

Effect of *Aspergillus niger* Inoculum Level and Incubation Period on Nutrient Composition and in Vitro Digestibility of Sugarcane Tops

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Abstract—This study aimed to evaluate the effects of *Aspergillus niger* inoculum level and incubation period on dry matter (DM), crude protein (CP), in vitro dry matter digestibility (IVDMD), and in vitro organic matter digestibility (IVOMD) of sugarcane tops. The experiment was arranged in a completely randomized design with a 3 × 3 factorial scheme, consisting of three inoculum levels of *A. niger* (10⁵, 10⁶, and 10⁷ CFU g⁻¹ sugarcane tops) and three incubation periods (10, 20, and 30 days), with four replicates per treatment. Dry matter and crude protein were determined using proximate analysis, while digestibility parameters were measured using the two-stage in vitro technique of Tilley and Terry. The results showed that *A. niger* inoculum level had no significant effect on DM and CP contents, whereas incubation period had a highly significant effect on both parameters. A significant interaction between inoculum level and incubation period was observed for DM content, but not for CP. The relatively best treatment was obtained at 10⁶ CFU g⁻¹ *A. niger* with a 30-day incubation period, resulting in DM and CP contents of 84.28% and 6.04%, respectively. For digestibility parameters, no significant interaction was observed between inoculum level and incubation period on IVDMD and IVOMD. However, increasing inoculum level and extending incubation period independently resulted in a highly significant improvement in digestibility values. Despite these improvements, IVDMD values remained relatively low, indicating that fermentation using *A. niger* alone was insufficient to fully optimize the digestibility of sugarcane tops.

Keywords— Sugarcane tops; *Aspergillus niger*; fermentation; incubation period; in vitro digestibility.

I. INTRODUCTION

During the dry season, ruminant farmers frequently face limited availability of high-quality fresh forage, which constrains livestock productivity. This condition has encouraged the utilization of agricultural by-products as alternative feed resources, one of which is sugarcane tops (*Saccharum officinarum*). The use of agro-industrial residues is not only a technical solution to feed shortages but also a strategic approach to improving the sustainability of ruminant meat production systems (Muwakhid et al., 2025). Sugarcane tops are abundantly available throughout the year; however, their utilization as animal feed remains limited due to low nutritional quality and poor digestibility. In Indonesia, sugarcane top production was reported to reach 56.54 million tons in 2018 and is projected to increase to 84.81 million tons, yet only approximately 22% is utilized as livestock feed, while the remainder is discarded or burned (Baharuddin et al., 2016; Pratama, 2019).

Nutritionally, sugarcane tops serve as a potential energy source because of their relatively high cellulose and hemicellulose contents. However, the high proportion of structural carbohydrates results in low digestibility, thereby limiting their efficient incorporation into ruminant diets (Widjaja and Setyo, 2012; Amin et al., 2016). The low digestibility of sugarcane tops is primarily attributed to the lignocellulosic complex, in which lignin acts as a major barrier to the enzymatic degradation of cellulose and hemicellulose.

Improving the feeding value of sugarcane tops can be achieved through chemical and biological treatments. Ammoniation using urea has been widely reported to disrupt lignocellulosic bonds, thereby increasing substrate accessibility for microbial degradation. Subsequently, biological fermentation using cellulolytic fungi, such as *Aspergillus niger*, has the potential to enhance fiber degradation through cellulase activity, leading to improvements in dry matter and organic matter digestibility. Previous studies have demonstrated that *A. niger* fermentation can improve nutrient profiles and reduce crude fiber content in various plant-based agricultural residues (Muwakhid et al., 2023; Muwakhid et al., 2023). Such fermentation processes are generally applied as pre-ruminal treatments to enhance feed utilization efficiency in ruminants.

Aspergillus niger is well known for its ability to produce a wide range of hydrolytic enzymes, including cellulases and proteases, which play important roles in the degradation of fibrous and protein components of feed materials. Extending the fermentation incubation period is expected to support fungal growth and metabolic activity, resulting in increased enzyme production and subsequent alterations in the chemical composition of the substrate. However, increasing inoculum levels does not necessarily lead to proportional improvements in feed quality, as microbial growth is also constrained by substrate availability and fermentation environmental conditions.

In addition to fermentation, urea supplementation during sugarcane top processing contributes not only to improved dry matter and organic matter digestibility but also to increased nitrogen availability for rumen microorganisms. The combination of ammoniation and biological fermentation is therefore expected to enhance the overall quality of sugarcane tops as ruminant feed. Nevertheless, the effectiveness of this combined approach is highly dependent on fermentation parameters, particularly inoculum level and incubation period.

Despite numerous studies on fungal fermentation of fibrous feedstuffs, information regarding the optimal balance between *A. niger* inoculum level and incubation duration for improving the nutritional quality and digestibility of sugarcane tops remains limited. Therefore, this study aimed to evaluate the effects of different *A. niger* inoculum levels and incubation periods on dry matter and crude protein contents, as well as in vitro dry matter and organic matter digestibility of sugarcane tops, and to assess the extent to which these treatments contribute to improving their feeding value for ruminants.

II. MATERIALS AND METHODS

Experimental Materials

The materials used in this study consisted of sugarcane tops (*Saccharum officinarum*), urea, and *Aspergillus niger* inoculum. Sugarcane tops were obtained from the Cening variety and chopped into approximately 3–5 cm lengths prior to treatment. *Aspergillus niger* was used as the fermentation agent, while urea was applied during the ammoniation process as a source of non-protein nitrogen.

Experimental Treatments

The experiment involved two treatment factors. The first factor was the inoculum level of *Aspergillus niger*, consisting of:

- T1: 10^5 CFU g^{-1} sugarcane tops
- T2: 10^6 CFU g^{-1} sugarcane tops
- T3: 10^7 CFU g^{-1} sugarcane tops

The second factor was the fermentation incubation period, consisting of:

- L1: 10 days
- L2: 20 days
- L3: 30 days

Fermentation was conducted following the ammoniation process to enhance the availability of lignocellulosic substrates for microbial activity during fermentation.

Experimental Design

The study was conducted as an experimental trial using a completely randomized design with a 3×3 factorial arrangement and four replicates per treatment, resulting in nine treatment combinations. This design was used to evaluate the effects of *A. niger* inoculum level, incubation period, and their interaction on the measured parameters.

In Vitro Digestibility Measurement

In vitro digestibility of sugarcane tops was determined using the two-stage technique described by Tilley and Terry (1963). Rumen fluid was collected from two rumen-fistulated Peranakan Friesian Holstein (PFH) bulls aged approximately 2.5 years. The digestibility parameters evaluated were in vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD).

Measured Parameters

The main parameters observed in this study were:

1. Dry matter content (DM, %),
2. Crude protein content (CP, %),

3. In vitro dry matter digestibility (IVDMD, %),
4. In vitro organic matter digestibility (IVOMD, %).

Statistical Analysis

The collected data were analyzed using analysis of variance (ANOVA). When significant treatment effects were detected, differences among means were further evaluated using the Least Significant Difference (LSD) test (Steel and Torrie, 1991). Statistical significance was declared at $P < 0.05$ and $P < 0.01$.

III. RESULTS AND DISCUSSION

Dry Matter Content

Analysis of variance followed by the Least Significant Difference (LSD) test showed that the inoculum level of *Aspergillus niger* had no significant effect ($P > 0.05$) on the dry matter (DM) content of sugarcane tops. In contrast, the incubation period had a highly significant effect ($P < 0.01$) on DM content. A significant interaction ($P < 0.05$) between inoculum level and incubation period was also observed for this parameter.

As illustrated in Figure 1, the DM content of sugarcane tops generally increased with increasing incubation period, although the response was not consistently linear across inoculum levels. The highest DM content was obtained at treatment T2 (10^6 CFU g^{-1} sugarcane tops), whereas further increasing the inoculum level to T3 (10^7 CFU g^{-1}) did not result in additional increases in DM content. Regarding the incubation period, the highest DM value was observed at L3 (30 days), reaching 83.34%, while the lowest DM content was recorded at L1 (10 days).

The lack of further increase in DM content at the highest inoculum level may be attributed to substrate limitation and the complex lignocellulosic structure of sugarcane tops. *Aspergillus niger* is a cellulolytic fungus capable of producing a cellulase enzyme complex that hydrolyzes cellulose into simple sugars (Sutopo et al., 2012). However, in plant materials, cellulose is tightly associated with lignin and hemicellulose, forming a lignocellulosic complex that restricts enzymatic accessibility. Lignin, composed primarily of phenylpropane units, is highly resistant to enzymatic degradation and therefore limits the effectiveness of cellulose hydrolysis, particularly under conditions of high microbial population density (Mohammed and Keikhosro, 2007).

These findings indicate that extending the incubation period plays a more important role in increasing DM content than increasing inoculum level. Longer fermentation periods allow microorganisms to adapt more effectively to the substrate and maximize enzymatic activity on available lignocellulosic components, whereas increasing inoculum concentration beyond an optimal level does not necessarily enhance substrate utilization.

Crude Protein Content

Analysis of variance indicated that the inoculum level of *Aspergillus niger* had no significant effect ($P > 0.05$) on the crude protein (CP) content of sugarcane tops, whereas the incubation period had a highly significant effect ($P < 0.01$). No

significant interaction ($P > 0.05$) between inoculum level and incubation period was observed for CP content.

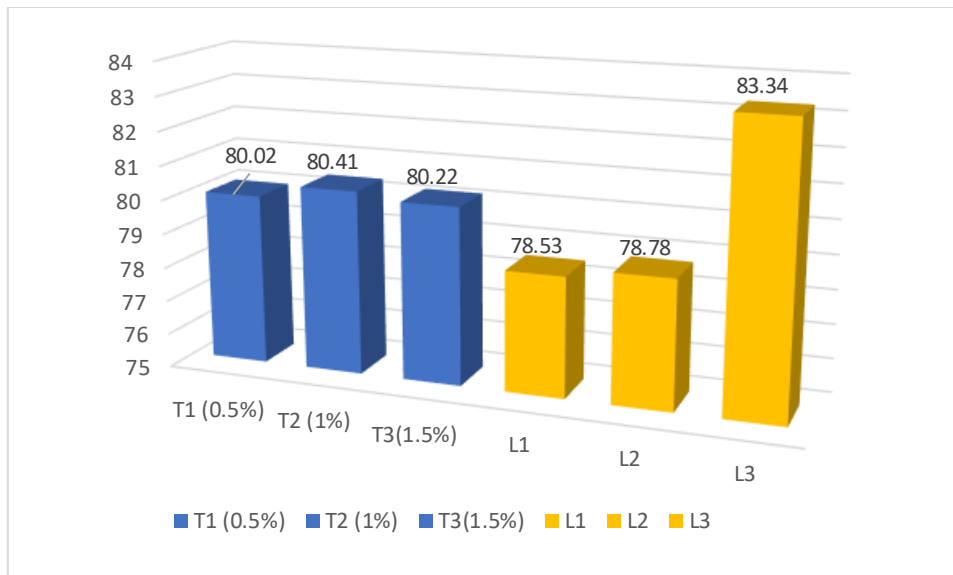


Figure 1. Dry matter content of sugarcane tops as affected by different *Aspergillus niger* inoculum levels and incubation periods.

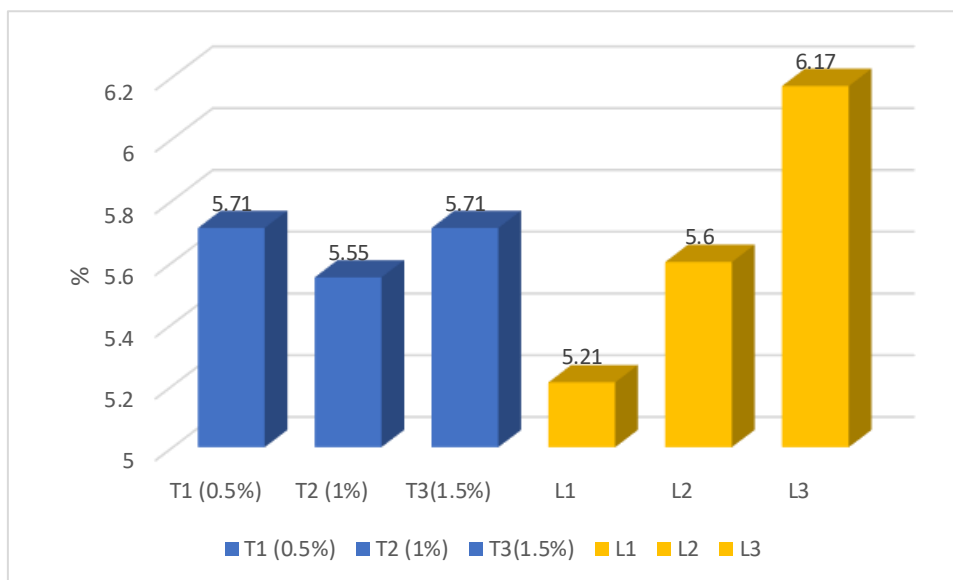


Figure 2. Crude protein content of sugarcane tops at different *Aspergillus niger* inoculum levels and incubation periods.

As shown in Figure 2, CP content varied within a relatively narrow range across treatments. Treatments T1 and T3 resulted in similar CP values (5.71%), while extending the incubation period to 30 days (L3) increased CP content to 6.17%. This value was higher than that reported for untreated sugarcane tops, which generally contain approximately 4% CP (Suningsih et al., 2019).

The increase in CP content with longer incubation periods is likely associated with fungal growth and metabolic activity during fermentation. *Aspergillus niger* is capable of producing proteolytic enzymes that degrade complex proteins into simpler nitrogenous compounds. In addition, the accumulation of fungal biomass during fermentation contributes to an increase in single-cell protein, thereby elevating the overall CP content

of the substrate (Has et al., 2017). This finding is consistent with the results of Otiento et al. (2013), who reported increased protein content in various feed materials following fermentation with *A. niger*.

The absence of a significant effect of inoculum level on CP content suggests that increasing fungal population density does not necessarily result in proportional protein enrichment. This observation indicates the presence of an optimal microbial growth threshold, which is constrained by substrate availability and fermentation environmental conditions. Consequently, incubation period appears to be a more critical factor than inoculum level in determining CP enhancement in fermented sugarcane tops.

TABLE 1. Dry matter and crude protein contents of sugarcane tops subjected to different *Aspergillus niger* inoculum levels and incubation period

Treatment	Dry matter content (%)	Crude protein content (%)
T1L1	77.87 ^a	5.3
T1L2	79.13 ^b	5.67
T1L3	83.06 ^c	6.17
T2L1	77.24 ^a	5.22
T2L2	79.71 ^b	5.39
T2L3	84.28 ^d	6.04
T3L1	80.48 ^{bc}	5.11
T3L2	80.5 ^{bc}	5.73
T3L3	82.7 ^c	6.3

(Values followed by different lowercase letters within the same column differ significantly; $P < 0.05$)

Based on **Table 1**, the average dry matter (DM) content of sugarcane tops across inoculum levels ranged from 80.02 to 80.41%, while CP content ranged from 5.55 to 5.71%. Statistical analysis confirmed that inoculum level had no significant effect ($P > 0.05$) on either DM or CP content, indicating that increasing inoculum concentration up to 10^7 CFU g^{-1} did not consistently enhance nutrient composition.

The lack of response to higher inoculum levels may be attributed to the complex lignocellulosic structure of sugarcane tops, which limits cellulose degradation by fungal cellulase enzymes. In addition, suboptimal ammoniation conditions may require greater energy expenditure and longer adaptation periods for fungal colonization before effective substrate utilization can occur.

In contrast, incubation period significantly affected ($P < 0.01$) both DM and CP contents. Dry matter values ranged from 78.53 to 83.54%, while CP content ranged from 5.21 to 6.17%. The LSD test at the 1% significance level indicated that a 30-day incubation period (L3) resulted in significantly higher DM and CP contents compared with shorter incubation periods (L1 and L2). This finding highlights fermentation duration as the primary factor influencing nutrient composition changes in sugarcane tops.

The significant effect of incubation period is likely related to the transition of *A. niger* from the adaptation phase to the logarithmic growth phase during extended fermentation, during which cellulase and protease production reaches its maximum (Kurniawati et al., 2019). This condition promotes fiber degradation and microbial biomass accumulation, thereby contributing to increased CP content.

Regarding the interaction between inoculum level and incubation period, DM content ranged from 77.24 to 84.28%, whereas CP content ranged from 5.11 to 6.30%. Statistical analysis revealed a significant interaction ($P < 0.05$) for DM content but not for CP content. The highest DM content was observed in the T2L3 treatment combination, indicating that an inoculum level of 10^6 CFU g^{-1} combined with a 30-day incubation period provided relatively optimal conditions for fermentation.

This condition likely reflects a balanced relationship between inoculum density and substrate availability, allowing efficient fungal growth and metabolic activity. Conversely, at higher inoculum levels (T3), increased microbial population did not translate into improved nutrient quality, suggesting substrate limitation or intensified microbial competition during fermentation.

Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD)

The analysis of variance showed that the interaction between *Aspergillus niger* inoculum level and incubation period had no significant effect ($P > 0.05$) on dry matter digestibility (DMD) and organic matter digestibility (OMD) of sugarcane tops. However, each factor independently namely inoculum level and incubation period had a highly significant effect ($P < 0.01$) on improving both DMD and OMD. The mean values of dry matter and organic matter digestibility under different treatments are presented in Figure 3.

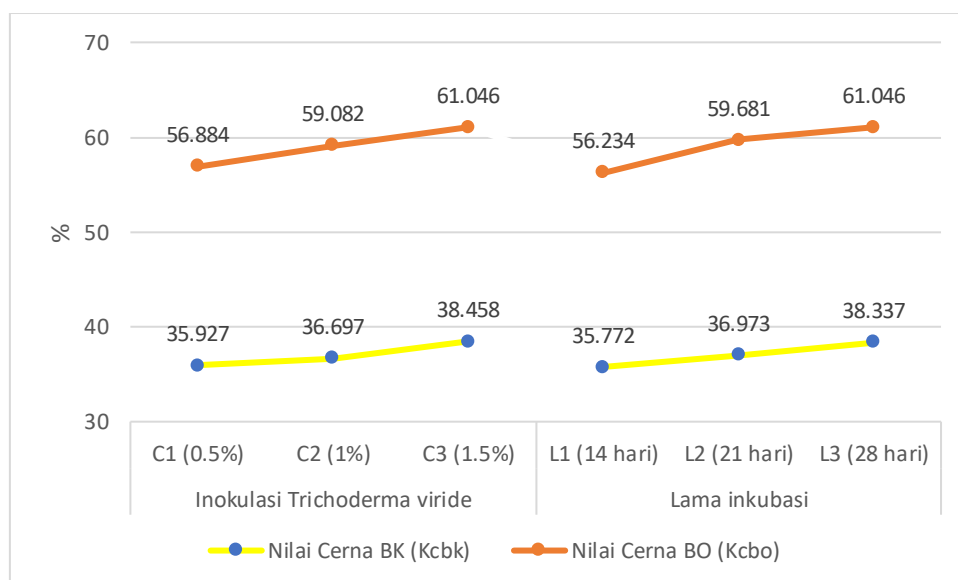


Figure 3. Mean values of dry matter digestibility (DMD) and organic matter digestibility (OMD) of sugarcane tops treated with different levels of *Aspergillus niger* inoculum and incubation periods.

As shown in Figure 3, the highest mean DMD among inoculum treatments was observed in T3 (10^7 CFU/g sugarcane tops), reaching 38.46%, whereas the lowest value was recorded in T1 (10^5 CFU/g sugarcane tops) at 35.93%. With respect to incubation period, the highest DMD value was obtained at L3 (30 days), amounting to 38.34%, while the lowest value occurred at L1 (10 days), at 35.77%. These results indicate that increasing both inoculum level and incubation period contributed to improvements in the dry matter digestibility of sugarcane tops.

A similar trend was observed for organic matter digestibility. The highest mean OMD among inoculum treatments was recorded in T3 (61.05%), while the lowest value was found in T1 (56.88%). Regarding incubation period, the highest OMD was achieved at L3 (30 days), reaching 61.05%, whereas the lowest value occurred at L1 (10 days), at 56.23%. Overall, increasing the inoculum level of *Aspergillus niger* and extending the incubation period enhanced both DMD and OMD of sugarcane tops, although no significant interaction between the two factors was detected.

The improvement in digestibility is presumably associated with microbial activity during fermentation, which partially degrades fiber components, particularly lignin and hemicellulose. The breakdown of lignin increases the accessibility of cellulose and hemicellulose to digestive enzymes, thereby improving dry matter and organic matter digestibility (Sidqi et al., 2015). These findings are consistent with those of Yalchi and Hajiegharari (2010), who reported that inoculation of wheat straw with *Trichoderma viride* improved fiber fractions and enhanced both dry matter and organic matter digestibility.

Nevertheless, the DMD values obtained in this study remained relatively low, ranging from approximately 35% to 40%, and did not reach the minimum level of dry matter digestibility considered normal ($\geq 60\%$) (Suparwi, 2000). This indicates that although fermentation using *Aspergillus niger* significantly improved digestibility, the complex lignocellulosic structure of sugarcane tops remains a major limiting factor. Therefore, a single fermentation treatment may not be sufficient to fully optimize digestibility, and additional processing strategies such as optimizing ammoniation conditions, applying microbial consortia, or extending fermentation duration are required to further enhance the digestibility of sugarcane tops.

TABLE 2. Mean values of dry matter digestibility (DMD) and organic matter digestibility (OMD) of sugarcane tops under different treatment combinations.

Treatment	DMD (%)	OMD (%)
T1L1	34.99	54.60
T1L2	35.89	57.09
T1L3	36.90	58.97
T2L1	35.44	56.61
T2L2	36.75	59.70
T2L3	37.90	60.93
T3L1	36.88	57.49
T3L2	38.28	62.25
T3L3	40.22	63.39

Note: Differences among treatments were highly significant ($P < 0.01$). Fermented treatments differed significantly from the control.

Based on Table 2, the dry matter digestibility (DMD) and organic matter digestibility (OMD) of sugarcane tops varied among combinations of *Aspergillus niger* inoculum levels and incubation periods. The lowest DMD value was observed in treatment T1L1, reaching 34.99%, whereas the highest DMD was obtained in treatment T3L3 at 40.22%. A similar pattern was observed for OMD, with the lowest value recorded in T1L1 (54.60%) and the highest value in T3L3 (63.39%).

The low digestibility values observed in treatment T1L1 were presumably due to the low inoculum level of *Aspergillus niger* combined with a relatively short incubation period, which limited fungal growth and development. Under these conditions, the microorganism was likely still in the adaptation (lag) phase, resulting in suboptimal production of cellulolytic enzymes and limited fiber degradation. In contrast, treatment T3L3, which combined a higher inoculum level with a longer incubation period, allowed for more extensive fungal growth and greater cellulase production, thereby enhancing fiber degradation and digestibility.

Despite these improvements, the pattern of increase in DMD and OMD across treatments was not entirely linear. This observation is consistent with the results of the analysis of variance, which indicated no significant interaction between inoculum level and incubation period on digestibility values. This finding suggests that increases in inoculum level and incubation duration exert independent effects on the digestibility of sugarcane tops rather than acting synergistically.

The application of *Aspergillus niger* inoculum to sugarcane tops was shown to improve both dry matter and organic matter digestibility compared to the control treatment. This improvement is associated with the activity of cellulolytic enzymes produced by the fungus, which are capable of reducing structural fiber fractions such as Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF). The reduction in these fiber fractions increases the proportion of dry matter that can be digested by rumen microorganisms. These results are in agreement with the findings of Lutojo (2005), who reported that fermentation using *Aspergillus niger* reduced NDF and ADF contents and improved the nutritional quality of fibrous feed materials.

Furthermore, the ammoniation process applied prior to fermentation played a critical role in loosening lignocellulosic bonds, thereby enhancing the accessibility of cellulose and hemicellulose to cellulase enzymes produced by *Aspergillus niger*. Increased cellulose degradation during fermentation led to higher digestible organic matter, which subsequently contributed to an overall improvement in dry matter digestibility. These findings support the conclusions of Murfiah (2014) and Omer et al. (2010), who reported that *Aspergillus niger* exhibits effective cellulolytic activity in improving the quality of fibrous feed resources.

Nevertheless, the DMD values of fermented sugarcane tops obtained in this study remained relatively low and did not reach the range considered optimal for dry matter digestibility. This indicates that although fermentation and ammoniation significantly improved digestibility, the complex lignocellulosic structure of sugarcane tops remains a major

limiting factor. Therefore, further processing strategies such as optimization of urea dosage, application of microbial consortia, or the integration of additional biological treatments are required to achieve more substantial improvements in the digestibility of sugarcane tops.

IV. CONCLUSION

Based on the results of this study, *Aspergillus niger* inoculum level had no significant effect on the dry matter and crude protein contents of sugarcane tops, whereas incubation period had a highly significant effect on both parameters. The interaction between inoculum level and incubation period significantly affected dry matter content but had no significant effect on crude protein content. The combination treatment T2L3, consisting of an *Aspergillus niger* inoculum level of 10^6 CFU/g sugarcane tops with an incubation period of 30 days, produced the best relative results, yielding a dry matter content of 84.28% and a crude protein content of 6.04%. Regarding digestibility parameters, the interaction between inoculum level and incubation period had no significant effect on dry matter digestibility (DMD) or organic matter digestibility (OMD). However, increasing *Aspergillus niger* inoculum level and extending the incubation period independently resulted in highly significant improvements in both DMD and OMD of sugarcane tops. Nevertheless, the dry matter digestibility values obtained in this study remained relatively low, indicating that fermentation using *Aspergillus niger* alone was not sufficient to fully optimize the digestibility of sugarcane tops. Overall, the findings indicate that incubation period plays a more critical role than inoculum level in improving the nutritional quality of sugarcane tops. These results also suggest that additional or combined treatment strategies are required to achieve more substantial improvements in digestibility.

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