COMPARATIVE ASSESSMENT OF SPENT GRAIN AND COW DUNG AS SUBSTRATES FOR GROWTH AND NUTRIENT CONVERSION IN HERMETIA ILLUCENS LARVAE

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COMPARATIVE ASSESSMENT OF SPENT GRAIN AND COW DUNG AS SUBSTRATES FOR GROWTH AND NUTRIENT

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Abstract**—** The growth and nutrient accumulation performance of *Hermetia illucens* larvae fed on a variety of common organic substrates, such as spent grain (SG) and cow dung (CD), were evaluated in the current study. In order to evaluate the performance of larvae fed on the two substrates for 12 days, a number of laboratory experiments were conducted, including measurements of body weight, survival rate, proximate composition, and Nutrient Enrichment Factor (NEF). At the end of 12 days, larvae given SG had a total sampled larval weight (30 individuals) of 1907.07 ± 242.45 g, a substantially higher body weight than those fed CD (425.61 ± 11.12 g, P = 0.012), despite equal survival rates. Furthermore, larvae fed SG had a considerably greater protein content (41.62%) and fat content (16.61%) than larvae given CD (25.32% protein and 6.83% fat, respectively). In a similar vein, larvae fed SG had a far higher energy content (2898.64 kcal/kg) than larvae given CD (1495.53 kcal/kg). Comparative analysis suggested that higher substrate protein, fat, and energy contents were associated with increased larval weight, while higher ash content appeared to limit growth. The results of this study provide a comparative evaluation of spent grain and cow dung as locally available substrates, highlighting differences in growth performance and nutrient conversion efficiency under controlled conditions.

Keywords **—***Hermetia illucens*, larval growth, spent grain, cow dung, nutrient enrichment

I. INTRODUCTION

The black soldier fly (*Hermetia illucens*), a member of the family Stratiomyidae and order Diptera, has drawn a lot of interest as a potential source for turning organic waste streams into very nutritious biomass with high protein and fat concentrations. This feature enables the use of this insect species as an alternative to conventional animal feed as well as for environmental sustainability in terms of waste management [1]. Since a meal heavy in protein, fat, and energy leads to weight gain and a superior nutritional profile, larval development and nutritional content are in fact heavily dependent on substrate availability [2, 3]. The *Hermetia illucens* insect is useful for circular bioeconomy reasons because it transfers nutrients from substrates to biomass, producing high-quality feed from agricultural waste for fish, chicken, and animal husbandry [4, 5]. While several studies have established that nutrient-rich substrates enhance larval growth, fewer studies have conducted direct, side-by-side comparisons of commonly available organic wastes such as spent grain and cow dung under controlled experimental conditions. This study therefore aims to provide a localized and comparative assessment of these substrates, with emphasis on nutrient enrichment efficiency and growth dynamics.

Larval growth and body composition are significantly influenced by the nutritional composition of their substrates [3, 6]. Cow dung has more ash and indigestible materials than spent grain, an industrial waste product from brewing that is typically rich in proteins and fermentable carbohydrates [7]. Studies have shown that larvae fed a high-nutrient diet grow more quickly and have a greater protein percentage than larvae fed a low-nutrient diet [8, 9]. Unfortunately, the majority of studies have concentrated on a single characteristic, like body composition or growth rates.

The Nutrient Enrichment Factor (NEF) is one of the most crucial instruments for determining how much of the nutrients in the substrate are converted by larvae. The ratio of a certain nutrient's concentrations in larvae to those in the substrate is known as the NEF. A NEF of unity indicates no enrichment, values over unity indicate a successful concentration of nutrients during growth, and values below unity indicate inefficient nutrient absorption [10, 11].

This study specifically investigates differences in growth efficiency, nutrient conversion, and enrichment patterns between spent grain and cow dung, providing context-specific data relevant to waste valorization strategies in Nigeria. Additionally, the protein, fat, fiber, moisture, ash, and metabolizable energy contents of the two meals and the larvae generated from them were determined in a comprehensive proximate study. NEF calculations were used to identify the nutrients that were enriched in the larvae.

To identify parameters impacting biomass output, additional correlation studies between substrate nutrients and larval growth characteristics were carried out. In addition to demonstrating the efficacy of cow dung and spent grains as larval substrates, the combined analysis highlights the potential of *H. illucens* as a nutrient-dense substitute feed source, facilitating the effective use of industrial waste products.

II. METHODOLOGY

A. *Study Design and Experimental Setup*

1) The purpose of this experiment was to assess the growth potential and nutrient utilization efficiency of H. illucens larvae on two distinct organic substrates: cow dung (CD) and spent grain (SG). In a controlled greenhouse at the University of Abuja, Biological Sciences Department, experiments were carried out with a photoperiod of 12:12 h, a temperature of 27 ± 2 °C, and a relative humidity of 65 ± 5%. After being gathered at the nearby farmers' market in Kuje Area Council, Abuja, the spent grain was left to stabilize for a full day at room temperature. Debris in the spent grain was removed, and the substrate was homogenized. Cow dung was purchased from the University of Abuja Animal Farms, fermented, and sieved to exclude rocks and long fibers. Key performance indicators such as feed conversion ratio (FCR), waste reduction efficiency, and dry matter conversion were not quantified in this study. The experimental focus was primarily on larval growth and nutrient composition; however, inclusion of these parameters would provide a more comprehensive evaluation of substrate utilization efficiency. Microbial composition and activity within the substrates were not characterized or controlled in this study. Substrates were used under natural conditions following standard preparation procedures.

B. *Larval Source and Rearing*

The larvae used in this investigation were taken from a laboratory colony at Ento'O'rganics Fly Farm in Iperu Remo, Ijebu, Ogun State, Nigeria (Plate 1). Each container had 600 larvae that were introduced into their respective substrates under regulated settings. For comparison, each treatment substrate (SG and CD) was replicated twice (N = 2). Although this number of replicates provided preliminary insights into larval growth and nutrient conversion, it is relatively low and may limit the statistical robustness and reproducibility of the findings. Future studies should incorporate a higher number of replicates to improve experimental reliability [12, 13]. Before the larvae were introduced into the containers, the substrates were weighed and packed. In order to prevent crowding, *ad-libitum* access to the substrate was made possible, guaranteeing uniform distribution on each substrate's surface. Every day, distilled water was used to keep the substrates' moisture level between 60% and 70%.

Figure 1: *Hermetia illucens* larvae.

C. *Sampling and Growth Measurement*

2) Data was gathered at five distinct pre-selected time points, including Day 1 (the starting point), Day 3, Day 6, Day 9, and Day 12. Growth was measured over 12 days. Larvae were carefully removed from the substrates and cleaned to get rid of any substrate components during the data collection process.

D. *Number of Larvae and Weight*

3) Each replicate's total number of surviving larvae was carefully tallied. Their survival and consistency in each therapy were assessed using the count. An analytical balance with an accuracy of 0.001 g was used to measure the weight of 30 randomly selected larvae per replicate. The recorded values represent the combined weight of the sampled larvae, as specified. All units were standardized accordingly to avoid ambiguity.

4) The selection of 30 larvae was guided by practical handling considerations and commonly used sampling practices in similar experimental setups; however, no formal sample size calculation was performed.

E. *Proximate Analysis*

The following process was carried out based on AOAC INTERNATIONAL (formerly the Association of Official Analytical Chemists) standard methods for the determination of proximates in order to measure the nutrition composition of each substrate and larvae:

• The Kjeldhal technique (N x 6.25) was used to calculate the crude protein content [14].
• Petroleum ether and a Soxhlet device were used to extract crude fat [15].
• An oven set at 105°C was used to measure moisture [16].
• Successive hydrolysis of acid and alkaline was used to analyze crude fiber [17].
• The ash content was measured at 550°C in a muffle furnace [18].

Factor of Nutrient Enrichment (NEF) The nutrient enrichment factor (NEF) was used to quantify the efficiency with which larvae assimilated nutrients from their rearing media.

NEF was calculated as:

NEF = Nutrient concentration in larvae/Nutrient concentration in substrate

A NEF value greater than 1 indicates nutrient accumulation, while a value less than 1 suggests nutrient depletion or limited assimilation [13].

F. Statistical Analysis and Results Presentation

SPSS version 26 (IBM, USA) was used for all statistical analyses. Descriptive statistics (mean ± standard deviation) were computed for all variables. Variations in larval weight and number across substrate types and time points were analyzed using a two-way repeated measures ANOVA, with F-values, degrees of freedom (df), P-values, and effect sizes (partial eta squared, η^2) reported. Due to the low number of replicates (N = 2), the statistical power is limited, and results should be interpreted with caution. Tukey's HSD test was applied for post-hoc comparisons at $P < 0.05$. An exploratory comparison was conducted between substrate nutrient composition and larval growth performance. However, formal correlation analysis using Pearson coefficients was not considered statistically robust due to the limited number of substrate conditions (n = 2). Therefore, relationships between variables are described qualitatively rather than inferred statistically. GraphPad Prism version 9 was used for visual comparison of nutrient enrichment.

III. RESULTS

The two-way repeated measures ANOVA demonstrated that substrate type and rearing duration significantly influenced larval weight, with a notable interaction effect between both factors. Effect size estimates (η^2) indicated that substrate type accounted for a substantial proportion of the variance in larval growth. In contrast, larval count showed no statistically significant variation across treatments, with low effect size values suggesting minimal biological impact.

Mean (± SD) larval body mass (based on a subset of 30 larvae per replicate) and total larval number of *H. illucens* grown on SG and CD are shown in Table 1. Body mass of larvae, estimated from the sampled subset, was observed to increase steadily with time in both treatments. However, in comparison with those grown on CD, larvae grown on SG appeared heavier starting from day 3 onwards ($P < 0.05$); however, this observed significance should be interpreted cautiously due to the limited number of replicates (N = 2). At day 12, the total weight of the sampled larvae (30 individuals) was significantly higher ($P = 0.012$) in SG (1907.07 ± 242.45 g) compared to CD (425.61 ± 11.12 g). This corresponds to an approximate mean individual weight of 63.57 mg and 14.19 mg for SG and CD, respectively. A substantially higher standard deviation was observed in the SG treatment (±242.45) compared to CD (±11.12), indicating greater variability in larval growth within the spent grain substrate. Conversely, there was no significant difference in larval numbers between the two treatments ($P > 0.05$).

Notably, variability in larval weight increased over time, particularly in the spent grain treatment, as reflected by larger standard deviation values. This suggests heterogeneity in growth rates among individuals reared on nutrient-rich substrates.

Table 1: Total Weight of Sampled Larvae (30 individuals) and Count of *H. illucens* Reared on Spent Grain and Cow Dung Substrates at Different Rearing Days

Days	SG Weight	CD Weight	SG Larvae Count	CD Larvae Count
1	0.50 ± 0.00^a	0.50 ± 0.00^a	531.5 ± 123.04^a	577 ± 230.31^a
3	53.44 ± 2.71^a	43.62 ± 8.66^b	530.5 ± 125.17^a	572 ± 226.27^a
6	102.52 ± 17.02^a	75.47 ± 21.21^b	542.5 ± 140.01^a	567 ± 228.64^a
9	116.64 ± 23.56^a	87.87 ± 33.73^b	542 ± 140.01^a	566.5 ± 226.73^a
12	1907.07 ± 242.45^a	425.61 ± 11.12^b	542.5 ± 140.01^a	566.5 ± 226.73^a

N = 2; values carrying different subscripts along the rows are significantly different at $P < 0.05$. Interpretation of statistical significance should be made cautiously due to the low number of replicates. Higher standard deviation values in SG indicate greater variability in larval growth, which should be considered when interpreting treatment differences.

The proximate composition of spent grain and cow dung substrate is presented in Table 2, together with the nutrient composition of *H. illucens* larvae grown on both substrates. Crude protein and crude fat content were higher in spent grain (22.52% and 2.39%, respectively) than cow dung, while the ash content was much higher in cow dung (32.00%) than in spent grain (4.26%). Similarly, the crude protein and crude fat content of larvae grown on spent grain was higher (41.62% and 16.61%, respectively) than that of larvae grown on cow dung (25.32% and 6.83%). The content of metabolizable energy in larvae obtained from the spent grain substrate was remarkably higher (2898.64 Kcal/kg) than in larvae obtained from cow dung (1495.53 Kcal/kg).

Table 2. Comparative Proximate Composition of Substrates and *H. illucens* Larvae Reared on Spent Grain and Cow Dung

Parameter	Spent Grain Substrate	Cow Dung Substrate	Larvae (Spent Grain)	Larvae (Cow Dung)
Crude Protein (%)	22.52	8.36	41.62	25.32
Crude Fat (%)	2.39	0.50	16.61	6.83
Moisture (%)	9.76	8.92	7.45	13.41
Crude Fibre (%)	14.80	14.26	9.10	9.30
Ash (%)	4.26	32.00	10.23	30.89
Metabolizable Energy (Kcal/kg)	1715.43	1558.82	2898.64	1495.53

The Nutrient Enrichment Factor (NEF) highlights how effectively *Hermetia illucens* larvae converted nutrients from spent grain and cow dung into larval biomass (Table 4). Protein levels increased in larvae from both substrates, with NEF values of 1.85 for spent grain and 3.03 for cow dung, indicating strong protein accumulation during larval growth. Fat showed the greatest apparent enrichment, particularly in larvae reared on cow dung (NEF = 13.66), indicating a strong capacity for lipid accumulation relative to the low initial fat content of the substrate. However, this high enrichment value should be interpreted in relation to the very low baseline lipid content of cow dung rather than absolute lipid gain alone.

Moisture content decreased in larvae reared on spent grain (0.76) but increased in those reared on cow dung (1.50). Crude fiber declined in larvae from both substrates (0.61–0.65), suggesting that fiber components were utilized during digestion. Ash content increased markedly in larvae reared on spent grain (2.40), indicating mineral concentration, while little change was observed in larvae reared on cow dung (0.97). Metabolizable energy also increased in larvae from spent grain (1.69) but remained slightly lower in those from cow dung (0.96). The results demonstrate the ability of *H. illucens* larvae to transform organic substrates into nutrient-rich biomass, particularly through the enrichment of protein and lipids, supporting their value as an alternative feed resource.

Table 3. Nutrient Enrichment Factor (NEF) of *H. illucens* Larvae Reared on Spent Grain and Cow Dung

Parameter	Spent Grain Substrate	Larvae (Spent Grain)	NEF (SG)	Cow Dung Substrate	Larvae (Cow Dung)	NEF (CD)
Crude Protein (%)	22.52	41.62	1.85	8.36	25.32	3.03
Crude Fat (%)	2.39	16.61	6.95	0.50	6.83	13.66
Moisture (%)	9.76	7.45	0.76	8.92	13.41	1.50
Crude Fibre (%)	14.80	9.10	0.61	14.26	9.30	0.65
Ash (%)	4.26	10.23	2.40	32.00	30.89	0.97
Metabolizable Energy (Kcal/kg)	1715.43	2898.64	1.69	1558.82	1495.53	0.96

Figure 1 illustrates a comparison between the enrichment factors of various nutrients for *Hermetia illucens* larvae fed with spent grain and cow dung. The highest enrichment factor for the nutrients is crude fat at 6.95 for spent grain larvae and 13.66 for cow dung larvae. Another nutrient that was enriched in both diets is protein, with values of 1.85 and 3.03 for larvae from spent grain and cow dung diets, respectively. On the other hand, crude fiber showed an enrichment factor lower than one (0.61 and 0.65) in both diets.

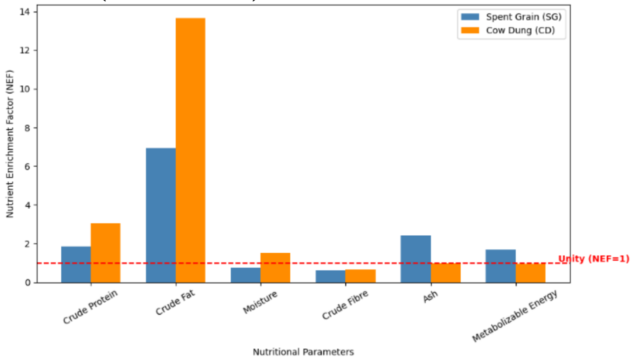

Figure 2: Comparative Nutrient Enrichment Factors of *Hermetia illucens* Larvae Reared on Spent Grain and Cow Dung.

A comparative assessment of substrate composition and larval growth patterns suggests that higher protein, fat, and energy contents in spent grain are associated with increased larval biomass, whereas higher ash content in cow dung appears to be associated with reduced growth. However, these observations are descriptive in nature, and no statistically valid correlation analysis was performed due to the limited number of substrate conditions.

Table 4. Descriptive Comparison of Substrate Nutrient Composition and Observed Larval Growth Trends.

Nutrient Parameter	SG Value	CD Value	Correlation with Larval Weight	Correlation with Larval Count	Interpretation
Crude Protein	22.52	8.36	+0.99	–0.96	Strongly enhances larval weight; slight reduction in count.
Crude Fat	2.39	0.50	+0.98	–0.94	Promotes larval weight gain; minor negative effect on count.
Moisture	9.76	8.92	+0.75	–0.68	Moderately supports larval growth.
Crude Fibre	14.80	14.26	+0.40	–0.35	Minimal influence; comparable across substrates.
Ash	4.26	32.00	–0.99	+0.97	High ash reduces growth but may support larval count stability.
Metabolizable Energy	1715.43	1558.82	+0.96	–0.92	Higher energy availability enhances larval growth.

IV. DISCUSSION

It has been demonstrated that the growth performance of *H. illucens* larvae was significantly influenced by the type of rearing substrate, as supported by the two-way ANOVA results showing significant main and interaction effects with moderate to high effect sizes [2-3, 19]. When compared to larvae raised on cow dung (CD), the weight gain of larvae raised on spent grain (SG) substrate was considerably higher ($P < 0.05$) starting on day 3. By the end of the trial, the highest average weights were achieved, with SG total sample larvae weighing 1907.07 ± 242.45 g and CD weighing 425.61 ± 11.12 g on day 12. Simultaneously, it has been demonstrated that there was no difference in larval survival rates between the various treatments based on the quantity of larvae. The aforementioned results imply that biomass development, rather than hatching rate or survivorship, is primarily linked to the impact of nutritional substrate composition on larvae. The high protein, lipid, and calorie content of spent grain substrate has been proposed as a possible explanation for this phenomenon.

It is commonly known that larvae reared on high-nutrient substrates gain more body weight than those raised on low-nutrient substrates. Therefore, compared to those grown on substrates like manure, larvae fed with formulated feedstuffs or other protein-based wastes exhibit better body weights ($p < 0.05$). In particular, it has been demonstrated that brewer's spent grain and other protein-rich residues support better H. illucens growth than substrates with lower nutritional content [20].

The current study provides more information about the nutritional differences between substrates for the larvae. Spent grain had much larger levels of crude protein (22.52%) and fat (2.39%) than cow dung (8.36% and 0.50%, respectively). However, it was shown that the ash content of cow dung was significantly higher (32%). As a result, larvae given spent grain had substantially higher crude protein (41.62%) and fat (16.61%) contents than those fed cow dung (25.32% and 6.83%, respectively). Additionally, SG larvae had nearly twice as much metabolizable energy (2898.64 kcal/kg) as CD larvae. Higher protein and energy content in substrates have been proven time and again to increase protein and lipid levels in BSF larvae [19, 21]. In contrast, lower-nutrient substrates, like manure, usually produce larvae that contain less protein and fat [22]. Protein and lipid-rich larvae are highly valuable as livestock feed due to their significant contributions to feed composition.

The results of the Nutrient Enrichment Factor (NEF) helped understand how effective the process of nutrient conversion from the substrate to larval biomass is. The enrichment factors for protein were relatively high for SG (1.85) but very high for CD (3.03). The reason for this is probably the fact that the CD substrate contained little protein at the beginning of the study, but the amount assimilated by larvae was considerable. The enrichment factors for fat were high both for SG (6.95) and especially for CD (13.66). The result shows that larvae can accumulate lipids effectively from substrates that originally contain small amounts of fats. At the same time, the ash enrichment factor was noticeable for SG (2.40) but insignificant for CD (0.97). It was metabolizable energy that increased considerably only for SG.

According to a previous study, BSF larvae may accumulate more fat than the substrate's fat content, suggesting that they are beneficial for producing bioenergy [22]. The patterns of protein enrichment seen here are in line with observations indicating the nutritional content of the substrate affects the biochemical makeup of larvae [21–24]. When constructing the larvae for particular applications, where protein-enriched larvae may be employed as feed, these tendencies in protein enrichment are crucial.

Larval weight appeared to increase with higher nutrient availability, particularly protein and lipid content, whereas substrates with higher ash content showed reduced growth performance. Except for a slight positive association with ash, larval numbers showed poor correlations with all dietary parameters. These findings show that while larval survival is less dependent on the type of substrate utilized, larval biomass gain is strongly dependent on the nutritional value of the substrate. It is well known that the amount of protein in the diet has a significant impact on the growth of larvae; the more proteins in the diet, the heavier the larvae and the quicker their development [25].

Despite the clear trends observed in larval growth and nutrient composition, a key limitation of this study is the low number of experimental replicates ($N = 2$). This may reduce the statistical reliability and generalizability of the findings. Consequently, while the results provide useful preliminary insights, further studies with increased replication are necessary to validate these outcomes and strengthen the conclusions.

Another limitation of the study lies in the sampling strategy used for weight determination. Only a subset of 30 larvae per replicate was measured, which may not fully represent the variability within the entire population. This approach, although practical, may introduce sampling bias and affect the accuracy of the estimated mean weights. Future studies should employ statistically validated sampling techniques or increase sample size to ensure more robust representation of the population.

Consistent with existing literature, the growth performance of *H. illucens* larvae was influenced by the type of rearing substrate. However, this study provides a direct comparison between spent grain and cow dung, highlighting substrate-specific differences in growth magnitude and nutrient accumulation under identical rearing conditions. Although the positive influence of nutrient-rich substrates on larval growth is well established, the present study contributes by providing a controlled, side-by-side evaluation of two widely available organic waste streams within a local context. The integration of growth performance, proximate composition, and nutrient enrichment factor (NEF) offers a more comprehensive assessment of substrate suitability than studies focusing on single performance metrics. This approach provides practical insights for optimizing substrate selection in small- to medium-scale insect production systems.

A notable observation in this study is the higher variability in larval weight within the spent grain treatment, particularly at later stages of development. This is evidenced by the substantially larger standard deviation compared to the cow dung treatment. Such variability may be attributed to uneven access to nutrients, differences in microhabitat conditions within the substrate, or density-dependent competition among larvae. Nutrient-rich substrates like spent grain may promote rapid growth in some individuals while others lag behind, resulting in a wider distribution of body weights.

The markedly high lipid enrichment factor (NEF = 13.66) observed in larvae reared on cow dung may be explained by the metabolic ability of *Hermetia illucens* larvae to synthesize and accumulate lipids de novo from non-lipid substrates. BSF larvae are known to convert carbohydrates and proteins into lipids through lipogenesis, primarily in the fat body, where excess dietary carbohydrates are metabolized via glycolysis and the acetyl-CoA pathway, ultimately leading to fatty acid synthesis. This process allows larvae to accumulate substantial lipid reserves even when reared on substrates with low initial fat content. In addition, microbial pre-digestion of organic matter in cow dung may enhance the availability of short-chain fatty acids and assimilable carbon sources, indirectly contributing to lipid biosynthesis in larvae. This observation is consistent with previous studies [13, 24] reporting that BSF larvae exhibit strong lipogenic capacity, converting carbohydrates and proteins into triacylglycerols stored in the fat body, particularly under nutrient-limited lipid conditions.

V. CONCLUSION

The current study provides preliminary evidence highlighting the importance of substrate selection for BSF larvae cultivation using nutrient-based methods. The best substrates for maximizing larval growth and nutritional value are those that are high in protein but low in ash, such as spent grain. These findings have an impact on the circular economy's ability to value agricultural waste and provide sustainable animal feed. This is due to the fact that the study provides information about the relative efficacy of substrates in comparison to cow dung and advances scientific knowledge of substrates and their effects on BSF larvae. These findings provide preliminary insights into the potential of agricultural waste valorization for sustainable animal feed, although conclusions should be interpreted with caution due to limitations in sampling representativeness. The findings as well contribute to ongoing efforts in agricultural waste valorization by offering comparative data that can inform substrate selection for sustainable insect-based feed production.

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CONFLICT OF INTERST

Authors declared that there is no conflict of interest

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A SUMMARY OF EACH AUTHOR'S CONTRIBUTION

A. Oboh: Conceived and designed the study, contributed to data collection and data analysis

D. Latseumso: Contributed to study design, data collection, and data analysis and wrote the manuscript.

A. DanKishiya: contributed to study design, data collection, and data analysis

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