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The Improvement of Maize Cobs Quality through Soaking in Firewood Ash Filtrate and Its Impact on *In Vitro* Rumen Fermentability and Digestibility

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Abstract*— Maize cobs can be used as a ruminant's feed, but low digestibility and its quality can be improved by soaking in firewood ash filtrate (FAF). The study aimed to test FAF treatments' effect on rumen fermentation and digestibility using a completely randomized design. Firewood ashes were obtained from Sumedang, Indramayu, and Bogor Regencies. The maize cobs were soaked by FAF at a ratio of 1:1 (w/v) for 3 hours at different concentration, i.e. 0.05 w/v (FA5), 0.10 w/v (FA10), 0.15 w/v (FA15), and 0.20 w/v (FA20). *In vitro* evaluation was done to measure the fermentability and digestibility of diets containing maize cobs soaked by FAF 0.20 w/v at the levels of 10% (MFA10), 20% (MFA20), 30% (MFA30), and 40% (MFA40). The results showed that firewood ash contained Calcium (Ca, 10.03%), Magnesium (Mg, 1.45%), Sodium (Na, 0.91%), and Potassium (K, 3.11%). The pH of FAF ranged from 11.46 to 12.16. The levels of neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose, hemicellulose, and crude fiber (CF) decreased significantly ($p < 0.05$) along with increased FAF concentration. The use of maize cobs soaked by FAF 0.20 w/v with 30% in the diets yielded the highest values of NH_3, volatile fatty acids (VFA), dry matter digestibility (DMD), and organic matter digestibility (OMD) ($p < 0.05$). The concentration of 0.20 w/v has resulted in the highest decrease in lignin content of maize cob while their uses up to 30% in the diets provided the best *in vitro* rumen fermentability and digestibility.

***Keywords*— ADF; cellulose; digestibility; hemicellulose; lignin; NDF; ruminant**

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I. Introduction

Agricultural waste is commonly widespread in Indonesia. Maize cob is an agricultural waste used as a fiber source of feed [1]. Its production reaches about 9 million tons per year. Maize cob has high cellulose and hemicellulose, with low protein content <5% [2], and has high lignin >10% [3]. The use of maize cobs for livestock required pretreatment because *in vitro* digestibility were <50% [4], resulting in low performance in ruminants. Several processing methods have been tried to improve maize cobs' quality, such as using NaOH, KOH, $Ca(OH)_2$, urea, *Trichoderma viridae*, *Aspergillus* or ensilage [4].

Maize cobs improvement for ruminant feeding can be processed using local organic waste ash and can improve their digestibility [5]. Processing in this way is more straightforward and cheaper as it is widely available in many rural areas and environmentally friendly compared to chemical treatments [6]. The use of wood as a household fuel in rural areas is still relatively high [7]. Bowe [8] estimates that about 55% of all wood harvested is used as fuel. Burning firewood produces ash that can treat maize cobs to improve its quality as the feed of ruminants.

II. Material and Method

A. Materials

Milled maize cobs used in this study were collected from maize farms in Bogor, while firewood ash was obtained from Sumedang, Indramayu, and Bogor Regencies. Native grass was harvested from the faculty-owned farm, and the feed ingredients to make a concentrate were purchased from a feed mill located in Sumedang. Chemicals and reagents used in this

experiment were purchased from a chemical store in Bandung city. All chemicals were categorized as an analytical grade.

B. Minerals Analysis Procedures

Firewood ashes were taken from three different locations (Sumedang, Indramayu, and Bogor Regencies). Firewood ash sample from each regency was subjected into several mineral analyses such as Ca, Mg, Na, and K.

For mineral analysis, each sample was initially prepared using a wet ash extraction method [9]. The sample was weighed in a 100 ml Erlenmeyer flask, then 5 mL of concentrated HNO_3 was added and kept for ±1 hour until it became clear. Next, the sample was heated for 4 hours on a hot plate. After 4 hours, the sample was cooled, and 0.4 mL of concentrated H_2SO_4 was added, then heated again for ± 30 minutes. When the color changed, the sample was added by 2-3 drops of a solution of a mixture of $HClO_4$ + HNO_3 (2: 1) and then heated again for ± 15 minutes. Finally, the sample was added with 2 mL of distilled water, and 0.6 mL of concentrated HCl was simultaneously added. After that, it was heated for ± 15 minutes until being dissolved. The sample was allowed to cool at room temperature and then dissolved in distilled water until 100 mL in a measuring flask. The wet ash extraction sample was added with 0.05 mL of Cl3La.7H2O solution, then centrifuged at 2500 rpm for 10 minutes. Finally, the absorbance was measured by atomic absorption spectroscopy (AAS) instrument Shimadzu AA-7000 at the wavelength according to the type of mineral to be read.

C. Soaking Maize Cobs with Firewood Ash Filtrate (FAF)

Dry maize cobs (dry matter/DM 88.94% or moisture content 11.06%) were milled using a grinding machine with a screen size diameter of 8-10 mm. Firewood ash (FA) was weighed and mixed with distilled water at ratio of 0.05 w/v (FA5), 0.10 w/v (FA10), 0.15 w/v (FA15), and 0.20 w/v (FA20). It was then stirred evenly and keep for twenty-four hours until the water becomes clear, then filtered with calico cloth (unbleached cotton) to be prepared as FAF. Each concentration of FAF was repeated five times, and the pH was measured by a pH meter.

The maize cobs were soaked by FAF as per treatment at a ratio of 1: 1 (w/v) and put in an airtight plastic container for three hours. After that, the substrate was dried using sunlight and an oven drying. Samples before and after soaking by FAF were prepared for crude fiber (CF) analysis, and fiber component analysis consisted of neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, and hemicellulose [10]. Cellulose content was calculated as ADF−Lignin, whereas hemicellulose content was calculated as NDF−ADF.

1) Neutral Detergent Fiber (NDF) Measurement Procedure: A sample of 0.5 g was weighed and put into Erlenmeyer flask (A), and 30 ml of α-amylase solution was added, then incubated at 40°C for 16 hours. The mixture was mixed with 200 ml of NDF solution and 0.5 g of Na_2SO_3 and boiled in an upright cooler for 60 minutes. Next, the mixture was filtered through a 2-G-3 glass filter and washed by hot distilled water several times and rinsed the sediment with acetone several times. The filter and precipitate were dried by oven at 105°C for at least 8 hours until a constant weight was obtained (B). Finally, the filter and sediment were burned in a furnace at 400-450°C for 3 hours until a constant weight (C) was obtained.

$$\% \text{ NDF} = (\text{B-C})/\text{A} \times 100\% \quad (1)$$

2) Acid Detergent Fiber (ADF) Measurement Procedure: A total of 1 g sample was weighed and put into an Erlenmeyer glass. About 100 ml of ADF solution was added and boiled in an upright cooler for 60 minutes. The mixture was filtered through a 2-G-3 glass filter, and the precipitate obtained was washed with hot distilled water several times, then washed again with acetone several times. The glass filter and precipitate were dried in an oven at 105°C for 8 hours until a constant weight (B) was obtained. Finally, the precipitate was burned in a furnace at a temperature of 400-450°C until a constant weight (C) was obtained.

$$\% \text{ ADF} = (\text{B-C})/\text{A} \times 100\% \quad (2)$$

3) Lignin Measurement Procedure: The sample was weighed as much as 0.5 g and put into Erlenmeyer flask (A), where about 100 ml of ADF solution was added. The mixture was boiled in an upright cooler for 60 minutes. Next, the mixture was filtered by filter paper, and the precipitate was washed with hot distilled water. The precipitate was taken and transferred to another Erlenmeyer, where about 25 ml of 72% H_2SO_4 was added. The mixture was shaken for 2 to 3 hours at 20 °C. The concentration of the mixture was diluted with distilled water until the concentration reached 3%. The mixture was heated in a water bath at 100°C for 2-4 hours. After finishing, the mixture was filtered with a 2-G-4 glass filter, and the sediment was washed with distilled water many times. The precipitate was rinsed with acetone several times. The filter and precipitate were dried in an oven at 105°C until a constant weight was obtained (B) and finally burned in a furnace 400-450°C until a constant weight was obtained (C).

$$\% \text{ Lignin} = (\text{B-C})/\text{A} \times 100\% \quad (3)$$

D. In Vitro Study

The best-soaked maize cobs by FAF (MFA) were used in the diet up to 10% (MFA10), 20% (MFA20), 30% (MFA30), and 40% (MFA40), replacing the field grass as a source of fiber. The experimental diets consisted of 60% forage fiber source and 40% concentrate on the DM basis. The concentrate was a mixture of feed ingredients such as coconut meal (11.3%), soy sauce waste (15.4%), corn gluten feed (6.0%), pollard (11.9%), corn (6.0%), rice bran (7.7%), wheat hull (15.8%), cassava meal (22.3%), molasses (1.2%), and mineral mix (2.4%) on the DM basis. The nutrient contents of each feed ingredient were determined using proximate analysis.

1) Dry Matter Measurement Procedure: The formula to calculated dry matter is 100% - % moisture content. Moisture content was determined by weighing a sample of about ±3 g in a dried aluminum plate which its weight was initially known. The plate containing the sample was then dried in an oven at 105°C for three hours. The plate was removed, cooled in a desiccator and weighed. Drying was continued, and every half hour it was cooled and weighed until a constant weight was obtained. The water content was calculated by Equation 4 below.

$$\% \text{ Moisture} = \frac{Initial\ weight - Constant\ weight}{Initial\ weight} \times 100\% \quad (4)$$

2) Ash Content Measurement Procedure: A total of ±3 g of a sample was carefully weighed in a dried porcelain crucible which its weight was known. The crucible containing the sample was then heated in a furnace at 600°C for four hours. The crucible was removed, cooled in a desiccator and weighed until getting a constant weight. The ash content was calculated by Equation 5 below.

$$\%\, Ash = \frac{(Weight\ of\ crucible+ash)-Empty\ weight)}{Sample\ weight} \times 100\% \quad (5)$$

3) Crude Protein (CP) Measurement Procedure: After being ground, the sample was weighed as much as 1 g and put into the Kjeldahl flask. Then, 7 g of K_2SO_4 and 0.8 g of $CuSO_4$ were added to the Kjeldahl flask containing the sample. About 12 ml of H_2SO_4 solution was added in a fume hood. The digestion process was carried out in the acid chamber by heating the sample in the Kjeldahl flask using an electric stove until it turned to tosca green. The Kjeldahl flask was allowed to stand for 20 minutes, then 25 ml of distilled water was added to the Kjeldahl flask containing the sample. After that, 50 ml of 40% NaOH and a few boiling stones were added into the Kjeldahl flask containing the sample. About 30 ml of H_3BO_3 was added into the Erlenmeyer with the addition of 3 drops of BCG-MR indicator to capture distillate from the distillation product. The distillate obtained from the distillation result was titrated using a standard solution of 0.1 N HCl until the color of the solution turned into pink. Perform the same procedure for calculating % N blank (sample was replaced by distilled water), as shown in Equation 6. The %Crude protein is calculated using Equation 7.

$$\%N = \frac{ml\ of\ HCl\ (sample-blank)\times N\ HCl \times 14.008\times 100\%}{Sample\ weight\ (g)\times 1000} \quad (6)$$

$$\%Crude\ protein = \%N \times 6.25 \quad (7)$$

4) Crude Fat (CFt) Measurement Procedure: The sample was weighed ±3 g and wrapped in a dried filter paper which its weight was known. Extraction was carried out using a Soxhlet connected to a back cooler, a fat flask containing several boiling stones and a hot plate. The solvent used was hexane with a volume of half the boiling flask volume or about 250 ml of hexane. Extraction was carried out for five to six hours. The sample that has been oil extracted was dried in the oven and weighed until a constant weight was obtained. Fat content was calculated by Equation 8 below.

$$\%Crude\ fat = \frac{Initial\ weight-Final\ weight}{Initial\ weight} \times 100\% \quad (8)$$

5) *Crude Fiber (CF) Measurement Procedure:* A total of ± 2 g of fat extracted samples were put into 500 ml Erlenmeyer and added 100 ml of 0.325 N sulfuric acid solution. The sample mixture was then brought to a boil in an upright cooling device for about 30 minutes, and 50 ml of 1.25 N NaOH solution was added and boiled again for 30 minutes. The mixture was then filtered through Whatman no. 41 which has been dried, and its weight was initially determined. The filter's rinsing was carried out successively with 0.325 N HCl solution, hot water and ethanol. The filter paper was dried in the oven for 1-2 hours, then cooled in a desiccator and weighed. Drying was repeated every half hour until a constant weight was obtained. Crude fiber content was calculated by Equation 9 below.

$$\%Crude\ fiber = \frac{Weight\ of\ dry\ deposits}{Initial\ weight} \times 100\% \quad (9)$$

Nitrogen free extract (NFE) was calculated by the formula, as follow:

$$\%\ 100\ DM\text{-}\%Ash\text{-}\%CP\text{-}\%EE\text{-}\%CF \quad (10)$$

Treatment diets were then tested for *in vitro* rumen fermentability and digestibility. Each sample was weighed at ± 0.5 g, then placed into a fermenter tube and mixed with 40 mL artificial saliva solution (McDougall solution) that consist of $NaHCO_3$ 9.8 g, KCl 0.57 g, $CaCl_2$ 0.04 g, Na_2HPO_4 9.30 g, NaCl 0.47 g, $MgSO_4.7H_2O$ 0.12 g and H_2O 1000 g and 10 mL of rumen fluid. The rumen fluid was from some sheep obtained from slaughterhouses which previously known to have been fed field-based grasses. It was then put into a water bath at a temperature of ± 39°C. Three hours after incubation, rumen fluid samples were taken to test the ammonia (NH_3) content by micro diffusion method of Conway and total volatile fatty acids (VFA) by steam distillation method, and then the pH value was measured with a pH meter. The remainder was cycled for 2 x 48 hours, then measured DMD and OMD as described [11].

6) Volatile Fatty Acid Measurement Procedure: Measurement of the total volatile fatty acid content was carried out using a Markham steam distillation device. A total of 5 ml of supernatant was put into a steam distillation tube heated with water vapor. The tube was immediately closed tightly after adding 1 ml of 15% H_2SO_4. Hot water vapor will carry volatile fatty acids through the cooling tube so that it would be condensed and accommodated in an Erlenmeyer containing 5 ml of 0.5 N NaOH until it reached a volume of about 300 ml. Furthermore, 2 drops of phenolphthalein indicator were added and titrated with 0.5 N HCl. The titration ended at the starting point of the color change from red to clear. Finally, 5 ml of 0.5 N NaOH was titrated and used as a blank. The total volatile fatty acid content was calculated using the formula:

$$\text{Total volatile fatty acid} = (b - s)\ \text{x N HCl x } 1000/5\ \text{mM} \quad (11)$$

Where,
b = vol. blank titrant
N = normality of HCl solution
s = vol. the sample titrant

7) Ammonia (N-NH_3) Measurement Procedure: Ammonia (N-NH_3) levels were determined using the Conway dish's micro diffusion method. 1 ml of supernatant was placed on the left of the Conway plate, and 1 ml of NaOH was placed near the right. The middle was filled with 1 ml of boric acid with methyl red indicator and bromine cresol green. Then the Conway plate was closed tightly with a vascular lid and then shaken it so that the supernatant was mixed with the NaOH solution. The mixed solution was kept for 24 hours at room temperature. Ammonia bound with boric acid was titrated with 0.005 N H_2SO_4 until the color turns reddish. The following formula calculated the level of N-NH3.

$$\text{N-NH3} = (\text{ml titration x N H2SO4 x } 1000)\ \text{mM} \quad (12)$$

8) The pH of the Rumen Fluid Measurement Procedure: The pH value of the rumen fluid was measured using a pH meter (AOAC, 2005) which has been standardized with a

buffer solution at pH 7 for ± 10 minutes, then measured in a buffer solution of pH 4 for ± 10 minutes. The cathode part was immersed in the solution for ± 10 minutes until the number in the pH-meter did not move, then the pH value was recorded.

9) *Digestibility Measurement Procedure:* In stage I, the fermenter tube containing the sample was incubated for 48 hours. After that, 0.25 ml of Hg_2Cl_2 was added to kill the microbes for 20 minutes and centrifuged at 10,000 rpm for 10 minutes. The supernatant was separated, and 5 ml of pepsin 0.2% solution was added to the precipitate in the fermenter under acidic conditions with pepsin activity 1: 10,000. Phase II, the fermenter tube was incubated back into the water bath for 48 hours. During incubation, the fermenter tubes were shaken every 3 hours. After the fermentation was complete, the precipitate was filtered with Whatman No. 41, then analyzed the dry matter (DM) and organic matter (OM) contents. As a blank rumen fluid without any treatment was used. The dry matter digestibility (DMD) and organic matter digestibility (OMD) was calculated using the following equation:

$$\text{DMD (\%)} = \frac{\text{initial DM} - (\text{DM residue} - \text{DM blank})}{\text{initial DM}} \times 100\% \quad (13)$$

$$\text{OMD (\%)} = \frac{\text{initial OM} - (\text{OM residue} - \text{OM blank})}{\text{initial OM}} \times 100\% \quad (14)$$

E. Data Analysis

The research was conducted experimentally using a completely randomized design consisting of 4 treatments and 5 replications. Data were subjected to one-way analysis of variance using SPSS 21 software package. Means of treatments were then compared by using Duncan's multiple range test when variance analysis was significant (p<0.05).

Description analysis was used to describe ash mineral content and pH of FAF. The relationship between treatments with fiber component and CF was measured by regression analysis, while the relationship among fiber component and CF was analyzed using Pearson correlation with a similar software of SPSS 21.

III. Results and Discussion

A. Minerals in Firewood Ash

The mineral plays an important role in maintaining overall plant body functions. Calcium (Ca), potassium (K), magnesium (Mg), and sodium (Na) are alkaline mineral and essential for the plant. The analysis of minerals of firewood ash from three regencies in West Java is presented in Figure 1. In general, Ca was higher than other minerals, followed by K, Mg, and Na. The highest Ca obtained from Sumedang Regency, while firewood ash with the highest Mg, K and Na were obtained from Indramayu Regency. Meanwhile, Bogor Regency had higher Na, but that others had lower than the average value. Calcium is high in firewood because this mineral is needed in plant stems for cell elongation, protein synthesis, and cell division [12].

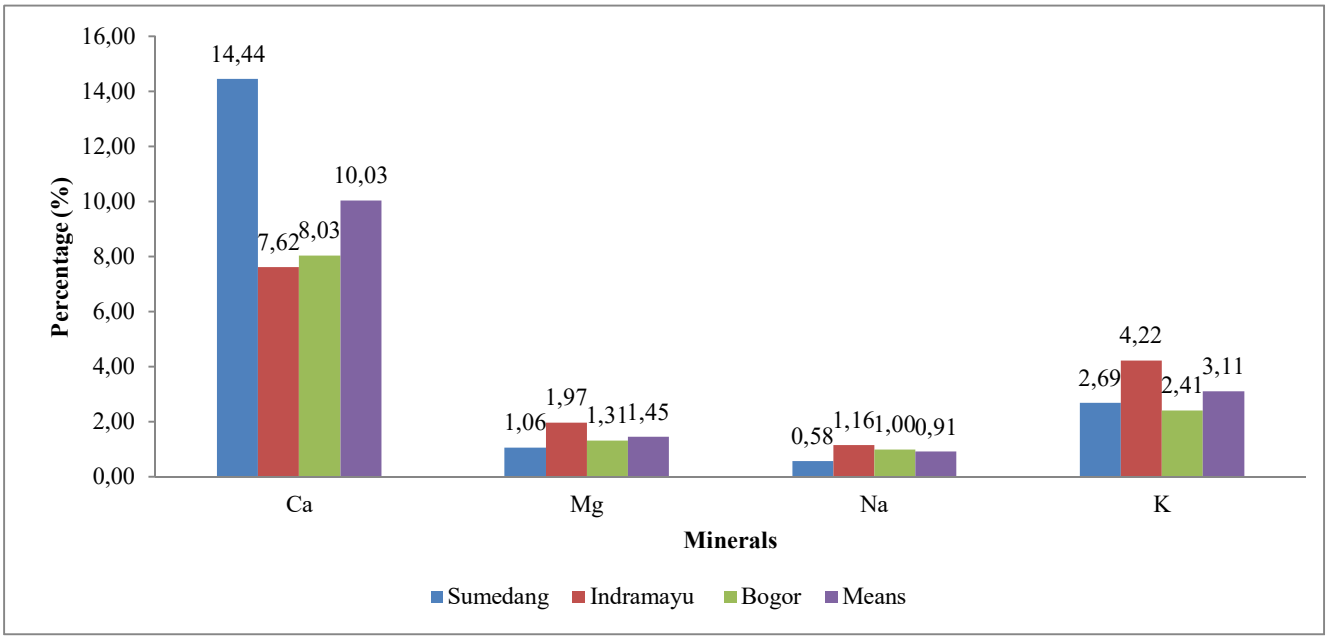

Fig. 1 The mineral in firewood ash (%) of three regencies in West Java, Indonesia. Values are expressed as mean (n=3).

Each regency contributed to the difference in the mineral content in each firewood ash. This condition was expected to result from the origin of fuelwood, mineral content and different soil conditions at each site. The content of alkaline minerals of firewood ash as a result of the research is higher than that of rice hull ash reported [13], where types of ash are also commonly found in rural areas. Rice plant are harvested faster than plants that are a source of firewood so that the alkaline mineral accumulation in their bodies is lower. The mineral elements are in mobilize on vegetation increases with maturity level [12]. Minerals and soil conditions, climate, vegetation phase, irrigation and fertilization will affect plant mineral content [14], [15].

B. The pH of the Firewood Ash Filtrate (FAF)

The purpose of measuring the pH of firewood ash filtrate (FAF) is to determine the solution's alkalinity. When dissolved in distilled water, firewood ash contains alkaline minerals (Figure 1), producing an alkaline solution. The base's strength depends on the base's ability to release OH

ions in the solution and the concentration of the base solution. The alkaline solution can damage the structure of organic compounds [4] so that they can be used to process fiber-rich feed. The average pH of the FAF was recorded in the range of 11.46-12.16 (Figure 2). The pH value increased along with the increasing amount of firewood ash dissolved in the distilled water.

The research results' pH value is higher than the pH value of filtrate rice husk ash 8.68-9.12 [16] and filtrate palm oil fruit bunch ash of 9.73-9.84 [17]. This result indicates that the pH of FAF is more alkalic/alkaline than the two filtrates and better used to process fiber-rich feed. The high content of alkaline minerals in firewood ash caused the cation in the solution to become higher so that it will increase the pH value of the filtrate. This finding proves that firewood ash can be used as a local alkaline mineral source.

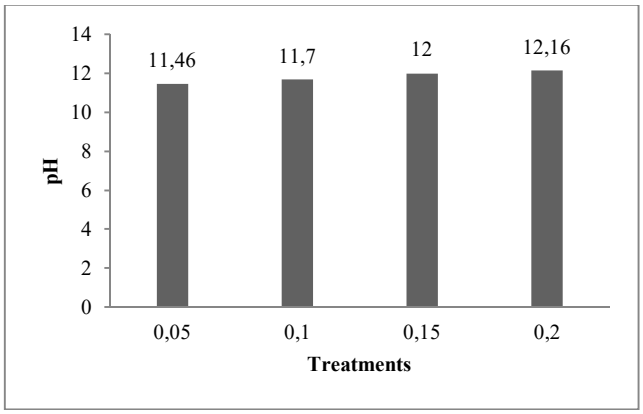

Fig. 2 The pH of FAF with various concentration (w/v). Values are expressed as mean (n=5).

The result of analysis of maize cobs before treatment showed that the CF was 39.52%, while NDF, ADF, lignin, cellulose, and hemicellulose were 86.26%, 60.21%, 10.37%, 49.84%, and 26.05% respectively. There was a decrease after soaked by FAF (Table 1).

TABLE I
FIBER COMPONENT AND CF OF MAIZE COB WHICH FAF HAS SOAKED

Variable	Treatments					
	FA5	FA10	FA15	FA20	SEM	P
NDF %	51.7^{d}	49.1^{c}	45.0^{b}	42.0^{a}	0.86	<0.01
ADF %	40.9 ^{d}	38.4^{c}	36.7^{b}	35.4^{a}	0.49	<0.01
Lignin %	8.02^{d}	7.13^{b}	6.18^{c}	4.82^{a}	0.28	<0.01
Cellulose (%)	32.8^{b}	31.3^{a}	30.5^{a}	30.6^{a}	0.27	<0.01
Hemi-cellulose (%)	10.8^{c}	10.7^{c}	8.38^{b}	6.58^{a}	0.45	<0.01
Crude Fiber %	35.2^{d}	33.7^{c}	31.4^{b}	28.6^{a}	0.58	<0.01

Values are expressed as mean (n=5), different letters within rows, represented significant differences (p <0.05)

The higher the FAF concentration caused, the lower the CF and fiber components in the maize cobs, shown by the linear regression pattern with a negative correlation (Figure 3). This is due to the alkaline action of FAF in dissolving phenol group or stretching/breaking lignin bond with cellulose and hemicellulose. According to principle, the alkaline power is (a) breaking some bonds between cellulose and hemicellulose with lignin and silica, (b) destroying acetyl groups by forming uronic acid, (c) overhauling the cell wall structure through the development of fiber tissue. Alkali treatment objectively may increase lignin's solubility, or phenol groups and other cell wall portions, especially hemicellulose [17].

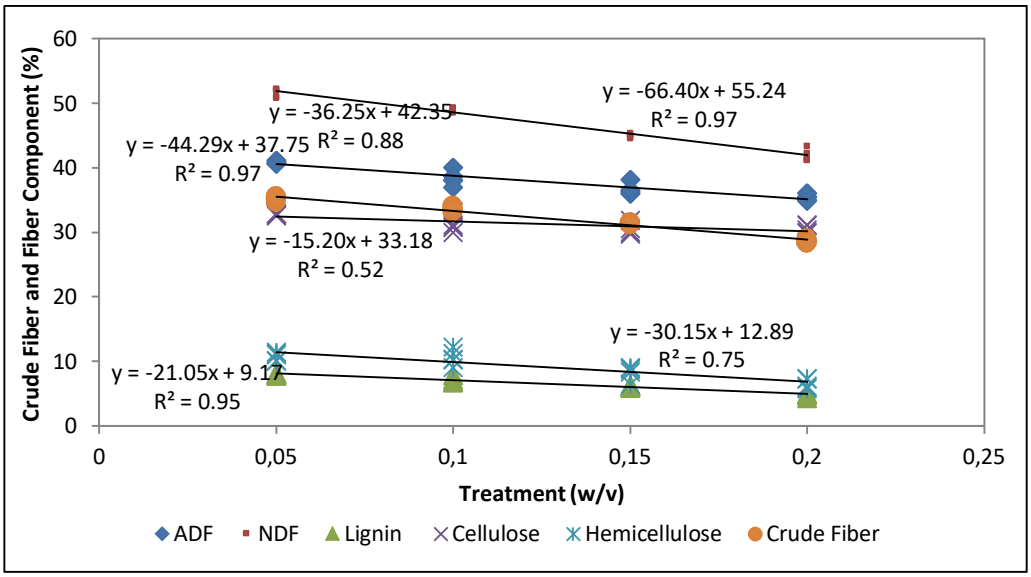

Fig. 3 The linear regression between the concentration of FAF and the content of fiber component and CF of maize cob

The solubility of lignin, cellulose, hemicellulose or phenol groups will affect the percentage of NDF, ADF, CF and have a positive correlation between them (Table 2). The percentage of ADF decreases with decreasing lignin and cellulose content since the fraction of the fiber comprises cellulose, lignin, and silica. The ADF composite component is particularly strongly binding to cellulose with lignin. If the lignin and its cellulose dissolve thus become low as the result of the FAF treatment, it would be followed by a low percentage of its ADF. Similarly, the percentage of NDF and

CF depends on the percentage of lignin, cellulose, and hemicellulose because the components contained therein include the feed fiber component. The loss of lignin, cellulose, and hemicellulose-induced components causes NDF and CF's value to decrease FAF.

TABLE II
PEARSON CORRELATION BETWEEN NDF, ADF, AND CF WITH LIGNIN, CELLULOSE, AND HEMICELLULOSE

Correlation		Lignin	Cellulose	Hemi-cellulose
NDF	Pearson Correlation	0.96*	0.70*	0.91*
	Sig. (2-tailed)	0.00	0.00	0.00
ADF	Pearson Correlation	0.90*	0.90*	-
	Sig. (2-tailed)	0.00	0.00	-
Crude Fiber	Pearson Correlation	0.86*	0.70*	0.65*
	Sig. (2-tailed)	0.00	0.00	0.00

* Correlation is significant at the 0.05 level. Data in parentheses are significant level

C. In Vitro Study

The *in vitro* study used maize cobs soaked by FAF with a concentration of 0.20 w/v (FA20), because this treatment has resulted in the lowest lignin content (Table 2). Lignin is an anti-nutrient that naturally binds against cellulose and hemicellulose; thus, the fiber component is difficult to be degraded by rumen microbes resulting in reduced feeding digestibility. Therefore, due to the decrease of lignin content. It is expected that the use of maize cobs is maximized. Hemicellulose and cellulose are components of cell walls that can be digested by microbes, while lignin itself cannot be digested by rumen microbes [18].

The *in vitro* diets contained crude protein (CP) with a range of 9.68%-11.50% and total digestible nutrient (TDN) with a range of 62.35%-63.76% (Table 4). Protein content decreased during the increasing of processed maize cobs due to the protein content of processed maize cobs was lower than field grass (Table 3). Whereas, the content of other substances was relatively similar. These diets were evaluated by *in vitro*.

TABLE III
INGREDIENTS AND THEIR CHEMICAL COMPOSITION

Nutrients	Native grass	Concentrate	MFA
Dry Matter (%)	38.3	85.9	89.4
Ash (%)	10.6	10.3	1.99
Crude protein/CP (%)	9.83	15.5	3.93
Crude fat/CFt (%)	1.53	9.52	1.50
Crude fiber/CF (%)	27.4	16.2	28.6
Nitrogen free extract/NFE (%)	50.7	48.7	64.0
TDN (%)[1]	58.9	72.2	58.9

[1]TDN was calculated using Sutardi equation as described in in Hernaman et al. [10]: (70.6+0.259%CP+1.01%CFt-0.76%CF+ 0.0991% NFE)
MFA = Maize Cobs Soaked by FAF

The results of *in vitro* fermentability evaluation and digestibility of the experimental diets are presented in Table 5. The pH of rumen fluid has a value of 7.12-7.24 and still within the physiology limits, i.e. 5.5-7.5 [19]. The pH of rumen fluid is an indicator of the concentration of VFA, NH_3, and buffer capacity [20]. The level of ammonia (NH_3) and VFA showed a significant effect ($p < 0.01$). This condition allowed for a difference in pH, especially VFA, which showed a large difference (85.6 mM versus 149 mM), but resulting in the same impact. This suggested that the concentrations of NH_3 and VFA were still low so that added artificial saliva solution as a rumen buffer in this *in vitro* experiment could maintain the pH condition closed to the normal pH of 7. A buffer solution is made to avoid rapid changes in pH when acid or alkali is added.

TABLE IV
INGREDIENTS, DIETS COMPOSITION AND NUTRIENT COMPOSITION OF COMPLETE DIETS

Feed Ingredients	Treatments			
	MFA10	MFA20	MFA30	MFA40
Native grass (%)	50	40	30	20
Maize cob soaked by FAF (%)	10	20	30	40
Concentrate (%)	40	40	40	40
Nutrients				
Ash (%)	9.66	8.90	8.13	7.37
Crude protein (%)	11.5	10.9	10.3	9.68
Extract ether (%)	4.68	4.63	4.59	4.54
Crude fiber (%)	23.3	23.8	24.3	24.8
Nitrogen free extract (%)	50.8	51.8	52.7	53.7
TDN (%)[1]	63.8	63.3	62.8	62.4

[1]TDN was calculated using Sutardi equation as described in Hernaman et al. [10] : (70.6+0.259%CP+1.01%EE-0.76%CF+ 0.0991% NFE).

TABLE V
IN VITRO RUMEN FERMENT ABILITY AND DIGESTIBILITY OF COMPLETE DIETS

Variables	Treatments				SEM	P
	MFA10	MFA20	MFA30	MFA40		
pH rumen fluid	7.18	7.20	7.22	7.24	0.01	0.37
NH_3 (mM)	2.27^b	2.47^c	2.73^d	2.18^a	0.05	<0.01
VFA (mM)	85.6^a	103^b	148^c	149^c	6.46	<0.01
DMD (%)	54.7^b	57.4^c	59.2^d	48.5^a	0.95	<0.01
OMD (%)	46.2^b	48.2^c	51.5^d	39.7^a	1.02	<0.01

Values are expressed as mean (n=5), different letters within rows represented significant differences ($p < 0.05$)

The increase of VFA concentration for diet treatments containing processed maize cobs was due to its lower lignin content, which was 4.82% (Table 1). It is also suspected that processing with FAF causes the bond between lignin with cellulose and hemicellulose to be more tenuous so that rumen cellulolytic microbes can penetrate and do more intensive fermentation of cellulose and hemicellulose in maize cobs into VFA.

Therefore, the VFA content is higher in concentration along with the use of processed maize cobs. Adesogan et al. [21] stated alkaline treatments are effective at breaking hemicellulose-lignin and lignocellulose bonds, hydrolyzing uronic and acetic acid esters, and disrupting cellulose crystallinity by inducing cellulose swelling. This condition

leads to penetration by more intensive microbial enzymes, making it easier to ferment the fiber component (cellulose and hemicellulose) to VFA.

The resulting VFA is utilized as a source of energy and protein synthesis for proteolytic bacteria to better develop and ferment the protein into NH_3. This result can be seen with an increase of VFA concentration followed by an increase in NH_3 concentration until MFA30 treatment, although the protein diets of treatment have decreased with the use of processed maize cobs (Table 3).

MFA40 treatment has resulted in a high VFA concentration but was not followed by high NH_3 values, even lower than other treatments. This result was due to the diet in the MFA40 treatment containing the lowest protein (Table 4) compared with other treatments. Rumen microbes will reform the protein in the diets into NH_3, so the diet protein affects the NH_3 rumen concentration. The concentration of NH_3 depends on the level of sample protein, the degree of degradability, dietary protein intake, the acidity (pH) of rumen fluid and feed duration in the rumen [22]. Diet protein also indicates the availability of N elements for rumen microbes which are very helpful in the growth and production of microbial protein synthesis [23].

High levels of digestibility will follow optimum rumen fermentability. Compared with other treatments, volatile fatty acid (VFA) and NH3 in the higher MFA30 treatment illustrate the easiness of the diet to be digested. Also, the high availability of VFA and NH_3 are expected to help rumen microbes growing and developing. The growth of rumen microbes will help in better-digesting diets [18]. This growth has resulted in the highest DMD and OMD, which are obtained at the MFA30 treatment. The processing of palm oil fiber using filtrate palm oil fruit bunch ash at a concentration of 15% had a significant effect on decreasing lignin and CF as well as an increase of DMD and OMD [17].

IV. Conclusion

Maize cobs soaked by FFA treatment at a concentration of 0.20 w/v results in the highest decrease in lignin content. The use of this treated maize cobs up to 30% in a diet provides the best *in vitro* rumen fermentability and digestibility.

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