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Effect of Honey Supplementation on Growth Performance, Microbial Load, and Nutritional Composition of Black Soldier Fly (*Hermetia illucens*) Larvae

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Abstract: Black Soldier Fly larvae (BSFL) have gained global attention as a sustainable alternative protein source for animal feed because of their rapid growth, high feed conversion efficiency, and ability to valorize organic waste. Optimizing larval nutrition is essential for maximizing biomass yield, improving feed safety, and enhancing nutritional quality. This study evaluated the effects of graded supplementation with commercial, unprocessed natural honey (0%, 5%, 10%, and 15% *v*/*w*) on growth performance, microbial load, and proximate composition of BSFL over a 21-day rearing period. A completely randomized design with triplicate treatments was employed. Growth parameters assessed included survival rate, average larval weight, feed conversion ratio (FCR), and total biomass yield. Microbial analyses measured total plate count, Escherichia coli, and lactic acid bacteria populations, while proximate composition was determined using standard AOAC methods. Honey supplementation significantly improved larval survival, weight gain, and biomass production ($p < 0.05$), with the 10% inclusion level yielding the most favorable overall performance. Honey-treated groups exhibited reduced total microbial and *E. coli* loads alongside increased lactic acid bacteria counts, although the mechanisms underlying these microbial changes were not directly investigated. Nutritional analysis revealed enhanced crude protein and lipid contents in supplemented larvae, particularly at 10%, without significant effects on ash or moisture. These findings indicate that moderate inclusion of natural honey may function as a nutritional and microbial-modulating feed additive for BSFL. However, because conventional honey is relatively high-value, its commercial sustainability will depend on the availability and safety of lower-value, surplus, downgraded, or otherwise non-marketable honey streams.

Keywords: *Hermetia illucens*; honey supplementation; growth performance; microbial load; nutritional composition

1. Introduction

The rapid growth of the global population and the corresponding increase in demand for animal-derived protein have intensified pressure on conventional feed resources such as soybean meal and fishmeal. These traditional protein sources are associated with environmental concerns, including deforestation, overfishing, greenhouse gas emissions, and competition with human food systems [1,2]. Consequently, there is growing interest

in sustainable and circular alternatives that can efficiently convert organic waste into high-quality protein. Insects have emerged as one of the most promising solutions to this challenge because of their high feed conversion efficiency, rapid growth rates, and relatively low environmental footprint [3].

Among edible insects, black soldier fly larvae (BSFL) have attracted significant attention because of their exceptional bioconversion capacity and nutritional profile [4]. BSFL can convert a wide range of organic substrates, including agricultural residues, food waste, and manure, into protein- and lipid-rich biomass suitable for livestock, poultry, and aquaculture feeds [5,6]. Their nutritional composition, growth performance, and conversion efficiency are strongly influenced by substrate source and nutrient composition [7–9].

Despite these promising attributes, optimizing larval diet composition remains essential for maximizing productivity, ensuring microbial safety, and improving nutritional consistency. Dietary supplementation strategies may modify nutrient availability and larval nutritional composition, but responses depend on the characteristics of both the basal substrate and the supplement. Targeted supplementation can alter the nutritional profile of harvested larvae, as demonstrated by recent mineral biofortification work in BSFL [10].

Honey is a natural carbohydrate-rich product composed primarily of fructose and glucose, together with water, organic acids, enzymes, minerals, and diverse phytochemicals [11]. Beyond its nutritional value as a readily available energy source, honey possesses documented antimicrobial activity associated with high osmotic pressure, low pH, hydrogen peroxide generation, and phenolic compounds [12]. These characteristics make honey a plausible source of readily available energy and a potential microbial-modulating dietary ingredient in insect diets. However, the sustainability of honey as a feed additive should not be assumed.

From a circular-bioeconomy perspective, surplus, downgraded, off-specification, or otherwise non-marketable honey could represent a more economically relevant feed resource than premium edible honey, provided that such materials meet applicable safety and regulatory requirements. Nevertheless, limited research has examined natural honey supplementation in BSFL, particularly with respect to simultaneous effects on growth, microbial populations, and larval composition.

Understanding how honey supplementation influences BSFL growth, microbial ecology, and nutrient composition is therefore relevant to the development of functional feeding strategies. The present study was designed as a controlled proof-of-concept using commercial, unprocessed natural honey rather than downgraded or surplus honey. We evaluated graded honey supplementation (0%, 5%, 10%, and 15% v/w) for effects on growth performance, microbial load, and proximate nutritional composition of BSFL under controlled rearing conditions. We hypothesized that moderate honey inclusion would enhance larval growth, improve measured microbial profiles, and increase protein and lipid deposition, with optimal performance occurring at an intermediate supplementation level.

2. Materials and Methods

2.1. Study Location and Experimental Conditions

The experiment was conducted in a controlled insect-rearing facility maintained under standardized environmental conditions. Rearing conditions were set at 27 ± 1 °C, 65 ± 5% relative humidity, and a 12:12 h light–dark photoperiod, which are within the reported range for BSFL development and feed conversion efficiency [5,13]. Environmental parameters were monitored daily using digital thermohygrometers throughout the 21-day experimental period.

2.2. Source of Insects and Egg Collection

BSFL eggs were obtained from an established laboratory colony maintained on an organic rearing substrate. Adult flies were housed in screened cages (60 × 60 × 60 cm) and provided with oviposition substrates consisting of corrugated cardboard placed near decomposing organic matter, following standard rearing protocols [14]. Egg clutches were collected within 24 h of oviposition and incubated at 27 °C until hatching (approximately 3–4 days). Newly hatched first-instar larvae were used for the feeding trial. The colony-maintenance substrate was distinct from the experimental basal diet described below.

2.3. Experimental Diet Preparation

A basal diet was formulated from vegetable waste, consisting primarily of cabbage and carrot residues, and wheat bran at a ratio of 70:30 (*w*/*w*). The vegetable residues and wheat bran were thoroughly mixed and mechanically homogenized to obtain a relatively uniform distribution of substrate components and moisture. The final substrate moisture content was adjusted to approximately 65%. The basal diet was not subjected to proximate analysis before the experiment; therefore, experimentally determined protein and lipid values for the basal

substrate are not reported. This limitation is considered when interpreting larval proximate composition because substrate macronutrient composition can strongly influence BSFL growth and nutrient deposition [7–9].

Commercial, unprocessed natural honey was sourced locally and analyzed for moisture and sugar content, including fructose, glucose, sucrose, and total sugars, before inclusion. The honey was not formally authenticated to a specific botanical or monofloral source; therefore, no floral denomination is assigned. This classification is reported to avoid introducing an unsupported varietal designation and should be considered when reproducing the experiment. Honey was incorporated into the basal diet at four levels:

Control (C): 0% honey
H5: 5% honey (*v*/*w*)
H10: 10% honey (*v*/*w*)
H15: 15% honey (*v*/*w*)

Before incorporation, honey was diluted in a small volume of distilled water to facilitate uniform mixing. The diluted honey was then thoroughly blended into the basal substrate using a mechanical mixer until the mixture appeared homogeneous. The diets were prepared fresh every three days to minimize fermentation and nutrient degradation. The same preparation procedure was used for all honey-supplemented treatments.

2.4. Experimental Design and Larval Rearing

The study employed a completely randomized design (CRD) with four dietary treatments and three replicates per treatment. Each replicate consisted of 500 first-instar larvae placed in plastic rearing containers (30 × 20 × 10 cm) containing 1 kg of the assigned diet. Larvae were fed ad libitum, and additional substrate was provided as needed to prevent feed limitation. Containers were covered with breathable mesh to allow ventilation while preventing escape. Substrate moisture was maintained by periodic spraying with distilled water to prevent desiccation. Larval development was monitored daily, and mortality was recorded throughout the experimental period. After 21 days, larvae were harvested for growth performance evaluation, microbial analysis, and proximate composition determination.

2.5. Growth Performance Evaluation

Growth performance indicators were assessed as follows:
Survival Rate (%):

$$\text{Survival Rate} = \left(\frac{\text{Number of live larvae at harvest}}{\text{Initial number of larvae}}\right) \times 100\ \%$$

Average Larval Weight (mg): Fifty larvae were randomly sampled from each replicate weekly and weighed using an analytical balance (±0.001 g accuracy).

Biomass Yield (g): Total weight of harvested larvae per replicate.

Feed Conversion Ratio (FCR):

$$\text{FCR} = \frac{\text{Total feed consumed (dry weight)}}{\text{Total larval biomass gained (dry weight)}}$$

Feed conversion ratio (FCR) = Feed consumed / Larval biomass gained. Feed intake was determined by subtracting residual substrate dry weight from the initial dry weight provided.

2.6. Microbial Analysis

Microbial analyses were conducted on harvested larvae to evaluate total microbial load and specific bacterial populations. Ten grams of larvae from each replicate were surface-sterilized with 70% ethanol for 30 s, rinsed with sterile distilled water, and homogenized in 90 mL of sterile buffered peptone water.

Serial dilutions (10^{-1} to 10^{-6}) were prepared and plated on selective media:

Total Plate Count (TPC): Plate Count Agar (PCA), incubated at 37 °C for 24–48 h.

Escherichia coli: Eosin Methylene Blue (EMB) agar, incubated at 37 °C for 24 h.

Lactic Acid Bacteria (LAB): De Man, Rogosa and Sharpe (MRS) agar, incubated anaerobically at 30 °C for 48 h.

Microbial counts were expressed as colony-forming units per gram (CFU/g) of fresh weight. Standard microbiological procedures were followed according to APHA guidelines [15].

2.7. Proximate Composition Analysis

Harvested larvae were washed, oven-dried at 60 °C for 48 h to constant weight, and ground into fine powder for proximate analysis. Nutritional composition was determined using standard AOAC methods [16]:

Moisture Content: Oven drying at 105 °C until constant weight.
Crude Protein: Kjeldahl method (N × 6.25 conversion factor).
Crude Lipid: Soxhlet extraction using petroleum ether.
Ash Content: Muffle furnace incineration at 550 °C for 4 h.
All analyses were conducted in triplicate to ensure accuracy and reproducibility.

2.8. Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among treatment means. When significant differences were detected ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) post hoc test was applied for pairwise comparisons. Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Results were expressed as mean ± standard deviation (SD).

3. Results

3.1. Honey Composition

The commercial natural honey used as the dietary supplement was characterized before incorporation into the experimental substrates. The honey contained 17.2% moisture, 38.2% fructose, 31.3% glucose, 0.7% sucrose, and 79.7% total sugars. Fructose and glucose constituted the predominant sugars, whereas sucrose occurred at a comparatively low concentration.

3.2. Growth Performance

Honey supplementation significantly affected all evaluated growth parameters (survival rate: $p = 0.003$; final larval weight, FCR, and biomass yield: all $p < 0.001$; Table 1). Larval survival rate increased progressively from the control (78.4%) to the H10 treatment (85.7%), before slightly declining at H15 (84.3%). The increase in survival may reflect improved substrate acceptability or other nutritional effects of honey, although these mechanisms were not directly tested. Average larval weight showed a statistically significant increase with honey inclusion. At day 21, larvae in the H10 group recorded the highest mean weight (172.3 ± 5.8 mg), representing an 18.3% increase compared with the control (145.6 ± 4.7 mg). Although the H15 group demonstrated higher weights than the control, performance did not exceed that of the H10 group, indicating a potential optimal supplementation threshold. Feed conversion ratio (FCR) improved significantly in honey-supplemented treatments. The lowest (most efficient) FCR was observed in H10 (1.96 ± 0.07), compared with 2.29 ± 0.11 in the control. Biomass yield followed a similar trend, with H10 producing the highest total biomass (141.2 ± 5.4 g), representing a 25.5% increase over the control.

Table 1. Effect of Honey Supplementation on Growth Performance of *Hermetia illucens* Larvae (Mean ± SD).

Parameter	Control (0%)	H5 (5%)	H10 (10%)	H15 (15%)	*p*-Value
Survival Rate (%)	78.4 ± 3.2 [a]	82.8 ± 2.8 [b]	85.7 ± 3.5 [c]	84.3 ± 3.9 [c]	0.003
Final Larval Weight (mg)	145.6 ± 4.7 [a]	158.9 ± 6.1 [b]	172.3 ± 5.8 [c]	167.1 ± 4.3 [c]	<0.001
Feed Conversion Ratio (FCR)	2.29 ± 0.11 [a]	2.10 ± 0.09 [b]	1.96 ± 0.07 [c]	2.01 ± 0.10 [c]	<0.001
Biomass Yield (g)	112.5 ± 5.1 [a]	128.4 ± 4.8 [b]	141.2 ± 5.4 [c]	136.8 ± 6.0 [c]	<0.001

Values represent mean ± standard deviation (n = 3). Means within the same row bearing different superscript letters (a–c) differ significantly at $p < 0.05$ according to Tukey's HSD test.

3.3. Microbial Load

Honey supplementation significantly affected total plate count (TPC), *Escherichia coli,* and lactic acid bacteria (LAB) counts ($p < 0.001$, $p < 0.001$, and $p = 0.002$, respectively; Table 2). Total Plate Count (TPC) decreased from 5.8×10^6 CFU/g in the control to 2.5×10^6 CFU/g in the H10 group. *Escherichia coli* counts were reduced by approximately 73% in the H10 treatment compared to the control.

Conversely, lactic acid bacteria (LAB) populations increased significantly in honey-supplemented groups, with the highest LAB count recorded in H10 (2.2×10^5 CFU/g). This shift in microbial counts is consistent with a change in the microbial profile associated with honey supplementation. However, because the study did not characterize the gut microbiome or directly test microbial mechanisms, the observed pattern should not be interpreted as proof of selective prebiotic activity.

Table 2. Effect of Honey Supplementation on Microbial Load of *Hermetia illucens* Larvae (CFU/g, Mean ± SD).

Parameter	Control (0%)	H5 (5%)	H10 (10%)	H15 (15%)	*p*-Value
Total Plate Count ($\times 10^6$ CFU/g)	5.8 ± 0.4 [a]	3.4 ± 0.3 [b]	2.5 ± 0.2 [c]	2.9 ± 0.3 [b,c]	<0.001
E. coli ($\times 10^4$ CFU/g)	2.1 ± 0.3 [a]	0.78 ± 0.1 [b]	0.56 ± 0.1 [c]	0.81 ± 0.2 [b]	<0.001
Lactic Acid Bacteria ($\times 10^5$ CFU/g)	1.2 ± 0.2 [a]	1.7 ± 0.2 [b]	2.2 ± 0.3 [c]	2.0 ± 0.2 [b,c]	0.002

Values represent mean ± standard deviation (n = 3). Different superscript letters (a–c) within the same row indicate significant differences at $p < 0.05$ (Tukey's HSD).

3.4. Proximate Composition

Honey supplementation significantly increased crude protein and crude lipid contents (both $p < 0.001$), whereas ash ($p = 0.412$) and moisture ($p = 0.278$) were not significantly affected (Table 3). Crude protein increased from 40.8% (control) to 45.2% (H10), representing a 10.8% relative increase (Table 3). Lipid content similarly increased from 28.1% to 31.8% in H10.

These results indicate that moderate honey supplementation positively influences nutrient deposition in larval tissues.

Table 3. Effect of Honey Supplementation on Proximate Composition of *Hermetia illucens* Larvae (% Dry Matter, Mean ± SD).

Parameter	Control (0%)	H5 (5%)	H10 (10%)	H15 (15%)	*p*-Value
Crude Protein (%)	40.8 ± 1.2 [a]	42.7 ± 0.9 [b]	45.2 ± 1.1 [c]	43.9 ± 1.3 [b,c]	<0.001
Crude Lipid (%)	28.1 ± 1.0 [a]	29.6 ± 0.8 [b]	31.8 ± 1.0 [c]	30.9 ± 1.1 [b,c]	<0.001
Ash (%)	8.2 ± 0.4 [a]	8.0 ± 0.5 [a]	7.9 ± 0.3 [a]	8.1 ± 0.4 [a]	0.412
Moisture (%)	6.3 ± 0.3 [a]	6.1 ± 0.2 [a]	5.9 ± 0.4 [a]	6.0 ± 0.3 [a]	0.278

Values represent mean ± standard deviation (n = 3). Means with different superscript letters (a–c) within the same row differ significantly at $p < 0.05$.

4. Discussion

The present study demonstrates that honey supplementation, particularly at 10% inclusion, significantly enhanced growth performance, altered microbial counts, and increased crude protein and lipid concentrations in BSFL. These findings contribute to emerging research on dietary modulation of BSFL production. Importantly, the present experiment used commercial, unprocessed natural honey as a proof-of-concept material; therefore, the results should not be interpreted as evidence that conventional food-grade honey is economically optimal for industrial BSFL production.

4.1. Growth Performance Enhancement

The present study demonstrates that honey supplementation, particularly at 10% inclusion, significantly enhanced growth performance, altered microbial counts, and increased crude protein and lipid concentrations in BSFL. Importantly, the present experiment used commercial, unprocessed natural honey as a proof-of-concept material; therefore, the results should not be interpreted as evidence that conventional food-grade honey is economically optimal for industrial BSFL production.

The significant improvement in larval weight gain, survival rate, biomass yield, and FCR observed in honey-supplemented groups may be partly associated with the high concentration of readily available monosaccharides, particularly glucose and fructose, present in honey [11]. Carbohydrates serve as immediate energy sources that support metabolic processes, tissue synthesis, and overall larval development. When sufficient carbohydrates are available, dietary proteins may be spared from catabolism for energy and instead utilized for structural growth and enzymatic functions [17].

Previous research demonstrates that substrate nutrient composition strongly influences BSFL growth efficiency and biomass yield [5,7,8]. The improved FCR observed in the 10% honey group is therefore consistent with more efficient conversion of the supplied diet into larval biomass, although nutrient digestibility and metabolic fluxes were not directly measured in the present study. The absence of proximate analysis of the basal diet also limits the extent to which the observed responses can be attributed specifically to changes in dietary carbohydrate supply.

Interestingly, while 15% supplementation improved performance relative to the control, it did not surpass the 10% group. This suggests that excessive sugar inclusion may alter substrate physicochemical properties or

microbial fermentation dynamics. High concentrations of soluble sugars may reduce water availability through osmotic effects [12]. Therefore, the results suggest the existence of an optimal supplementation threshold beyond which benefits plateau or decline.

4.2. Microbial Modulation and Food Safety Implications

A major finding of this study was the significant reduction in total microbial counts and Escherichia coli populations in honey-treated groups, alongside increased LAB counts. Honey's antimicrobial activity is well documented and is attributed to multiple mechanisms, including high osmotic pressure, low pH, hydrogen peroxide generation, and phenolic compounds [11,12]. These antimicrobial properties may have contributed to the reduction in culturable microbial counts observed in the honey-supplemented treatments.

Microbial safety is a critical issue in insect farming, particularly when larvae are reared on organic waste streams [18]. The observed reduction in E. coli suggests that honey supplementation may potentially improve the microbiological quality of harvested biomass for subsequent feed applications. However, reductions in culturable microbial counts alone do not establish complete product safety.

The increase in LAB populations is noteworthy because beneficial microbial communities may contribute to nutrient utilization and competitive exclusion of undesirable microorganisms. However, the present study measured culturable LAB and E. coli rather than the complete gut microbiome. Therefore, the increase in LAB should be interpreted as an observed microbial-count response rather than evidence that honey acted as a prebiotic. The observed microbial shift may have contributed to improved larval performance, but this mechanism requires confirmation using microbiome profiling and functional assays.

4.3. Nutritional Composition Enhancement

Honey supplementation significantly increased crude protein and lipid content in larval biomass. Nutritional composition of BSFL is known to vary widely depending on substrate composition [2]. The elevated protein levels in the H10 treatment may reflect improved nitrogen retention and protein deposition efficiency due to enhanced energy availability. When sufficient carbohydrates are present, amino acids may be preferentially directed toward tissue synthesis rather than oxidation [17].

The increase in lipid content observed in honey-supplemented larvae may be consistent with carbohydrate-driven lipogenesis, in which excess dietary carbohydrates can contribute to fatty-acid synthesis and energy storage. However, this mechanism was not directly measured in the present experiment. Thus, the observed changes in larval composition should be interpreted as responses to the complete dietary treatment rather than evidence of a single metabolic pathway.

Notably, ash and moisture contents were not significantly affected by honey inclusion, indicating that mineral balance and water retention remained stable across treatments.

4.4. Implications for Sustainable Insect Farming

The results have potential implications for BSFL production because improved FCR and biomass yield indicate greater conversion efficiency under the experimental conditions. Nevertheless, the use of conventional commercial honey requires careful economic and sustainability assessment because honey is a relatively high-value food product.

From a circular-bioeconomy perspective, the use of locally available surplus, downgraded, off-specification, or otherwise non-marketable honey could potentially improve the economic rationale for supplementation if such materials are safe and legally permitted for feed use. However, honey production itself has environmental inputs, and sustainability advantages should therefore be evaluated at the level of the specific honey stream rather than assumed solely from its natural origin.

Economic feasibility must therefore be considered explicitly. Future studies should compare the performance and cost of food-grade honey with lower-value or surplus honey streams, characterize their chemical and microbiological safety, quantify their availability, and conduct life-cycle and cost–benefit assessments at production scale.

4.5. Study Limitations and Future Research

The present study was conducted under controlled laboratory conditions using a specific vegetable-waste/wheat-bran basal diet and commercial, unprocessed natural honey. The botanical origin of the honey was not formally authenticated, and the proximate composition of the basal substrate was not experimentally

determined. Consequently, responses may differ with honey floral source, processing history, substrate nutrient composition, or rearing system.

Future research should characterize both basal and supplemented diets for protein, lipid, fibre, ash, carbohydrate, dry matter, and energy; compare different honey types and lower-value honey streams; investigate amino acid and fatty acid profiles; characterize the gut microbiome using molecular sequencing; assess immune and antioxidant responses; evaluate long-term feeding effects in livestock or aquaculture species; and conduct economic and environmental assessments under commercial production conditions.

5. Conclusions

The present study demonstrates that supplementation with commercial, unprocessed natural honey positively influenced growth performance, microbial counts, and nutritional composition of BSFL under controlled laboratory conditions. Among the tested inclusion levels (0%, 5%, 10%, and 15%), 10% supplementation produced the most favorable overall outcomes, with significantly higher survival, final body weight, improved feed conversion efficiency, and greater biomass yield than the control group. These findings provide preliminary evidence supporting the potential of moderate honey supplementation as a functional dietary strategy, while recognizing that the mechanisms responsible for the observed responses were not directly measured.

From a microbial perspective, honey supplementation significantly reduced total plate counts and E. coli populations while increasing culturable LAB. These results indicate a favorable shift in the measured microbial profile, but they do not establish a comprehensive improvement in gut health or product safety because the broader microbiome and specific antimicrobial mechanisms were not investigated.

Nutritionally, honey inclusion increased crude protein and lipid contents without significantly affecting ash or moisture. Because the basal diet was not subjected to proximate analysis, the mechanisms responsible for these compositional changes cannot be attributed solely to altered carbohydrate supply.

Overall, this study identifies natural honey as a potentially multifunctional dietary supplement for BSFL, with the potential to improve productivity and alter measured microbial and nutritional characteristics under controlled conditions. However, the sustainability and commercial feasibility of the approach should not be inferred from the use of honey alone. Future work should prioritize surplus, downgraded, off-specification, or otherwise non-marketable honey streams while establishing their safety, consistency, availability, cost, and environmental footprint. Large-scale validation, comprehensive substrate characterization, gut microbiome analysis, nutrient profiling, and life-cycle and economic assessment will be necessary before recommending honey supplementation for industrial BSFL production.

Author Contributions

Conceptualization, T.E.A. and C.F.A.; methodology, T.E.A. and C.F.A.; investigation, T.E.A.; formal analysis, T.E.A.; data curation, T.E.A.; writing—original draft preparation, T.E.A.; writing—review and editing, T.E.A., C.F.A. and R.H.; supervision, C.F.A. and R.H. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

Ethical review and approval were not required for this study because it involved the rearing and experimental evaluation of black soldier fly (*Hermetia illucens*) larvae and did not involve humans or vertebrate animals.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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