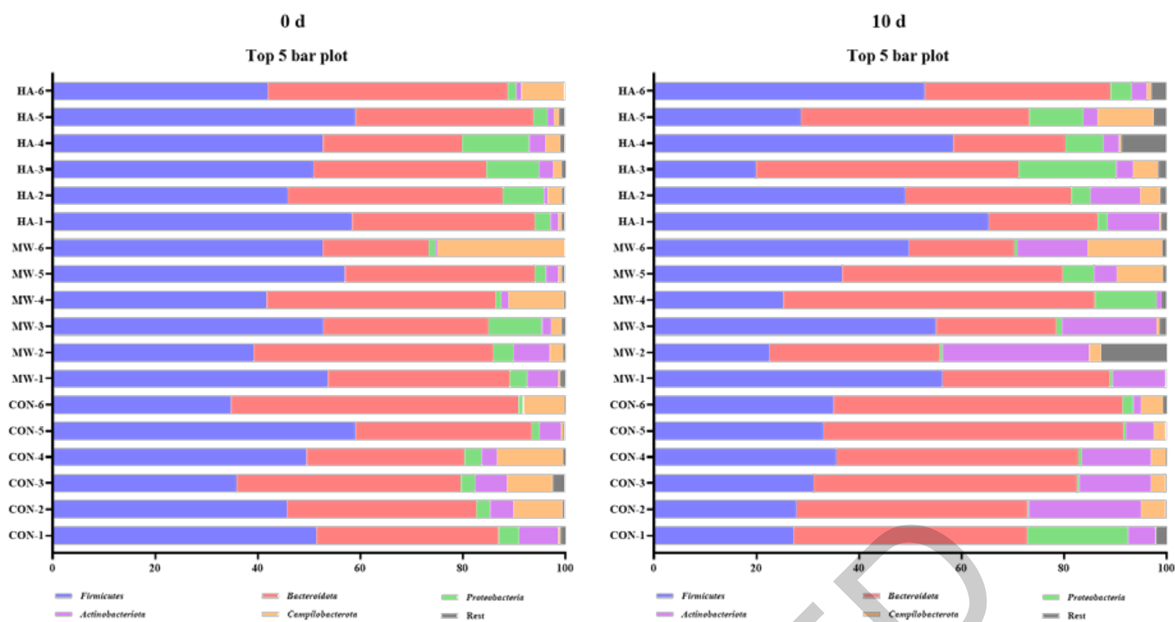

677 **Figure 3. 16S rRNA gene analysis revealed the relative abundance of fecal microbiome at the phylum**
678 **level in replacing PM with differently processed HI in cats at day 0 and day 10.** CON, basal diet; MW,
679 basal diet with 3% PM replaced by microwave drying HI; HA, basal diet with 3% PM replaced by hot-air drying
680 HI. PM, poultry meal; HI, *Hermitia illucens* larvae.

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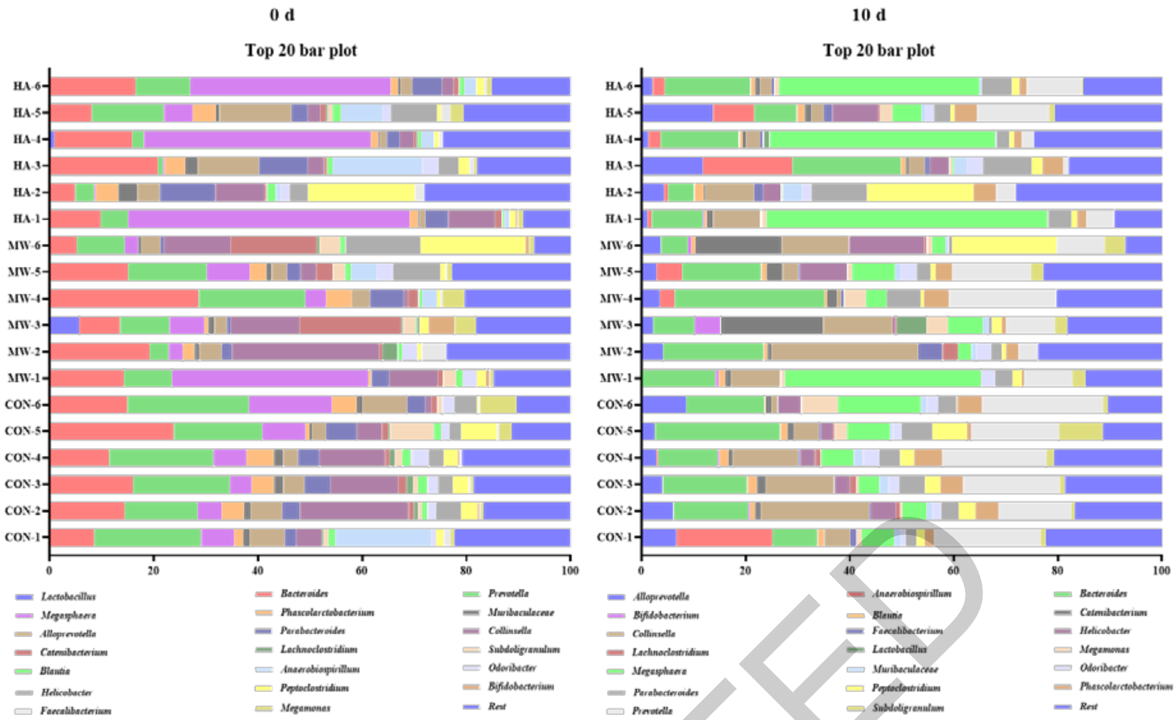

683

684 **Figure 3. 16S rRNA gene analysis revealed the relative abundance of fecal microbiome at the genus level**
685 **in replacing PM with differently processed HI in cats at day 0 and day 10.** CON, basal diet; MW, basal diet
686 with 3% PM replaced by microwave drying HI; HA, basal diet with 3% PM replaced by hot-air drying HI. PM,
687 poultry meal; HI, *Hermitia illucens* larvae.

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