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Sustainability Spotlight Statement

Large quantities of pre-consumer food waste, particularly spent coffee grounds, are generated globally as residues, posing environmental challenges and representing lost nutritional resources. Addressing this issue is important for reducing organic waste, creating value-added products, and potentially improving food security.

This study utilises mealworm larvae to convert both untreated and decaffeinated spent coffee grounds, transforming this waste into a sustainable, protein- and oil-rich source of edible insects suitable for human and animal consumption. Our results demonstrate that green solvent-based decaffeination pretreatment of spent coffee grounds enhances insect growth and optimises nutrient recovery, thereby promoting a circular food economy.

Accordingly, this work aligns with UN Sustainable Development Goal 2 (Zero Hunger) and Goal 12 (Responsible Consumption and Production).

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Pretreatment of Spent Coffee Grounds for Edible Insect Farming: Impacts on Mealworm (*Tenebrio molitor*) Development and Gut Microbiome

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Spent coffee grounds (SCGs) are among the most widely produced food and beverage residues. Due to their nutritional value, negligible cost, and considerable availability as pre-consumer food waste that does not compete with human food, using SCGs as feed for edible insects is highly attractive for developing a circular food economy. In this study, we investigated both the fundamental and practical aspects of using untreated and decaffeinated SCGs as feed for rearing mealworms (*Tenebrio molitor*), one of the most established edible insect species. Our results showed that incorporating a decaffeination step using green solvent ethanol as the extractant had a positive impact on mealworm larvae, significantly enhancing their body mass and pupation rates. Additionally, the protein content of mealworm larvae fed a wheat bran/SCG blend were higher compared to those reared on an unblended wheat bran diet. Gut microbiome analysis revealed that larvae fed untreated SCGs exhibited a microbial community structure similar to that of the starvation group, whereas decaffeinated SCGs resulted in a distinctly different community. The beneficial bacterial genus *Spiroplasma*, which degrades plant biopolymers in SCGs via secretion of carbohydrate-degrading and proteolytic enzymes, was significantly inhibited by phytochemicals such as caffeine present in untreated SCGs. In contrast, *Enterococcus* displayed strong tolerance to these phytochemicals and was also abundant in the starvation group. These findings provide a scientific basis for phytochemical-directed pretreatment of food waste in insect-mediated upcycling and support the application of decaffeinated SCGs in industrial-scale edible insect production.

Introduction

The rapid growth of the global population, together with the substantial environmental burdens associated with conventional livestock production, has intensified efforts to identify sustainable and resource-efficient protein sources. Edible insects have emerged as a promising alternative due to their high protein content, favourable essential amino acid profiles, and significantly lower land, water, and greenhouse gas requirements compared with traditional animal agriculture.[1-3] Among commercially relevant species, the yellow mealworm (*Tenebrio molitor*) has received particular attention following its authorization as a "Novel Food" for human consumption in frozen, dried, and powdered forms within the European Union.[4] In addition to their nutritional value, mealworms can function as biological agents for organic waste valorisation by converting low-value residues into high-quality biomass, thereby supporting circular bioeconomy models.

Spent coffee grounds (SCGs) are among the most abundant food-processing residues worldwide and represent a growing challenge for sustainable waste management.[5] Coffee is one of the most traded commodities globally, ranking second only to petroleum, and its processing generates substantial quantities of residual biomass.[6] Approximately six million tonnes of wet SCGs are produced annually, with instant coffee production alone generating about two kilograms of SCGs per kilogram of product.[7] Conventional disposal pathways, particularly landfilling, pose environmental risks due to the slow degradation of SCGs and the release of bioactive compounds such as caffeine and tannins into surrounding ecosystems.[8-10] As a result, the development of sustainable strategies for SCG valorisation has become a priority within circular bioeconomy frameworks.

The use of SCGs as substrates for rearing edible insects offers a dual benefit by reducing organic waste streams while producing protein-rich insect biomass. Several insect species, including *Hermetia illucens*, *Zophobas morio*, and *Tenebrio molitor*, have been evaluated for their capacity to bio-convert food and agricultural residues.[11-13] However, not all insects are equally suited to SCG-based diets. Black soldier fly larvae exhibit limited capacity to degrade lignocellulosic materials and often require prolonged developmental periods on recalcitrant substrates,[14] while superworms show relatively low conversion efficiency despite broad feeding capabilities.[15] In contrast, *Tenebrio molitor* demonstrates strong adaptability to diverse substrates and has therefore emerged as a promising candidate for SCG-based bioconversion systems.[16,17] From a process and nutritional perspective, SCGs contain appreciable levels of protein and dietary fibre contents,[18] which are key nutritional components also found in wheat bran,[19] can support mealworm growth, indicating their potential to partially substitute conventional substrates such as wheat bran in edible insect production systems. Their utilisation

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contributes to sustainable waste valorisation by converting nutrient-rich residues into insect biomass. However, SCGs also contain phytochemicals such as caffeine and tannins, bioactive compounds whose influence on insect physiology, gut microbiota, and development remains insufficiently characterised, representing a key bottleneck in SCG-based bioconversion processes.

Caffeine is known to direct physiological effects, it exhibits antimicrobial activity that may kill gut microbial community structure and function, potentially impairing nutrient assimilation and digestion.[20] Additionally, insects often have very alkaline midguts, which make tannins oxidise into semiquinone radicals, quinones, and other reactive oxygen species, and damage midgut tissues and cause lesions and necrosis of epithelial cells.[21] Given the central role of gut microbiota in insect digestion and adaptation to complex substrates, such disruptions may directly constrain bioconversion efficiency.[22] Despite increasing interest in insect-based waste valorisation, limited attention has been paid to the role of bioactive phytochemicals inherent in waste substrates and their interactions with insect gut microbiota during bioconversion. In particular, the extent to which phytochemicals mediate changes in gut microbial composition and thereby influences mealworm growth and substrate conversion remains poorly understood. Addressing this knowledge gap is essential for optimising SCG-based feeding strategies and improving the reliability of insect-driven circular bioeconomy systems.

In this study, we investigated the feasibility of using untreated and ethanol-decaffeinated SCGs as feed substrates for *Tenebrio molitor*. We hypothesised that certain dietary phytochemicals impose antimicrobial stress on the gut microbiota, leading to reduced digestive efficiency and impaired larval development, whereas decaffeination using ethanol mitigates these inhibitory effects. To test this hypothesis, we evaluated larval mass gain, survival, and pupation rates under diets containing varying proportions of SCGs. We further characterised gut microbial communities using high-throughput sequencing and analysed the chemical composition of mealworm biomass under SCG-based, wheat bran, and starvation diets. Our results indicate that ethanol decaffeination markedly enhances the efficiency of SCG bioconversion into mealworm biomass. By integrating insect performance metrics, gut microbiome dynamics, and substrate transformation outcomes, this study provides mechanistic insight into the role of phytochemicals in insect-mediated SCG valorisation and highlights decaffeination as a critical step for enabling effective and sustainable waste-to-protein conversion.

Experimental

Materials and Methods

Spent Coffee Grounds and Feed Preparation. SCGs were obtained from a Hong Kong coffee shop, Bistro NTT by Attic and dried in a forced-air oven at 60 °C for 48 hours to remove residual moisture. For the decaffeination treatment (DeCA), caffeine was extracted from dried SCGs using a Soxhlet apparatus with absolute ethanol as the solvent, following AOAC guidelines.[23] Extractions were conducted in batches for 16 h, after which the decaffeinated SCGs were oven-dried to remove residual ethanol. Untreated dried SCGs were used as the control. To assess the effects of caffeine and other soluble phytochemicals on mealworm-based bioconversion, both caffeinated and decaffeinated SCGs were incorporated into wheat bran–based diets at two inclusion levels (10% and 25%, w/w). The resulting diets were designated as CA10%, CA25%, DeCA10%, and DeCA25%. A wheat bran–only diet (WB) served as the control, and a starvation group was included as a blank reference.

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Decaffeination Extract Characterisation. The decaffeination extract was concentrated *in vacuo*. The dried residue was extracted with hexane for separating the bulk of lipids and other components, then both fractions were dried at high vacuum at 30°C for 24 hours to ensure complete evaporation of solvents. Samples were then dissolved in DMSO-d6 for NMR analysis. 1H NMR spectra were recorded in a Bruker AVANCE NEO NMR 600MHz with BBFO and TXI probe at 25°C using zg30 in the standard library of Bruker NMR pulse sequences. The number of scans was 256, the recycle delay was 1 seconds, and the Fourier transformation was performed after using the exponential window function with line broadening factor equal to 0.3 Hz, followed by manual phase correction, and polynomial (9th order) baseline correction using the MestReNova software version 14.0.0. DMSO-d5 residual peak at 2.5ppm was used as reference for all other peaks. These were carried out in part in the Advanced Life Sciences and Mass Spectrometry Laboratory (LSMS) of Hong Kong Baptist University. The hexane insoluble fraction was compared to known spectrums of roasted coffee extracts[24,25] to identify the phytochemicals extracted, and was also compared to known literature spectrums of individual compounds (Fig. S1 in Supplementary Information). The hexane soluble fraction was confirmed to be mainly composed of lipids (Fig. S2 in Supplementary Information) by comparison with similar extracts.[26] Caffeine was quantified by HPLC, with an Agilent 1100 HPLC system, coupled with a binary pump, autosampler, and a diode array detector (DAD), using a Waters SymmetryR C18 3.9 x 150 mm column. The mobile phase used was a mixture of 6:4 v/v ACN:H_2O with isocratic elution. A calibration curve was set up using known quantities of standard and the DAD was set to measure the frequency of 273 nm[27] (Fig. S3 in Supplementary Information). A test portion of 1g of SCG, and a test portion of 5 g of decaffeinated SCG were each stirred in 40ml of

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mobile phase at 60 ℃ for 90 minutes, then centrifuged and the supernatant was collected and topped up to 100ml in a volumetric flask for HPLC.[28]

Mealworm Rearing and Feeding Experiments. *Tenebrio molitor* larvae were sourced from a commercial mealworm supplier in Foshan, Guangdong, China, and acclimated under controlled laboratory conditions (continuous darkness, 24.0 ± 1 °C, and 56 ± 5% relative humidity). Prior to experimentation, the larvae were fed wheat bran for 72 h, followed by a 48 h starvation period to minimise the influence of prior diet.[29] Feeding experiments were conducted using 50 larvae per replicate housed in rectangular aluminium containers (100 × 80 × 46 mm), with four biological replicates per treatment. Container openings were covered with cotton mesh to allow ventilation. The ratio between mealworms and feedstocks followed the study by Kotsou et al.;[16] each container initially received 8 g of the assigned diet. Substrate levels were inspected daily; when the substrate was nearly depleted, 2 g of the same diet was added, following the same procedure.[16] This practice was only applied to DeCA groups, with supplementary feeding conducted on days 21 and 25. The larvae were reared under constant environmental conditions throughout the experiment. Larval survival, mortality, and pupation were recorded weekly. Surviving larvae were separated from the substrate by sieving, counted, and weighed collectively to determine total biomass. Residual substrate and frass were also collected and weighed. For following biochemical analyses, a larger scale batch experiment was reared in stainless steel trays (27 × 20 × 4.8 cm), each containing 2000 larvae and 320 g of the assigned substrate. After 35 days, larvae were processed, fasted for 48 h, euthanised by freezing at −20 °C, oven-dried for 24 h, ground into powder, and stored in sealed plastic bags at room temperature.

Proximate Composition Analysis. Mealworm body samples were analysed for dry matter, crude fat, crude protein, crude fibre, and crude ash. Dry matter, crude fat, crude protein, and crude fibre were determined according to the AOAC Methods of Analysis, with some modifications.[23] To determine dry matter using method 930.15, a sample was dried at 60 °C for 24 hours, maintaining the compositional integrity while removing more than 99% of moisture.[30] Crude fat was determined according to method 920.39, using anhydrous diethyl ether. Crude protein was determined by Beijing Zhong Ke Bai Ce Technology Co., Ltd. via the Kjeldahl method to measure the total nitrogen content, which was then converted to crude protein using an experimentally obtained conversion factor of 4.76 for *Tenebrio molitor* larvae, following Janssen et al.[31] Other studies cited also utilise this conversion factor for mealworms, and utilise 6.25 for SCG and wheat bran.[32,33] Crude fibre was determined according to AOAC Official Method 978.10, with acidic and then alkali washes and then ashing. Crude ash was determined according to ISO 936:1998.

Gut Microbiome Sequencing and Bioinformatic Analysis. Gut microbiome composition was analysed using 16S rRNA gene sequencing following established protocols.[34] Representative larvae (n = 7 per replicate) were randomly selected from each treatment group and surface-sterilised with 75% ethanol for 1 min, followed by rinsing in sterile phosphate-buffered saline (PBS, pH 7.4). Entire gut tracts were aseptically excised under a stereomicroscope in a laminar flow hood, pooled by replicate, and stored at −80 °C until analysis. We initially set more than four biological replicates for each group. After repeated sample processing and library preparation, only a smaller number of CA group samples satisfied the quality requirements for 16S rRNA sequencing. Microbiome sequencing was conducted for the following treatments: CA10% (n = 2), CA25% (n = 2), DeCA10% (n = 3), DeCA25% (n = 2), WB (n = 3), and starvation (n = 4). DNA extraction, PCR amplification, and sequencing were performed by Novogene Co., Ltd. (Beijing, China). Genomic DNA was extracted using the TIANamp Stool DNA Kit (TIANGEN BIOTECH, China), and the V3–V4 region of the bacterial 16S rRNA gene was amplified using primers 341F and 806R.[35] Amplicons were sequenced on an Illumina NovaSeq 6000 platform. Sequencing data was processed using QIIME2 (version 2023.2). Amplicon sequence variants (ASVs) were generated using the DADA2 pipeline, and taxonomic assignment was performed using the classify-sklearn algorithm against the SILVA 138.1 reference database. Paired-end reads were merged and quality filtered using FLASH (version 1.2.11).

Statistical Analysis. All statistical analyses were performed using R (version 4.5.0). Data preprocessing involved importing Excel datasets using the readxl package and cleaning with dplyr functions[36] including filter () and mutate(). For each experimental metric, summary statistics such as mean, standard deviation, and standard error were calculated using group_by () and summarise functions from the dplyr package. Statistical assumptions were systematically evaluated: normality was assessed with the shapiro_test () function from the rstatix package, and homogeneity of variances was tested with the bartlett.test () function from base R. Based on the fulfillment of these assumptions, either a parametric approach using ANOVA with the aov () function followed by Tukey's HSD post-hoc test, or a non-parametric approach using the Kruskal-Wallis test with the kruskal.test () function and Dunn's test via the dunn_test () function from the rstatix package was applied.
Visualisation was implemented using the ggplot2 package. For endpoint comparisons including average individual mass gain, pupation rate, cannibalism rate, and mortality rate, boxplots were generated using geom_boxplot() to display

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data distribution across six treatment groups. Individual replicate values were overlaid using geom_jitter() with horizontal jitter only to preserve exact measurements. ANOVA was applied to test overall group differences, followed by Tukey's honest significant difference (HSD) post-hoc test using the multcomp package. Alpha diversity was calculated by using a rarefied phyloseq object (10,000 reads per sample). Beta diversity was evaluated using Bray-Curtis dissimilarity and visualised via PCoA, with group differences tested using PERMANOVA (999 permutations). Taxa showing differential abundance were identified using DESeq2. The colours and shapes in the plot, derived from a palette provided by the RColorBrewer package, differentiated between treatments and types, offering a clear comparison of variability.

Results and Discussion

Mealworm Growth Performance

The HPLC quantification revealed the untreated SCG had a caffeine concentration of 3.147mg/g, which matches literature values for commercial shops percolated SCGs.[37] The decaffeinated SCG had a concentration of 0.142 mg/g, revealing the decaffeination procedure removed approximately 96% of caffeine in the SCGs.

The growth performance of mealworm larvae was markedly influenced by the presence of caffeine in dietary spent coffee grounds (SCGs) over the 5-week feeding trial (Fig. 1a). Larvae fed caffeinated SCGs (CA10% and CA25%) showed significant growth inhibition or net mass loss: the CA10% group gained only 10.33 mg per larva, while the CA25% group lost 2.39 mg per individual (Fig. S4 in Supplementary Information; ANOVA, $p < 0.05$). Starved larvae exhibited the greatest mass loss, at −8.78 mg. In contrast, larvae fed decaffeinated SCGs (DeCA10%, DeCA25%) or wheat bran alone (WB) showed substantial weight gains, averaging 28.78 mg, 21.01 mg, and 32.63 mg, respectively (Fig. S4 in Supplementary Information). Weekly growth trajectories further highlighted differences among treatment groups. Each group contained 50 larvae. Larvae fed the wheat bran (WB) diet showed steady and consistent weekly weight gain. In comparison, larvae fed the 10% decaffeinated coffee grounds diet (DeCA10%) exhibited slightly lower but comparable weekly weight gain, at 5.76 mg per larva per week (11.18% of initial body mass). Larvae fed the 25% decaffeinated coffee grounds diet (DeCA25%) showed noticeably lower growth than the DeCA10% group, although still achieved a total weight gain of 21.01 mg per larva over the five-week period.

Our findings are consistent with previous studies reporting that phytochemicals such as caffeine negatively affects insect growth: as previously demonstrated, caffeine exerts anorexigenic effects[38] and disrupts metabolic[49], thereby suppressing feeding, nutrient assimilation, and energy storage in insects. In addition to caffeine, several phytochemicals present in SCGs, including tannins and trigonelline derivatives (Fig. S1 in Supplementary Information), may also contribute to the impaired growth of mealworm larvae: tannins act as antinutritive compounds that reduce protein digestion and utilisation, thereby inhibiting larval growth,[40,41] while trigonelline derivatives, another group of alkaloids detected in SCGs, may act synergistically with caffeine to exert oxidative and antimicrobial effects, serving as a potential chemical anti-insect substance in coffee[42] although their specific functions in mealworms remain unclear. Moreover, caffeine and these phytochemicals may disrupt synthesis or release of brain hormone, potently inhibiting the development of *Hyalophora cecropia*,[43] and as plant secondary metabolites often have paralytic and toxic effects on insects by inhibiting phosphodiesterase activity and increasing the intracellular cyclic AMP level,[44] caffeine, tannins, and trigonelline derivatives may together produce additive or synergistic inhibitory effects, leading to poor growth in mealworm larvae.

Pupation Performance

Regarding the pupation response of mealworms, significant differences were observed among different dietary treatments in the present five-week feeding trial (Fig. 1b). Larvae fed diets containing untreated coffee grounds, which contained natural phytochemicals, showed strongly inhibited pupation. The pupation rates of CA10% and CA25% were 4.5 ± 1.2% and 2.0 ± 1.6%, respectively, and these values were similar to that of the starvation group (5.5 ± 1.9%) (Fig. S5 in Supplementary Information). By comparison, larvae fed the wheat bran control diet (WB) achieved the highest pupation rate of 28.5 ± 4.1%, while those fed decaffeinated coffee grounds showed intermediate values (DeCA10%: 20.5 ± 5.5%; DeCA25%: 15.5 ± 11.4%). These results indicated that untreated phytochemicals in coffee grounds markedly reduced the pupation rate of mealworm larvae, to a level comparable to starvation.

Caffeine is a methylxanthine alkaloid that exerts dose-dependent physiological effects on insects, including growth inhibition and developmental disruption.[45] Increasing dietary concentrations of coffee grounds also cause reduced insect growth and pupation,[46] which supports the present findings. Caffeine has been widely reported to delay pupation time and decrease pupation rate in insects.[47] In this study, the gradual decrease in pupation with increasing caffeine concentration further confirmed that caffeine negatively affected larval development, probably by disturbing normal physiological processes and nutrient utilisation. Besides caffeine, tannins may also contribute to impaired metamorphosis. Successful pupation depends on the precise regulation of ecdysteroids and juvenile hormones.[48] Tannins can disrupt these endocrine pathways in insects.[49] Since the treated groups showed much higher pupation rates than the untreated coffee ground groups, tannins in SCGs may act synergistically with caffeine to

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inhibit pupation. However, direct evidence for the effects of tannins on mealworm pupation is still insufficient. The observed synergistic inhibitory effects are consistent with the mixture synergy framework proposed by Mithöfer & Boland.[44] Analysis of survival data showed that CA25% and DeCA25% had relatively higher mortality values, and the average mortality was similar across CA25%, DeCA10% and the starvation group. ANOVA verified no significant difference in mean mortality among all treatments ($p > 0.05$; Fig. S7) This indicates that phytochemicals from SCGs have little influence on larval survival. The adverse effects of these compounds are mainly manifested as restricted body weight gain and blocked development, rather than lethal toxicity.

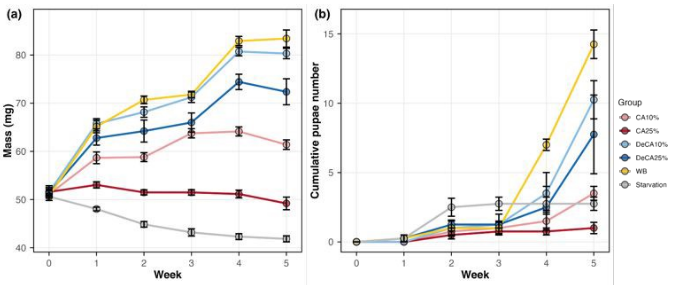

Fig. 1. Analysis of mealworm growth under different diets (n = 4). (a) Mass of individual mealworm along the time; (b) Cumulative pupae number along the time of mealworm. Abbreviations: CA10% = 10%CA + 90%WB, CA25% = 25%CA + 75%WB, DeCA10% = 10%DeCA + 90%WB, DeCA25% = 25%DeCA + 75%WB, WB = 100%WB.

Mealworm Proximate Compositions

The proximate composition of the mealworms in conjunction with other parameters such as pupation and cannibalism provide insight into their development and health. It was observed that starvation led to cannibalism, weight loss, and inhibited pupation (Table 1 and Fig. S5 in Supplementary Information). This is reflected in their composition, which showed a markedly lowered dry mass, and crude fat which could be the result of conversion to energy to maintain metabolic functions, which in turn would cause a higher protein, fibre and ash contents relative to dry mass. Other studies such as Kopecká et al.'s work[33] on temperature influence in mealworm growth and Andreadis et al.'s[50] found similar compositions of wheat bran control groups, with slight variations attributed to feed source or other environmental conditions. Both treated groups DeCA10% and DeCA25% showed similar composition, albeit slightly lower in fat than the WB control group, corroborating the other findings indicating they did not suffer significant development impairments. Remarkably, the larvae of these two groups exhibit noticeably higher protein content than WB group after biodegradation. Although they exhibited little or no weight gain, the untreated groups both showed slightly higher protein content than the other groups, but differed in fat content. The CA25% group showed lower fat content, which might be explained by a similar mechanism to the starvation group, where energy deficiency caused lipids to be used for metabolic energy, which is supported by their weight loss throughout the experiment. Wheat bran and SCGs exhibit similar nutritional profiles that support mealworm growth. Wheat bran contains crude protein in the range of 16.45–19.75%, crude fat of 3.31–5.41%, and crude ash of 3.31–5.41%.[32] In comparison, SCGs contain crude protein ranging from 13.87–16.12%, crude fat from 8.58–18.55%, and crude ash from 2.16–3.61%.[33] Despite SCGs containing higher fat content, the pretreatment performed in this work removes ethanol-soluble lipids, making it closer to wheat bran in fat content. Therefore, feed crude nutritional composition was not the main factor in the observed differences between groups.

Table 1. Proximate composition of mealworm body mass for different groups. Values expressed as g/kg of dry matter. The bottom two rows contain data from the literature.

Group	Dry Mass	Crude Fat	Crude Protein	Crude Fiber	Crude Ash
CA10%	433.02	283.26	427.62	63.57	37.47
CA25%	412.75	206.77	415.78	74.55	49.57
DeCA10%	426.94	286.82	384.07	62.02	34.76
DeCA25%	434.58	275.66	389.29	76.19	27.58
Starvation	360.68	79.38	494.59	113.13	67.02
WB	430.86	311.36	376.07	81.55	34.31
Kopecká et al.[33]	*323.5*	*273.2*	*433.4*	*N/A*	*28.5*
Andreadis et al.[50]	*393*	*252*	*390*	*N/A*	*55*

Gut Microbiome Compositions

A total of 1,368,982 high-quality sequences were obtained from 16S rRNA gene amplicon sequencing across all six dietary groups (CA10%, CA25%, DeCA10%, DeCA25%, WB, and starvation), with 100% sample coverage (Table 2). ASV-based analysis (a proxy for microbial species richness) revealed that groups fed caffeine- and phytochemical-containing diets (CA10%, CA25%) harboured markedly fewer ASVs (both 30) than decaffeinated (DeCA10%: 44; DeCA25%: 211) and wheat bran (WB: 144) controls, indicating a significant reduction in gut microbial richness in response to dietary phytochemicals. Previous research has verified that

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caffeine, a key phytochemical in coffee, exhibits broad-spectrum microbial toxicity and can inhibit microbial physiological activity.[20] However, Shannon and Simpson diversity indices showed a contrasting trend (Table S1). DeCA10% had lower Shannon (0.33) and Simpson (0.12) values compared with the CA groups. The pattern is driven by the extreme dominance of *Spiroplasma* and resultant differences in overall community evenness between groups (Fig. 2).

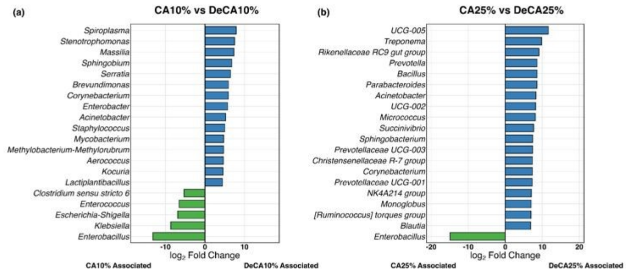

Fig. 2. Differential abundance analysis of gut microbial ASVs between experimental diets. Only the 20 ASVs with the highest relative abundance are shown. The log2 fold change indicates the diet with which each ASV is more strongly associated (labelled on the x-axis). (a) Microbiome of CA10%-fed mealworms versus DeCA10%-fed mealworms. (b) Microbiome of CA25%-fed mealworms versus DeCA25%-fed mealworms.

PCoA also supported the same pattern observed in our overall microbiome analysis (Fig. 3). The first two principal components accounted for 58.37% and 19.5% of the total variation, respectively, indicating reliable discrimination among groups. The gut microbial community of larvae in the starvation group clustered closely with those fed untreated phytochemical-containing SCGs. PERMANOVA further confirmed a significant difference among groups (p = 0.001). These findings suggest that phytochemicals in untreated coffee grounds place larvae in a starvation-like physiological state, which represents a new observation from this study. In addition, the microbial communities of larvae fed wheat bran differed distinctly from those fed treated coffee grounds with reduced phytochemicals. Although DeCA10% exhibited relatively low Shannon and Simpson diversity values, alpha diversity metrics should not be directly equated with microbiome functionality. Differential abundance analysis identified *Corynebacterium* as a taxon shared by both decaffeinated treatment groups (DeCA10% and DeCA25%) but absent from the untreated SCG groups, suggesting a consistent community shift associated with decaffeination (Fig. 2). In addition, spent coffee grounds remain a fibre-rich and lignocellulosic substrate after decaffeination,[51] and previous studies have shown that lignocellulose-rich diets can reshape mealworm gut microbiota and favour a narrower subset of adapted microorganisms.[52] Therefore, the lower alpha diversity observed in DeCA10% may reflect substrate-driven community specialisation rather than reduced microbiome functionality. Further studies integrating metagenomics, microbial isolation, and functional assays will be required to determine the ecological significance of *Corynebacterium* and the mechanisms underlying these community shifts.

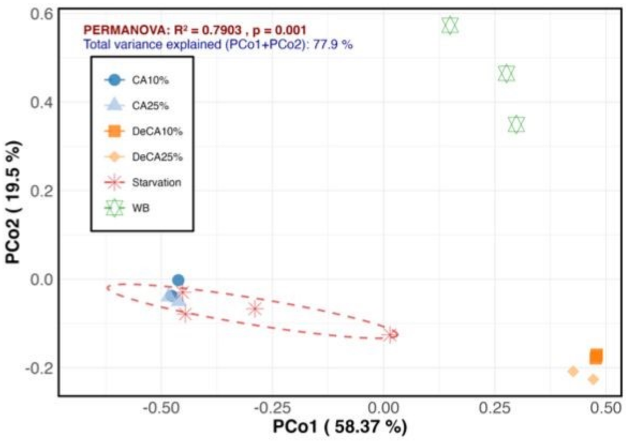

Fig. 3. Principal Coordinates Analysis (PCoA) of gut microbial diversity in mealworms with different diets based on Bray-Curtis dissimilarity.

Relative abundance analysis revealed significant differences in the dominant microbial phylum and family between the CA and DeCA feed groups (Fig. 4). In the CA10% and CA25% diet treatments, the dominant phylum in mealworm larval gut was Proteobacteria, which was consistent with the starvation treatment group, while the DeCA10% and DeCA25% diet groups were dominated by phylum, Firmicutes, showing the same pattern as the WB control group. Notably, a consistent microbial variation trend was detected across all treatment and

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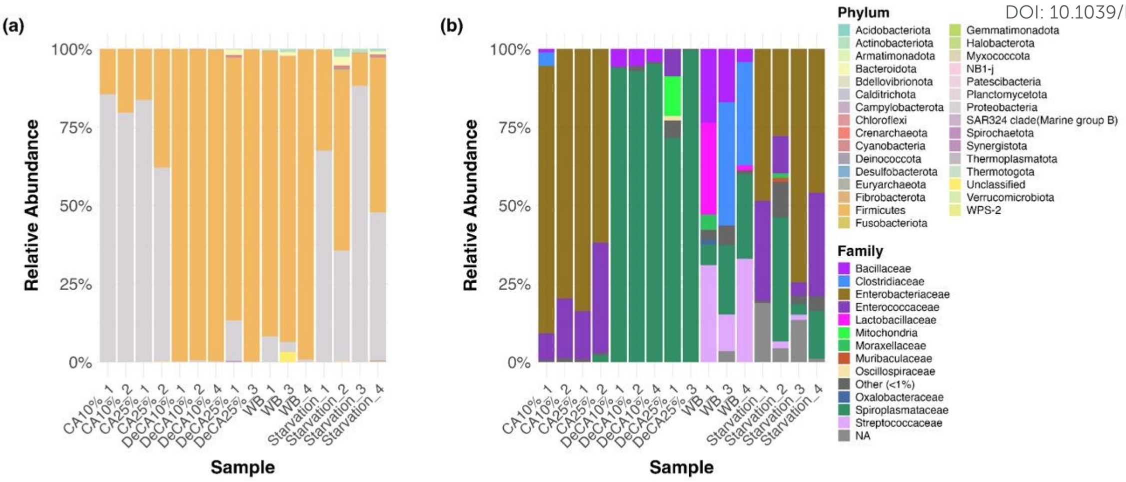

Fig. 4. The relative abundance of intestinal microbiota in different treatment groups and control groups of mealworms. (a) The relative abundance by phylum. (b) The relative abundance by family.

control groups, with stable and uniform community differences at both phylum and family levels. Recent studies have demonstrated that Firmicutes participate in dietary fibre fermentation and promote host nutrient absorption,[53] and Proteobacteria is closely associated with insect gut detoxification functions,[54-56] which fully supports the present findings that gut microbiota shifts in response to different dietary treatments, and the regulatory effect of diets on mealworm gut microbial community presents a consistent and regular pattern among all groups.

Further analysis at the genus level showed significant gut microbial differences among untreated phytochemical-containing CA groups, decaffeinated DeCA groups, WB control group and starvation group, with *Spiroplasma* and Enterobacteriaceae being the core differential genera (Fig. 5). Differential abundance analysis and log2 fold change analysis clearly validated the distinct enrichment patterns of these two taxa: *Spiroplasma* was significantly enriched in DeCA groups, while Enterobacteriaceae dominated in CA and starvation groups, with obvious log2 fold change differences between these groups (Fig. 2). *Spiroplasma* was the dominant genus in DeCA10% and DeCA25% groups, with relative abundances of 94.06% and 85.61% respectively, and also accounted for 18.58% in the WB control group, while it was nearly absent in CA and starvation groups. As a key beneficial symbiont in mealworm gut, *Spiroplasma* possesses proteolytic capacities related to protein digestion.[57-59] Based on the observed results, it can be hypothesised that *Spiroplasma* also contributes to the degradation of the carbohydrate fraction of the feed, and that this involvement is closely associated with normal larval growth and development. Conversely, Enterobacteriaceae (dominated by *Enterobacillus*) was the predominant taxon in CA10%, CA25% and starvation groups, with *Enterobacillus* relative abundances of 82.44%, 72.77% and 49.02% respectively. This family, Enterobacteriaceae, is associated with tolerance to phytochemicals like caffeine and tannins.[60-63] Specifically, regarding tannins, *Enterobacter* cloacae[61] and *Enterobacter* ludwigii[63] produce tannase that hydrolyses tannic acid to gallic acid and glucose, thereby mitigating the anti-nutritional effects of tannins on the host. For caffeine, while direct degradation by Enterobacteriaceae has not been extensively documented, their tolerance to caffeine is supported by the presence of caffeine-tolerant isolates such as *Enterobacter*-like bacteria from coffee berry borer guts.[60] Meanwhile, *Enterococcus* in CA and starvation groups has both cellulase and protease activities to degrade cellulose and proteins, and tolerates plant toxins;[64-67] specifically, *Enterococcus* faecalis produces tannase that hydrolyses tannic acid to monomeric compounds,[68] and exhibits tannin tolerance up to 4% (w/v).[69] Additionally, *Enterococcus* hirae has demonstrated tannin tolerance up to 5 g/L.[70] A small amount of *Bacillus* with cellulolytic ability was found in DeCA10% group.[71] All these microbial differences are derived from dietary phytochemical stress, shaping the functional traits of mealworm gut microbiota. A limitation of this study is a small number of biological replicates (n = 2) for the CA10% and CA25% groups, which may reduce statistical power. Yet PERMANOVA and DESdq2 can be applied to datasets with 2–3 replicates in microbiome research.[72-74] The replicates within each group yielded consistent results.

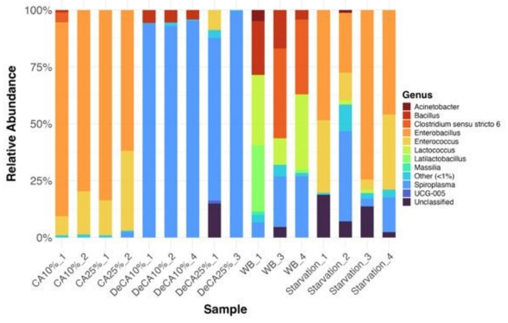

Fig. 5. The relative abundance of intestinal microbiota in different treatment groups and control groups of mealworms by genus level.

Conclusions

Upcycling food industry byproducts and kitchen waste by using them to rear edible insects is a practical strategy for developing a circular food economy. As one of the largest sources of pre-consumer food processing residue, SCGs are being explored as a key feed ingredient for rearing these novel protein sources. In our study, mealworms were used as model organisms to investigate the feasibility of utilising untreated and ethanol-decaffeinated SCGs as feed ingredients. Our results revealed that untreated SCGs, which contain anti-nutritional phytochemicals, induced detrimental effects on mealworms, including larval mass loss, inhibited pupation, and severe gut microbial dysregulation. Specifically, untreated and decaffeinated SCGs were associated with distinct gut microbial profiles. Differential abundance analysis identified *Corynebacterium* as a taxon shared by both decaffeinated treatment groups, whereas it was not enriched in the CA groups, suggesting that pretreatment may reshape the mealworm gut microbiome in a treatment-dependent manner.

In contrast, green ethanol decaffeination effectively mitigated these adverse impacts, enhancing the suitability of SCGs as feed and promoting normal larval development and nutrient accumulation. Larvae fed with decaffeinated SCG-blended diets showed enhanced body mass, as well as favourable protein content in the insect biomass, confirming the suitability of pretreated SCGs as a supplementary feed.

Overall, this study verifies the inhibitory effects of SCG phytochemicals, which reduce the viability of SCGs as insect feed, and highlights the importance of the solvent decaffeination pretreatment step, introducing a feasible method for SCG upcycling. Future studies can further explore the impacts of SCGs mixed with other organic waste on insect growth to boost the practical application of SCGs in the industrial rearing of edible insects.

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Author contributions

Ruoxin Du: Data Curation, Investigation, Methodology, Visualisation, Writing – Original Draft; Felipe Nunes Henriquez: Data Curation, Investigation, Methodology, Visualisation, Writing – Original Draft; Tiantian Zhang: Investigation, Writing – Original Draft; Xinbo Wang: Investigation; Chi Man Leong: Formal Analysis, Resources, Supervision, Writing – Review & Editing; Matthew Y. Lui: Conceptualisation, Formal Analysis, Resources, Supervision, Writing – Review & Editing

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the Supplementary Information.

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Data Availability Statement

The data supporting this article have been included as part of the Supplementary Information.