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Research Article Use of Fiber Cracking Technology to Improve the Nutritive Quality of Corn and Sugarcane By-products for Ruminant Feeds

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Abstract

Background and Objective: Both corn and sugarcane by-products have high fiber content but low nutritive quality, which leads to decreased livestock productivity. This study aimed to evaluate the use of fiber cracking technology (FCT) to improve the nutritive quality of corn and sugarcane by-products. Feeds used in this experiment included corn straw, corn husks, corn cobs, sugarcane tops and bagasse. Materials and Methods: The feeds were combined with 5% urea and added to the FCT operated at a temperature of 135°C and a pressure of 2.30 atm for 2.5 h. The treatments tested in this study included the following: T1 (untreated corn straw), T2 (corn straw+FCT+5% urea), T3 (untreated corn husk), T4 (corn husk+FCT+5% urea), T5 (untreated corn cob), T6 (corn cob+FCT+5% urea), T7 (untreated sugarcane top), T8 (sugarcane top+FCT+5% urea), T9 (untreated bagasse) and T10 (bagasse+FCT+5% urea), with each one replicated 4 times. A Van Soest analysis was performed to determine the fiber fraction and in vitro analysis was used to measure the digestibility. Scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) were performed to confirm the decrease in the fiber fraction in the samples. Results: The FCT+5% urea treatment decreased the fiber fraction (NDF, ADF, cellulose and lignin) content of corn and sugarcane by-products. Through SEM, XRD and FTIR analysis, it was shown that the FCT+5% urea treatment destroyed the cell wall structure, decreased the cellulose crystallinity index and broke down the fiber fraction bonds. In addition, the treatment generally increased the IVDMD, IVOMD and ruminal ammonia concentration (p<0.05), decreased the propionate proportion (p<0.05) and elevated the methane emission and the ratio of acetate to propionate (p<0.05). However, the treatment did not affect the acetate, butyrate, valerate or total VFA concentrations. **Conclusion:** The combination of FCT+urea treatment can effectively improve the quality of corn and sugarcane by-products.

Key words: Fiber cracking, infra red, electron microscopy, urea, crystallinity index

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The availability of fodder for livestock feeding is reduced during dry seasons. Agricultural by-products are often used to overcome feed limitations during dry seasons, including corn and sugarcane by-products such as corn straw¹, corn husks², corn cobs³, sugarcane tops⁴ and bagasse⁵. The main obstacle that occurs with the use of agricultural by-products as ruminant feed is their low nutritive quality. These by-products are typically characterized by high fiber content (lignocellulosic components), which is difficult to digest and contributes to the low production performance of livestock⁶. Therefore, it is necessary to perform certain processing techniques on agricultural by-products in order to improve their quality and potentially increase the productivity of livestock.

The processing techniques for agricultural by-products consist of physical, chemical and biological processing. The ammonia treatment is able to destroy lignocellulosic bonds⁷ and urea is commonly used for ammonia treatment, since the utilization of urea is safe, easy to obtain and easy to use. The use of urea in the diet improves dietary crude protein content as well and may be converted to microbial protein in the rumen that in turn enhances nitrogen utilization of ruminants⁸. Combinations of different processing techniques are more effective to break down lignocellulosic components, such as the combination of high temperature, high pressure, steam and the addition of urea. Combinations of these processing techniques have been studied previously for enhancing the nutritive values of rice straw⁹ and oil palm tree empty fruit bunches¹⁰.

This study aimed to evaluate the use of fiber cracking technology (FCT) to improve the nutritive quality of corn and sugarcane by-products, (corn straw, corn husks, corn cobs, sugarcane tops and bagasse). Scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) analyses were employed to confirm the effects of FCT on the corn and sugarcane by-products. An *in vitro* rumen fermentation study was also conducted to determine their digestibility.

MATERIALS AND METHODS

Experimental setup: The corn and sugarcane by-products were analyzed by Van Soest to determine fiber fraction content. SEM, XRD and FTIR methods were used to prove the lignocellulosic bond termination. *in vitro* analysis was conducted to determine the biological digestibility of the

by-products. These analyses were conducted from November, 2016 to February, 2018. The fiber cracking technology (FCT) analysis was performed at the Laboratory of Science and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Indonesia. The Van Soest analysis was conducted at the Laboratory of Proximate, Livestock Research Center, Ciawi, Bogor, Indonesia. The scanning electron microscopy (SEM) and X-ray diffraction (XRD) analyses were performed at the Nanotechnology Laboratory, Center for Agricultural Post-Harvest Research and Development, Cimanggu, Bogor, Indonesia. The Fourier transform infrared spectroscopy (FTIR) was conducted at Center for Tropical Biopharmaca Studies, Bogor Agricultural University, Indonesia. The in vitro analysis was conducted at the Laboratory of Dairy Nutrition, Faculty of Animal Science, Bogor Agricultural University, Indonesia. Partial VFA analysis was performed at the Laboratory of Center for Food and Nutrition Studies, Gadjah Mada University, Indonesia.

Sample collection and preparation: Samples were collected from two different places: corn straw, corn husks and corn cobs were obtained from a corn field in Jati Asih, Bekasi, Indonesia, while sugarcane tops and bagasse were obtained from a sugarcane field in Bogor, Indonesia. The samples were chopped and then oven-dried at 60°C for 48 h. All samples were subsequently ground by a hammer mill to pass through a 5 mm screen for fiber cracking technology (FCT) incubation and a 1 mm screen for chemical composition determination, *in vitro* incubation and SEM, XRD and FTIR analyses. The samples were divided into the following treatments:

 T1: Untreated corn straw (control), T2: Corn straw+FCT+ 5% urea, T3: Untreated corn husks (control), T4: Corn husks+FCT+5% urea, T5: Untreated corn cobs (control), T6: Corn cobs+FCT+5% urea, T7: Untreated sugarcane tops (control), T8: Sugarcane tops+FCT+5% urea, T9: Untreated bagasse (control) and T10: Bagasse+FCT+ 5% urea. The fiber cracking technology (FCT) used in this experiment is an incubator that has a 50 L capacity, can reach a temperature of 200°C and can be pressurized up to 5 atm. The system was set to run at 2.30 atm and 135°C for 2.50 h. Urea (CO(NH₂)₂) was solubilized in the water before being added to the samples, according to Jayanegara¹⁰. The samples were sprayed by the urea solution, mixed thoroughly and then put into the FCT. This experiment was conducted in four replicates

Chemical component analysis: All samples were analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF) and

lignin content according to Van Soest *et al.*¹¹. The value of NDF was the result of a 1 g sample boiled in 100 mL neutral detergent solution (consisting of EDTA, sodium tetraborate, SDS, mono glycol ether, sodium dihydrogen phosphate and distilled water) for 1 h; the residue was rinsed with Aqua Dest and acetone and oven-dried at 105°C. The determination of ADF is similar to that of NDF, except that the solution used for ADF is an acid detergent solution (consisting of CTAB, sulfuric acid and distilled water). The NDF and ADF content values were expressed minus the residual ash. The lignin was measured by adding 72% sulfuric acid to the ADF residue and stored for 3 h before being rinsed with hot water and acetone. The residue was then oven-dried at 105°C for 4 h and burned in a 600°C furnace. The cellulose value was determined by the difference between the NDF and the ADF. The NDF, ADF, cellulose and lignin analyses were performed in duplicate.

Scanning electron microscopy (SEM) analysis: The samples were prepared as dry powder in specimen holders (stubs) that were coated with carbon tabs. The sample was affixed to the surface of the specimen holder in as a thin layer as possible. The coating process was performed by a Quorum type Q150R ES sputter coater device and employed gold material (gold coating) with a sputter current of 20 mA and a sputter time of 60 sec. Furthermore, the sample that had been coated in the specimen holder was mounted in-stage for SEM analysis. The sample stages were inserted into the chamber and the image was taken by the ZEISS SEM with the EVOMA 10. The SEM image was taken by a secondary electron detector, WD (working distance) 8.0 mm, EHT 16.00 kV with 500, 1000 and 2000 magnification.

X-ray diffraction (XRD) analysis: The samples were placed in holders and then flattened and inserted into the Bruker D8 ADVANCE ECO diffractometer, which was operated in reflection mode (40 kV, 35 mA), using Cu-K α radiation (| 1 = 1.54 Å and | 2 = 1.54 Å) and a step-scan mode that started at (°2 θ) of 5.00°, with a step size (°2 θ) of 0.02° (76.8 sec per step) and ended at (°2 θ) of 80.1°.

Fourier transform infrared spectroscopy (FTIR) analysis:

Approximately 200 mg KBr was put into mortar with a 2 mg sample and quickly mixed until homogeneous raw pellets were produced. The pellet was made by a pelletizer and stored in a dry place. The samples were inserted into the Bruker TENSOR 37 Fourier transform infrared spectrometer.

In vitro rumen incubation: Rumen fluid and McDougall buffer were mixed according to Tilley and Terry¹² to incubate

the samples. Rumen inoculum (from fluid and solid particle) was obtained from three fistulated Ongole crossbred cattle at the Biotechnology Research Center, Indonesian Academy of Sciences (Lembaga Ilmu Pengetahuan Indonesia, LIPI), Cibinong, Bogor, Indonesia. In the first step, a 0.75 g sample was inserted into a 125 ml serum bottle to which 75 ml buffered rumen fluid (ratio rumen fluid:buffer was 1:4 v/v) was added. The allocation of treatments into the serum bottles as experimental units followed a randomized complete block design. Incubation was performed in three replicates and each treatment was represented by two serum bottles. The serum bottles were immediately sealed with butyl rubber stoppers and aluminum crimp seals to start the incubation at 39°C. The supernatant was collected for determination of the ammonia (NH₃) concentration as well as partial and total volatile fatty acid (VFA) after 24 h, according to Jayanegara et al.¹³. The residue was incubated with a 75 mL pepsin-HCl 0.20 N solution for another 24 h to measure the in vitro dry matter digestibility (IVDMD) and the in vitro organic matter digestibility (IVOMD) and corrected for blanks. The partial VFA consisted of C_2 (acetate), C_3 (propionate), C_4 (butyrate) and C₅ (valerate) and was analyzed using gas chromatography. Methane (CH₄) production was estimated by a partial VFA through a stoichiometric equation, according to Moss et al.14, where:

$$CH_4 = (0.450 \times C_2) - (0.275 \times C_3) + (0.4 \times C_4)$$

The equation had been shown to be very accurate through a comparison of methane estimated with methane measured using an infrared methane analyzer¹⁵.

Data analysis: Chemical composition data were descriptively tabulated. *In vitro* data were analyzed by a two-way analysis of variance (ANOVA) by following a randomized complete block design. The blocks represented the number of different rumen fluid batches taken at different weeks. The following statistical model was used:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

Where:

 $Y_{ij} = Observed value for ith treatment$

- jth = Replicate
- μ = Overall mean
- τ_i = Treatment effect
- $\beta_i = \text{Block effect (replicate)}$
- ϵ_{ii} = Random residual error

The significance of the result was indicated when the ANOVA showed p<0.05. Any significant difference among the treatments within a parameter was examined through a post hoc test, namely, Duncan's multiple range test. Data were checked for outlier values before the ANOVA; any values lower than -2 or higher than 2 of their standardized residuals were categorized as outliers. SPSS software version 21.0 was used for statistical analysis.

RESULTS

Chemical composition: Table 1 shows high NDF, ADF, cellulose and lignin values in all untreated samples. Lower NDF, ADF, cellulose and lignin content values were found in all samples treated with FCT+5% urea (T2, T4, T6, T8, T10) compared to those of the untreated samples (T1, T3, T5, T7, T9).

Scanning electron microscopy (SEM) analysis: Figure 1 shows that the cross-section of the cell wall in an untreated corn cob indicates a normal cell wall structure. After FCT+5% urea treatment, the corn cob showed damage in the cross-section of the cell wall. Similarly, the cross-section of the cell wall in the untreated bagasse showed a normal cell wall structure, but it was destroyed after the FCT+5% urea treatment (Fig. 2). These results confirm the breakdown of cell walls in corn and sugarcane by-products due to the FCT+5% urea combination.

X-ray diffraction (XRD) and crystallinity index: The effects of the X-ray diffractometer on both the untreated and the treated corn cobs and bagasse were presented in Fig. 3. Samples treated with FCT+5% urea (T2, T4, T6, T8) had a lower percentage of crystallinity structure than that of the untreated samples (T1, T3, T5, T7), except in T9 and T10 (Table 2). Conversely, the percentage of amorphous structure of samples treated with FCT+5% urea was higher than that of the untreated was used to examine the degree of cellulose crystallinity of corn and sugarcane by-products.

Fourier transform infrared spectroscopy (FTIR) analysis:

Figure 4 shows that transmittance of both the untreated corn cobs and the corn cobs treated with FCT+5% urea was different in some wave number regions. The treated corn cobs had higher transmittance, especially at 900-1,400 cm⁻¹. The treated bagasse had a higher transmittance at 900-1,400 and 2,800-3,000 cm⁻¹. However, both the treated and untreated corn cobs had relatively similar transmittance at 1.800-2.400

Table 1: Neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose and
lignin (% dry matter) contents of untreated and treated corn and
sugarcane by-products with fiber cracking technology (FCT) and 5%

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Treatment	NDF	ADF	Cellulose	Lignin
T1	69.2	44.8	27.6	7.26
T2	45.7	22.0	14.5	4.63
T3	69.4	33.4	27.2	4.88
T4	45.7	12.2	11.1	1.06
T5	83.8	43.5	33.9	7.43
T6	61.5	21.2	20.4	2.22
T7	73.0	41.2	31.8	3.87
Т8	49.5	20.7	17.7	0.85
Т9	83.8	51.7	43.2	2.11
T10	56.5	29.8	25.1	0.57

T1: Untreated corn straw (control), T2: Corn straw+FCT+5% urea, T3: Untreated corn husk (control), T4: Corn husk+FCT+5% urea, T5: Untreated corn cob (control), T6: Corn cob+FCT+5% urea, T7: Untreated sugarcane top (control), T8: Sugarcane top+FCT+5% urea, T9: Untreated bagasse (control), T10: Bagasse +FCT+5% urea, NDF: Neutral detergent fiber, ADF: Acid detergent fiber

Table 2: Percentage of crystallinity and amorphous of treatments by using X-Ray diffraction (XRD) method

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Crystallinity (%)	Amorphous (%)				
39.5	60.5				
37.4	62.6				
33.8	66.2				
32.7	67.3				
34.7	65.3				
34.4	65.6				
48.7	51.3				
47.5	52.5				
40.6	59.4				
41.0	59.0				
	Crystallinity (%) 39.5 37.4 33.8 32.7 34.7 34.7 34.4 48.7 47.5 40.6 41.0				

T1: Untreated corn straw (control), T2: Corn straw+FCT+5% urea, T3: Untreated corn husk (control), T4: Corn husk+FCT+5% urea, T5: Untreated corn cob (control), T6: Corn cob+FCT+5% urea, T7: Untreated sugarcane top (control), T8: Sugarcane top+FCT+5% urea, T9: Untreated bagasse (control), T10: Bagasse +FCT+5% urea

and 2.800-3.000 cm⁻¹, while the treated and untreated bagasse had similar transmittance at 1.800-2.400 cm⁻¹.

In vitro rumen fermentation characteristics: Samples treated with FCT+5% urea generally had higher IVDMD, IVOMD and ammonia levels than those of the untreated samples (p<0.05), although some data were insignificant due to the high variability (Table 3). However, treatment of FCT and urea tended to increase the methane emission. The ammonia concentration in the untreated samples ranged from 7.8-12.1 mM, while the range of ammonia concentration obtained in the treated samples with FCT+5% urea was from 74.3-96.0 mM.

In vitro ruminal VFA profile: The FCT+5% urea treatment generally decreased the propionate and increased the ratio of acetate to propionate (Table 4, p<0.05),



Fig. 1(a-c): Scanning electron microscopy (SEM) of untreated (left) and treated with FCT+5% urea (right) on corn cob at (a) 500x, (b) 1000x and (c) 2000x magnification

Table 3: Parameters of *in vitro* ruminal incubation on untreated and treated corn and sugarcane by-products with Fiber Cracking Technology (FCT) and 5% urea at 24 h incubation period

Treatment	IVDMD (%)	IVOMD (%)	NH₃(mM)	CH ₄ (mM)
T1	39.4±17.63 ^{ab}	34.5±15.96 ^a	10.1±1.42 ^{bc}	7.0±1.93ª
T2	$46.8 \pm 16.59^{\text{abc}}$	46.7±15.29 ^b	80.3±1.8 ^e	11.9±3.33℃
T3	50.8±12 ^{bc}	48.5±11.66 ^b	9.1±1.4 ^{ab}	8.5 ± 1.54^{abc}
T4	52.1±11.64 ^c	52.2±9.47 ^b	76.0±1.51 ^d	7.0±1.55ª
T5	37.5±13.11°	35.1±12.8ª	7.8±2.03ª	7.5 ± 0.52^{ab}
T6	53.0±15°	51.2±13.9 ^b	74.3±2.13 ^d	10.9±0.57 ^{bc}
T7	50.8±11.29 ^{bc}	50.5±11.2 ^b	8.4±1.02 ^{ab}	6.6±1.77ª
Т8	51.0±7.38 ^{bc}	51.8±7.69 ^b	75.6±2.85 ^d	8.5 ± 3.74^{abc}
Т9	43.8±8.03 ^{abc}	44.7±8.03 ^{ab}	12.1±1.21°	5.7±1.51ª
T10	49.9±4.55 ^{bc}	50.4±4.31 ^b	96.0±1.11 ^f	7.3±2.89 ^{ab}

Different superscripts within the same column are significantly different at p<0.05. T1: Untreated corn straw (control), T2: Corn straw+FCT+5% urea, T3: Untreated corn husk (control), T4: Corn husk+FCT+5% urea, T5: Untreated corn cob (control), T6: Corn cob+FCT+5% urea, T7: Untreated sugarcane top (control), T8: Sugarcane top +FCT+5% urea, T9: Untreated bagasse (control), T10: Bagasse+FCT+5% urea, IVDMD: *In vitro* dry matter digestibility, IVOMD: *In vitro* organic matter digestibility, pH: Potential of hydrogen, NH₃: Ammonia, CH₄: Methane

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Fig. 2(a-c): Scanning electron microscopy (SEM) of untreated (left) and treated with FCT+5% urea (right) on bagasse at (a) 500x, (b) 1000x and (c) 2000x magnification

Table 4: In vitro ruminal volatile fatty acid (VFA) profiles of untreated and treated corn and sugarcane by-products with fiber cracking technology (FCT) and 5% urea at 24 h incubation period

Treatment	C ₂ (%)	C ₃ (%)	C ₄ (%)	C ₅ (%)	C ₂ /C ₃	Total VFA (mM)
T1	40.5±0.33	24.6±1.02 ^{ab}	15.0±2.28	2.99±0.39	1.65±0.06 ^{bc}	40.1±0.3
T2	44.5±1.78	22.8±1.91ª	14.2±3.04	2.81±0.57	1.96±0.15 ^d	61.1±0.38
Т3	40.6±3.39	24.9±0.56 ^{ab}	13.8±0.04	2.26±0.12	1.63±0.1 ^{bc}	50.0±0.18
T4	39.8±2.44	25.0±2.43 ^{ab}	13.4±1.99	3.70±1.19	$1.60 \pm 0.18^{\text{abc}}$	42.6±0.86
T5	39.9±2.47	27.0±1.79 ^{bc}	14.5±1.6	2.25±0.46	1.48±0.01 ^{ab}	46.1±0.25
T6	43.1±1.81	24.5±0.65 ^{ab}	14.1±2.88	2.51±0.36	1.76±0.04°	60.0±0.24
T7	40.7±2.83	28.7±1.33°	13.6±3.32	2.26±0.37	1.42±0.04ª	41.2±0.36
Т8	42.3±2.04	24.4±1.77 ^{ab}	17.2±4.07	2.72±0.03	1.74±0.2°	44.4±0.56
Т9	40.0±2.28	26.5±3.32 ^{bc}	14.2±4.94	2.12±0.27	1.52±0.12 ^{ab}	35.7±0.7
T10	40.1±3.52	25.5±1.85 ^{ab}	14.0±2.18	2.15±0.29	1.57±0.02 ^{abc}	43.9±0.23

Different superscripts within the same column are significantly different at p<0.05. T1: Untreated corn straw (control), T2: Corn straw+FCT+5% urea, T3: Untreated corn husk (control), T4: Corn husk+FCT+5% urea, T5: Untreated corn cob (control), T6: Corn cob+FCT+5% urea, T7: Untreated sugarcane top (control), T8: Sugarcane top +FCT+5% urea, T9: Untreated bagasse (control), T10: Bagasse+FCT+5% urea, C₂: Acetate, C₃: Propionate, C₄: Butyrate, C₅: Valerate, C₂/C₃: Ratio of acetate to propionate, VFA: Volatile fatty acid



Fig. 3(a-b): X-ray diffractogram (XRD) of untreated (black color) and treated with FCT+5% urea (red color) of (a) Corn straw and (b) Sugarcane top

but the treatment had no significant difference on acetate, butyrate, valerate and total VFA concentrations (Table 4).

DISCUSSION

The high temperature and pressure generated by the FCT apparently induces the release of the acetyl group from the fiber structure, leading to an enhancement of substrate acidity and an increase in fiber solubility¹⁰. Further, both urea

and ammonia can damage the lignin structure¹⁶. The combination of FCT+5% urea increases the effectivity of fiber degradation in corn and sugarcane by-products. According to Tsabitah *et al.*⁷, a combination of two or more processing techniques such as those in this experiment would be more efficient and effective in decreasing the fiber components and the breakdown of the lignocellulosic bonds.

According to El Abed *et al.*¹⁷, SEM has the combination of higher magnification, greater resolution and greater field

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Fig. 4(a-b): Fourier transform infra-red spectroscopy (FTIR) untreated (blue color) and treated with FCT+5% urea (red color) on (a) Corn cob and (b) Bagasse

depth. SEM uses a beam of electrons to form a specimen image from the sample and produces a good representation of a three-dimensional sample. The decrease in the cellulose crystallinity index of the samples treated with FCT+5% urea is apparently due to the breakdown of cellulosic hydrogen bonds¹⁸. In this study, FTIR spectroscopy was used to show the breakdown of lignocellulosic bonds. FTIR has been used to study the characterization of hydrogen bonding in cellulose¹⁹. According to Adapa *et al.*²⁰, FTIR spectroscopy is able to determine changes in the composition of cellulose,

hemicellulose and lignin before and after treatment on agricultural biomass. The samples treated with FCT+5% urea showed a higher peak than that of the untreated samples. A higher peak indicates a transmittance increase due to the breakdown of the lignocellulose C-O-C bond²¹.

The treatment of FCT+urea of fibrous feed led to an increase in IVDMD and IVOMD¹⁰. An increase in digestibility (IVDMD and IVOMD) is caused by the breakdown of lignin bonds with cellulose and hemicellulose; this enables rumen microbes to access and degrade fiber more easily. Urea may

be converted to ammonia and further utilized by rumen microbes as a substrate of microbial protein synthesis²². Methane emission increased along with an increase of IVDMD and IVOMD values after the treatment with FCT and urea. The main components of VFA in rumen fluid from the largest to the smallest proportion are acetate, propionate and butyrate. Formate, isobutyrate, valerate, isovalerate and caproate are present in small proportions in the VFA component. The small proportion of butyrate is due to butyrate being more easily absorbed than acetate and propionate are²³. The treatment of FCT and urea was previously shown to increase the total VFA concentration, a result that did not align with the present study¹⁰.

CONCLUSION

Fiber cracking technology combined with the addition of 5% urea is able to reduce the fiber fraction in both corn and sugarcane by-products and increase their *in vitro* digestibility. This effect is possible through the breakdown of lignocellulose bonds and the destruction of cell walls as confirmed by SEM, XRD and FTIR analyses. Fiber cracking technology can be an excellent processing technology to improve the quality of fibrous feeds.

SIGNIFICANCE STATEMENT

This study discovers the potential use of fiber cracking technology that can improve nutritive values of agricultural residues by effectively degrading their fiber components. This study will help the researcher to uncover the critical area of fiber degradation by combination of different processing techniques that many researchers were not able to explore. Thus, a new theory on fiber structural and functional group changes, may be arrived at.

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