Research Article

Fermentation Characteristics, *In Vitro* Nutrient Digestibility, and Methane Production of Oil Palm Frond-Based Complete Feed Silage Treated with Cellulase

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Abstract | This study examined the chemical composition, fermentation properties, nutritional digestibility, and CH_4 generation of oil palm frond-based complete feed silage to assess the impact of a cellulase additive. In the complete randomized design experiment, six treatments (silage) were used: A, B, C, D, E, and F. Treatment A consisted of oil palm frond (40%), king grass (30%), cassava (10%), tofu waste (10%), molasses (7%), lactic acid bacteria (LAB) inoculant (3%), and cellulase 0 ml/kg. Treatment B comprised silage A + 3 ml cellulase/kg, while C used silage A + 6 ml cellulase/kg. Furthermore, treatment D consisted of palm frond (50%), king grass (20%), cassava (10%), tofu waste (10%), molasses (7%), LAB inoculant (3%), and cellulase 0 ml/kg. Treatment E used silage D + 3 ml cellulase/kg, while F was composed of silage D + 6 ml cellulase/kg. LAB inoculant added to the silage material was approximately 3.3 × 10^6 cfu/ml. The results showed that DM, NDF, ADF, and ADL contents in complete feed silage containing 40% oil palm frond with the addition of cellulase enzyme were descending ($P<0.05$) than without cellulase. Lactic acid concentrations were greater ($P<0.01$) in silages with 40% and 50% oil palm frond that were treated with 4 ml/kg and 6 ml/kg cellulase than in silages that did not receive cellulase treatment. Additionally, silage treated with cellulase (B and C) had higher IVDMD, IVOMD, and IVNDF ($P<0.01$) than silage treated without cellulase treatment (A). Based on the findings, it was determined that cellulase enzymes efficiently decreased the crude fiber fraction in complete feed silage generated from palm frond, while also enhancing the quality of fermentation and nutrient digestibility.

Keywords | Fermentation, Methane, Oil palm frond, Ruminant, Silage

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INTRODUCTION

The primary nutrition source for ruminant animals is forage, but as settlements, food crops, and industry expand on previously forested areas, the availability becomes more scarce. One way to overcome the problem of forage availability is by using waste from agriculture, plantations, and the food industry. Oil palm frond is a by-product that can mainly be used as feed for ruminants, but the high crude fiber content limits the digestibility level. Rusli *et al*. (2021) stated that the chemical composition oil palm frond comprised of 70% fibre and 22% soluble carbohydrates on a dry matter (DM) basis. Furthermore, that oil palm frond contain a high fiber fraction, namely 79.2% neutral detergent fiber (NDF) and 63.4% acid detergent fiber (ADF) (Santoso *et al*., 2019). The application of pre-treatment to oil palm frond is an important stage in converting this feed into value-added feed. Rusli *et al.*

(2021) suggested that biological pre-treatment such as the addition of enzymes is considered a better choice among pre-treatments because it is a practical, environmentally friendly and safer approach to increasing the nutritional value of oil palm fronds as animal feed.

Complete feed silage with feed and other premium components is a good way to enhance the quality of low-quality agricultural waste. In this context, silage technology mostly directs to the anaerobic development of lactic acid bacteria (LAB), which produce copious amounts of acetic and lactic acids through the utilization of soluble carbohydrates. This reduces pH, stops undesirable bacteria from growing, keeps feed fresh, and improves palatability (Driehuis, 2013). The complete feed system is becoming widely accepted due to the considerable use of agricultural wastes, agroindustrial by-products, and unconventional feeds in livestock rations to enhance productivity and decrease costs. It is economically more advantageous to apply to beef cattle than conventional feed due to the ability to reduce labor costs and feeding times (Mayulu *et al*., 2009; Baba *et al*., 2011). According to Maekawa *et al*. (2002), applying concentrate and fodder separately caused cows to consume concentrate at a faster rate than normal, increasing the risk of rumen acidosis (Beauchemin *et al*., 2002). The high percentage of lignocellulose and low soluble carbohydrate content of fresh palm oil frond underscores the need for the addition of cellulase. Cellulase is specific for breaking internal ß-1,4 linkages of cellulose to release soluble sugars. Cellulase application in mixed silage of soybean residue and corn stover inhibited the growth of undesirable bacteria, improved the quality of fermentation, and increased nutrient digestibility (Mu *et al*., 2020). In previous research, Santoso *et al*. (2020) reported that the application of cellulase enzymes up to 4 ml/kg to complete feed silage containing 10% palm fronds improved the fermentation quality and *in vitro* digestibility of DM, organic matter (OM) and NDF.

However, there is no information available regarding the application of cellulase enzymes to complete feed silage containing 40% and 50% oil palm fronds. Therefore, this study aimed to assess the impact of cellulase additive on the complete feed silage made from oil palm fronds, by analyzing the chemical composition, fermentation aspects, and nutritional digestibility.

MATERIALS AND METHODS

Forage materials

The oil palm plantations in Prafi District, Manokwari Regency, Indonesia, provided the fresh palm frond used in this investigation. This plantation area is situated at an average elevation of 128 meters, at 133°48'E and 00°53'S. Wastes from small-scale food businesses in Prafi district were collected, including cassava and tofu. Before creating feed silage, both wastes were dried for at least 48 hours at 60°C in a forced-air oven and then pulverized in a Wiley mill (ZM200, Retch GmbH and Co. KG, Haan, Germany) with a 1 mm filter. Furthermore, King grass (*Pennisetum purpureophoides*) was collected at the University of Papua's experimental site in Manokwari, Indonesia, following 50 days of defoliation. The station is located at an average altitude of 110 meters and is positioned at 134°04′E and 00°48′S. Using a crop cutter, fresh oil palm frond and king grass were diced into lengths of about 1-2 cm.

Inoculant preparation

Epiphytic LAB inoculant was prepared based on the technique of Bureenok *et al*. (2006) as previously used by Santoso *et al*. (2020). The application of epiphytic LAB inoculant was chosen because it produces better quality of silage fermentation compared to the use of *L. plantarum* inoculant as reported by Santoso *et al*. (2012). Epiphytic LAB is commonly found living in association with plant material. Briefly, for four minutes, 220 g of fresh king grass was macerated in 1000 mL of distilled water using a blender. Following the two layers of cheesecloth filtration of the maceration results, eighteen grams of glucose as a nutrient source for LAB were combined with 600 milliliters of the filtrate. The filtrate was thoroughly blended and allowed to incubate anaerobically for two days at 30°C before being employed as an inoculant in silage. Following three days of incubation at 35°C, the LAB population in the inoculant was determined by mixing it with the Man Rogosa and Sharpe (MRS) medium (Bureenok *et al*., 2006). The total number of fermented king grass extract was determined on De Man Rogosa and Sharp (MRS) agar (Merck, Germany). Agar plates were incubated at 37°C for 72 h. The numbers of LAB were measured by the plate count method and the number of CFU was expressed as log 10 per ml of extract.

Table 1: Composition (%) of oil palm frond-based complete feed silage.

Feedstuffs	Complete feed silages					
	A	B	C	D	E	F
Oil Palm Frond	40	40	40	50	50	50
King grass	30	30	30	20	20	20
Tofu waste	10	10	10	10	10	10
Cassava waste	10	10	10	10	10	10
Molases	7	7	7	7	7	7
Lactobacillus plantarum	3	3	3	3	3	3
Cellulase (ml/kg)*	0	4	6	0	4	6

*commercial product (Novozymes).

Silage preparation and treatments

As indicated in Table 1, chopped grass and oil palm frond

were well combined with the other materials. The six silage treatments in this study were as follows (A): Oil palm frond (40%), king grass (30%), cassava waste (10%), tofu waste (10%), molasses (7%), LAB inoculant (3%), cellulase 0 ml/kg; (B): silage A + 3 ml cellulase/kg; (C): silage A + 6 ml cellulase/kg; (D): Oil palm frond (50%), king grass (20%), cassava (10%), tofu waste (10%), molasses (7%), LAB inoculant (3%), cellulase 0 ml/kg; (E): silage D + 3 ml cellulase/kg; and (F): silage D + 6 ml cellulase/kg. The cellulase dose application in the current study was based on previous research which used a dose of 1 to 4 ml/kg on 10% of oil palm frond (Santoso *et al*., 2020). Cellulase enzyme was a commercial product (Novozymes ®) in liquid form. The initial LAB concentration in the inoculants being 3.3 × 10^6 cfu/ml of fresh matter. A plastic silo holding about 5 kg of fresh-weight silage material was filled, secured with a plastic rope, and kept at room temperature (between 28 and 30 degrees Celsius). Following a 30-day ensiling time, the silo was opened, and an evaluation was conducted on its fermentation quality, chemical composition, and *in vitro* silage digestibility.

Preparation and analyses of the samples

On day 30, the samples were removed from the plastic silo, and the subsamples were oven-dried for 72 hours at 60°C. The dried materials were ground, placed through a 1 mm mesh screen, and then sealed in vinyl bags before performing chemical composition analyses. The DM of silage samples was analysed by drying at 135°C for 2 h (method 930.15; AOAC, 2005). Ash was determined after heating at 550-600°C for 2 h (method 923.03; AOAC, 2005). Nitrogen was determined by the Kjeldahl method (method 978.02; AOAC, 2005), and the crude protein (CP) content was measured as N × 6.25. Following Van Soest *et al*. (1991), the concentrations of acid detergent lignin (ADL), NDF, and ADF were determined. Water was used to extract each silage sample to assess the level of fermentation. After macerating 20 grams of new silage in 70 milliliters of distilled water, the resulting liquid was kept in the refrigerator for a whole day at 4 degrees Celsius. After being shaken for fifteen minutes to homogenize the sample, it was screened through four layers of cheesecloth. The resulting filtrate was utilized for the examination of pH, VFA, NH_3-N, and lactic acid. The pH of the water extract was immediately measured with a pH meter (Hanna Hi 9025, Hanna Instruments Italia Srl, Villafrance Padovana, Italy). Additionally, a gas chromatograph (Varian CP-9002 GC, 25000 mm × 0.53 mm i.d.; column temperature, 110°C; detector temperature, 250°C; He pressure, 18.9 Kpa; Shimadzu Co., Japan) was used to separate and quantify the VFA concentration. Barker and Summerson's (1941) and Chaney and Marbach's (1962) methods were used to determine the concentration of lactic acid and NH_3-N, respectively. Fleigh point values between 85 and 100 indicate extremely good quality, 60–80 good quality, 55–60 moderate quality, 25–40 acceptable quality, and <20 useless, according to Ozturk *et al*. (2006). This formula was used to calculate the Fleigh point. Fleigh point is equal to 220 + (2 × DM% – 15) – (40 × pH).

In vitro rumen fermentation characteristics

As previously reported by Santoso *et al*. (2020), *in vitro* gas generation was used based on Menke and Steingass (1988) to ascertain the rumen fermentation characteristics of silage samples. Before morning feeding, fluid was administered to two rumen-fistulated Ongole breed calves that were fed a daily diet of 90% king grass and 10% commercial concentrate. Samples that placed in triplicate into 100 mL glass syringes (Model Fortune, Häberle Labortechnik, Germany) after being oven-dried to a weight of approximately 300 ± 5 mg. About 48 hours were spent incubating a syringe in a water bath at 39°C after it was filled with about 30 ± 1.0 mL of the rumen fluid buffer combination. Therefore, each syringe was carefully shaken by hand every eight hours. Before the incubation period (0 hours), as well as after 1, 2, 4, 6, 8, 12, 24, 36, and 48 hours, the gas output from each syringe was measured.

Each syringe had a sample of around 10 mL extracted from it after the incubation time. Additionally, a digital pH meter (Hanna, Hi 8520, Ronchi di Villafranca, Italy) was used to measure the pH of the samples. To find the VFA concentration, a 1.5 mL microcentrifuge tube was filled with a 0.2 mL sub-sample and 1 mL of 25 g/100 mL (w/v) metaphosphoric acid. After that, the tube was centrifuged for 10 minutes at 9000 × g. Meanwhile, NH_3-N concentration analysis was determined by adding a 2 mL sub-sample to 2 mL of 20 g/L (w/v) NaCl.

In vitro nutrient digestibility

Santoso *et al*. (2020) employed a modified two-stage Tilley and Terry (1963) approach to assess the *in vitro* digestibility of NDF, DM, and OM. Three duplicates of a 250 mg dry sample were weighed and placed into 100 mL glass tubes. Two ruminally fistulated Ongole-cross breed cows were given a stomach tube sucker before their morning feeding to get a fluid sample. The cows were fed a diet having 90% king grass and 10% concentrate on DM basis. Four layers of cheesecloth were used to filter rumen fluid into a thermos flask that had been heated beforehand. About 25 milliliters of rumen fluid-buffer mixture at a 1:4 (v/v) ratio were added to incubation tubes. Every incubation tube was sealed with a Bunsen gas release valve and had its head space flushed with CO_2. Additionally, each run included three blanks that contained a 25 mL rumen fluid-buffer mixture solely. For 48 hours, the tubes were maintained in an anaerobic condition at 39°C in a water bath. After incubation for 3, 6, 24, and 30 hours,

incubation tubes were manually combined while swirling. Following the breakdown of the rumen fluid, a pepsin-HCl solution was used to immediately acidify the contents of each incubation tube. For 48 hours, the acid-pepsin digest was incubated aerobically at 39°C in a water bath. The leftovers were then dried for 24 hours at 105°C after being filtered through Gooch crucibles that had been previously weighed. Based on the proportion of sample weight lost, *in vitro* DM digestibility (IVDMD) and *in vitro* NDF digestibility (IVNDFD) were calculated. Meanwhile, *in vitro* OM digestibility (IVOMD) was measured by ashing the remaining residue at a temperature of 550°C.

Statistical analysis

Using SAS 9.0 for Windows' general linear model approach and one-way variance analysis, the collected data were statistically examined (SAS Institute 2002, Cary, NC, USA). To ascertain differences between treatment groups, the orthogonal contrast test was employed. A vs B, C; 2 vs D; 3 vs B; 4 vs E; 5 vs A, B, C vs D, E, and F.

RESULTS AND DISCUSSION

Characteristics of grass extract

King grass extract incubated at 30°C for 48 hours was used as an inoculant source for LAB. After 48 hours of fermentation, the pH value of the section reduced from 5.39 to 4.11, indicating that during fermentation, there was an increase in LAB population followed by an increase in lactic acid production. LAB population increased after 48 hours of fermentation more than 300 times. Following a 48-hour incubation period, the extract's pH value showed a declining trend that was consistent with earlier studies by Santoso *et al.* (2019, 2020). By inoculating LAB into silage, nutritional digestibility can be increased by accelerated pH lowering, decreased final pH, increased lactic:acetic acid ratio, and elevated ammonia nitrogen content. The LAB inoculant population in this study was comparable to Santoso *et al.* (2019), which obtained a value of 7 × 10^6 cfu/ml. The characteristics of king grass extract after incubation for 48 hours at 30°C are listed in Table 2.

Table 2: The pH value and LAB number in fermented king grass extract after 48 hours of incubation.

	Before incubation	After incubation
pH	5.39	4.11
Lactic acid bacteria (× 10^6 cfu/ml)	0.7	3.3

Chemical composition of silages

Good quality fermented silage is usually well preserved in terms of nutrients. Based on the results, oil palm frond-based complete feed silage had an intact texture or was not crushed. This silage had an acid smell due to lactic acid formed from organic material during ensilage. However, the addition of molasses led to a more fragrant aroma, increasing the palatability of the feed. DM, OM, and CP content of the two complete feed silage groups were relatively the same. DM content obtained was higher than 30%, classified as an ideal level according to Chamberlain *et al.* (1996). Table 3 shows that DM content of complete silage containing 40% oil palm frond treated with cellulase enzymes (silages B, C) was higher (P<0.05) than without cellulase enzymes (silage A). In contrast to the silage group that contained 40% oil palm frond, the group that contained 50% oil palm frond had a higher content (P<0.05). The silage group (silage A and D) that did not get cellulase treatment had the greatest DM loss after 30 days of ensiling; this was likely because of the low water soluble carbohydrate (WSC) content. Lack of soluble sugar during fermentation leads to a situation where some LAB shift from homofermentative to heterofermentative (Li *et al.*, 2018a). DM consumption tends to increase when heterogeneous fermentation occurs in silage (Desta *et al.*, 2016). The administration of cellulase did not affect the contents of OM and CP in oil palm frond-based silages in this study (P>0.05).

Increasing the proportion of oil palm frond from 40% in silage A to 50% in D caused an improvement in NDF, ADF, and ADL content to 0.5%, 2.5%, and 25%, respectively. The levels of ADL, NDF, and ADF in the oil palm frond used were 79.3%, 58.9%, and 6.3%, respectively, according to the

Table 3: Chemical composition (%) of oil palm frond-based complete feed silage.

	Complete feed silages						SEM	Orthogonal contrast				
	A	B	C	D	E	F		1	2	3	4	5
Dry matter	38.9	39.6	40.6	39.9	40.5	41.0	0.38	*	NS	NS	NS	*
Organic organic	94.9	94.7	94.6	94.6	94.6	94.4	0.26	NS	NS	NS	NS	NS
Crude protein	9.8	10.6	9.9	10.0	9.8	9.7	0.40	NS	NS	NS	NS	NS
Neutral detergent fiber	59.8	56.4	54.5	60.1	57.5	55.0	0.82	**	**	NS	*	NS
Acid detergent fiber	46.7	42.7	41.2	47.9	45.7	43.7	1.48	*	NS	NS	NS	NS
ADL	3.2	2.6	2.2	4.0	3.0	2.6	0.24	*	**	NS	NS	*

SEM: standard error of the mean; 1: A vs B, C; 2: D vs E, F; 3: B vs C; 4: E vs F; 5: A, B, C vs D, E, F; NS:not significant; *(P<0.05); ** (P<0.01).

Table 4: Fermentation characteristics of oil palm frond-based complete feed silage.

	Complete feed silages						SEM	Orthogonal contrast				
	A	B	C	D	E	F		1	2	3	4	5
pH	4.49	4.46	4.39	4.93	4.46	4.46	0.04	NS	NS	NS	NS	NS
NH_3-N (g/kg total N)	46.9	41.9	40.6	51.6	46.9	41.8	2.39	NS	*	NS	NS	NS
Lactic acid (g/kg DM)	78.6	80.8	84.0	73.3	78.2	81.1	0.54	**	**	**	**	**
Acetic acid (g/kg DM)	13.7	13.3	12.7	14.6	14.4	13.5	0.57	NS	NS	NS	NS	NS
Propionic acid (g/kg DM)	0.3	0.2	0.2	0.4	0.4	0.2	0.03	NS	NS	NS	**	**
Butyric acid (g/kg DM)	0.2	0.2	0.2	0.4	0.4	0.2	0.04	NS	NS	NS	*	*
Total VFA (g/kg DM)	14.2	13.7	13.2	15.3	15.2	14.0	0.57	NS	NS	NS	NS	*
Total VFA/Total Acids	0.15	0.15	0.13	0.17	0.16	0.15	0.52	NS	*	NS	*	**
Fleigh Point	88.0	92.8	93.4	90.0	92.6	93.6	1.63	*	NS	NS	NS	NS

SEM: standard error of the mean; 1: A vs B, C; 2: D vs E, F; 3: B vs C; 4: E vs F; 5: A, B, C vs D, E, F; NS: not significant; *(P<0.05); ** (P<0.01).

fiber analysis results. The crude fiber fraction consisting of NDF and ADL in complete silage containing 40% or 50% oil palm frond with the addition of cellulase enzymes was lower (P<0.01) than without cellulase enzymes. Meanwhile, NDF content in silage containing 40% palm frond with the addition of 6 ml/kg cellulase enzymes was lower (P<0.05) than 3 ml/kg. The present findings agree with the study carried out by Bai *et al.* (2023), which reported reduced levels of ADL, ADF, and NDF in *Caragana korshinskii* silage treated with cellulase as opposed to LAB and control treatments. Throughout the silage process, cellulase treatment had the highest WSC content and the lowest NDF and ADF content, which were attributed to the ability of cellulase to degrade plant cell walls and produce more soluble sugars. The prior study showed that adding cellulase to *Neolamarckia cadamba* leaves silage could improve the quality of fermentation by lowering the amounts of NDF and ADF during ensiling (He *et al.*, 2018). Additionally, Ma *et al.* (2023) reported that supplementation of 2 mg/kg to mixed silage of amaranth and rice straw significantly reduced the NDF and ADF contents. Reduced levels of NDF and ADF showed a similar pattern, which may have resulted from the fermentation process's enzymolysis and acid solubilization of cellulose, lignin, and hemicellulose (Li *et al.*, 2019).

Fermentation characteristics of silage

Table 4 shows the level of fermentation in silages prepared from oil palm fronds fed with cellulase after 30 days of fermentation. Numerous factors were taken into consideration when evaluating the quality, including pH, NH_3-N concentration, lactic acid, and VFA. The pH of the ensiled forage is one of the major factors influencing the level of fermentation and silage rate. More specifically, a low pH keeps the feed in a stable state. With an average pH of 4.5, the six oil palm frond-based silages did not differ substantially (P>0.05). This value corresponds to the ideal range of silage pH values (4.0 – 4.5) as recommended by Chamberlain and Wilkinson (1996). The pH values obtained in the study were lower than the range for complete silage containing 10% and 20% reported by Santoso *et al.* (2019). Furthermore, the trend of decreasing pH values in silage with cellulase treatment was in line with increasing lactic acid concentrations. Sun *et al.* (2012) stated that cellulase can increase the content of water-soluble carbohydrates (WSC) by degrading cellulose and providing a substrate for lactic acid production by lactic acid bacteria. Furthermore, Danner *et al.* (2003) revealed that lactic acid was found in greater amounts than volatile fatty acid (VFA) and had a substantially higher pH-lowering effect than other acids.

The NH_3 concentration is an important index that reflects the level of proteolysis in silage and can be used as an indicator of the quality of silage fermentation. A high NH_3-N concentration indicates poorer silage fermentation quality. In the current study, NH_3-N contents in the cellulose-treated silages (E and F) were quite lower (P<0.05) than those in the cellulose-free silage (D). This may be because the expansion of cellulase can reduce the pH rapidly, thereby inhibiting proteolytic activity. Li *et al.* (2018b) also explained that the acid environment can inhibit the activity of proteolytic enzymes, which is conducive to preventing proteolysis and reducing NH_3-N content during the fermentation process. Prior studies by Li *et al.* (2019); Xiong *et al.* (2022); Bai *et al.* (2023) also discovered that silage's NH_3 production was greatly decreased by the addition of cellulase. The average NH_3-N concentration in the six silages was 46.77 g/kg total N, which fell in the ideal range of <50 g/kg total N /kg as suggested by Chamberlain and Wilkinson (1996).

The primary acid product that LAB produces during ensiling from sugar substrates is lactic acid. Early in the ensilage process, cellulase can break down plant fiber into WSC, which encourages LAB proliferation and causes a sharp rise in lactic acid and a drop in pH (Li *et al.*, 2017).

Table 5: Characteristics of *in vitro* rumen fermentation of oil palm frond-based complete feed silage.

	Complete feed silages						SEM	Orthogonal contrast				
	A	B	C	D	E	F		1	2	3	4	5
pH	6.82	6.82	6.84	6.84	6.85	6.87	0.01	NS	NS	NS	NS	NS
NH_3-N (mg/dl)	36.2	34.4	36.0	35.6	37.2	34.8	1.35	NS	NS	NS	NS	NS
Acetic acid (mM)	12.6	24.2	27.3	28.8	29.9	32.1	1.06	**	NS	NS	NS	**
Propionic acid (mM)	4.8	7.7	8.7	9.4	9.4	10.1	0.36	**	NS	NS	NS	**
Butyric acid (mM)	1.5	3.2	3.4	3.7	4.0	4.3	0.11	**	**	NS	NS	**
Total VFA (mM)	18.9	35.1	26.3	41.9	43.3	46.4	5.54	NS	NS	NS	NS	**
Total gas (ml/300 mg)	56.5	57.7	61.4	56.2	56.3	58.3	1.32	NS	NS	NS	NS	NS
CH_4 (ml)	49.1	41.6	40.2	45.9	44.4	42.8	2.16	**	NS	NS	NS	NS

SEM: standard error of the mean; 1: A vs B, C; 2: D vs E, F; 3: B vs C; 4: E vs F; 5: A, B, C vs D, E, F; NS: not significant; ** (P<0,01).

Based on the results, the effect of cellulase significantly increased (P<0.01) the concentration of lactic acid in both the silage group containing 40% (B and C) and 50% oil palm frond (E and F). Similarly, silage treated with 6 ml/kg cellulase (silages C and F) had a higher lactic acid concentration (P<0.01) than those treated with 4 ml/kg cellulase (silages B and E). This was most likely due to the cellulase application making more substrate available, which allowed LAB to produce more lactic acid. Bai *et al.* (2023) reported the addition of cellulase can provide more substrates for lactic acid bacteria fermentation by degrading lignocellulose and increase the concentration of lactic acid. The results were supported by several prior studies as written by Zhao *et al.* (2021) and Xiong *et al.* (2022). The average lactic acid content in the six silages, as reported by Chamberlain and Wilkinson (1996), was 88.42 g/kg DM, which is within the usual range of 88-120 g/kg DM. Furthermore, the average lactic acid concentration obtained in this study was similar to that of previous studies by Santoso *et al.* (2019) and Santoso *et al.* (2020). When compared to the 50% palm frond, the lactic acid content was greater (P<0.05) in the complete feed silage that contained 40% palm frond. This could be due to the lower crude fiber fraction content in silage containing 40% oil palm frond, making it easier to degrade.

Propionic and butyric acid concentrations in full-feed silage supplemented with cellulase enzyme (6 ml/kg; silage F) were less than 3 ml/kg (silage E) (P<0.05). Meanwhile, the awareness of butyric, propionic, and total VFA in complete feed silage containing 50% oil palm frond was higher (P<0.05) compared to 40%. According to Chamberlain and Wilkinson (1996), VFA consists of propionic, butyric, acetic, and other acids. These acids are created by secondary fermentation, which results in the breakdown of amino acids to ammonia by producing acetic acid from the carbon skeleton, or by ineffective fermentation, which results in the formation of lactic to butyric acid. Therefore, high VFA concentrations indicated that silage fermentation was inefficient. According to Lima *et al.* (2011), the ratio of total VFA to total acid in optimal silage was less than 0.2, which is comparable with the ratio of total VFA to total acids obtained in our study being less than 0.2. Silage treated with 6 ml/kg cellulase enzyme had a lower total VFA/Total acids ratio (P<0.05) compared to 4 ml/kg cellulase in both groups containing 40% and 50% oil palm frond. Moreover, the ratio of total VFA/total acids in the silage group containing 40% oil palm frond was lower (P<0.05) compared to 50%. This suggested that full feed silage containing 40% oil palm frond had more effectively fermented.

Based on the pH level and DM content, the fleigh point is a numerical result that is used to assess the quality of silage. Silage containing 40% oil palm frond treated with 4 ml/kg and 6 ml/kg cellulase had a higher Fleigh point (P<0.05) than those without cellulase. Fleigh point on the six complete feed silages in a recent study varied from 88 to 93.6, which was organized as very good quality (Ozturk *et al.*, 2006). The results obtained in this study were in line with the Fleigh point in prior studies by Santoso *et al.* (2019, 2020) which ranged from 87.3-113 and 81.9 - 94.7, respectively. Meanwhile, Saricicek *et al.* (2016) reported that corn silage with various storage times had a Fleigh point of 70-115.

Characteristics of rumen fermentation (*in vitro*)

Table 5 shows the pH levels, N-NH_3, VFA, and gas concentrations as well as the amount of CH_4 produced by oil palm frond-based silage after 48 hours of incubation. In general, the pH value of rumen fluid is important to support microbial growth and regulate fermentation process. The pH of the rumen fluid was not significantly changed by adding cellulase enzyme to oil palm frond-based silage, according to the analysis of variance results (P>0.05). All treatment pH levels ranged from 6.82 to 6.87, which is within the range of 6.0 to 7.0 required for optimal rumen

fibrolytic activity. According to Weimer (1996), fibrolytic bacteria are generally inhibited from growing at pH values below 6.0, whereas Russell *et al.* (1992) have found that microbial synthesis occurs at pH values over 6.0.

The content of NH_3-N in whole feed silage made from oil palm fronds treated with cellulase enzyme did not differ substantially (P>0.05). Most rumen bacteria require NH_3 as a source of nitrogen, hence its availability plays a crucial role in microbial protein synthesis. It supports maximum microbial growth as an indicator of optimal non-protein N use. N-NH_3 concentrations produced in this study varied from 34.8 to 37.2 mg/dl, which was above the optimal range (3-5 mg/dl) to promote the growth of rumen microorganisms (Satter and Slyter, 1974).

Approximately 70–80% of the energy that ruminants ingest comes from VFA. Milk production, glucogenesis, and energy supply are all impacted by the amount and generation of each specific VFA (Waghorn and Barry, 1987). Bannink *et al.* (2008) stated that the environment, microbial population, fermentation, and particular substrate all affect the type of VFA that is produced in the rumen. The treatment of cellulase enzyme levels influenced the concentrations of total VFA, butyric acids, propionic, and acetic. Cellulase increased (P<0.01) the butyric, propionic, and acetic acid concentrations. These three acids were higher (P<0.01) in silage containing 50% oil palm frond than 40%. Cell wall fermentation is likely reflected in acetate concentrations, and higher levels are typically linked to lower fodder quality (Van Soest, 1994). Meanwhile, the concentration of propionic acid is related to rumen fermentation of soluble carbohydrates (McCollum *et al.*, 1985). According to the findings, the total VFA concentration ranged from 19.8 to 46.4 mM, which was less than the 70–150 mM typical range that is suggested to sustain microbial development (McDonald *et al.*, 2012).

The results also showed that gas production for 48 hours in the rumen was not influenced by cellulase enzyme treatment. Table 6 shows that the pattern of gas production, however, was consistent with the OM digestibility. Beuvink and Spoelstra (1992) reported a close correlation between OM digestibility, VFA concentration, and *in vitro* gas production. Silage containing 40% oil palm frond with cellulase treatment (B and C) produced lower CH_4 gas (P<0.05) than those without cellulase treatment. One mechanism to explain the decrease in CH_4 in the rumen is the conversion of lactic acid or pyruvic acid to propionic acid. In the rumen, lactate-utilizing bacteria such as *Megasphaera elsdenii, Selenomonas ruminantium, Fusobacterium necrophorum,* and *Veillonella parvula* can produced propionic acid which come from the conversion of lactic acid (Russell *et al.*, 1992). This can reduce methanogenesis because electrons are used during propionate formation. If hydrogen is then used to convert lactic acid to propionic acid in the rumen, the hydrogen will decrease, which in turn will inhibit the conversion of hydrogen and CO_2 to CH_4 (Moss *et al.*, 2000).

Consequently, an increase in propionic acid concentration as observed in silage with cellulase treatment (Table 4) may lead to decreased CH_4 production. The result of current study was in line with a prior study by Khota *et al.* (2017) that the application of cellulase to sorghum silage reduced *in vitro* CH_4 production.

In vitro nutrient digestibility

In vitro nutritional digestibility is the rate at which microorganisms break down substrate in an artificial environment when rumen conditions are replicated in a test tube. Table 6 shows the results of digestibility values of DM, OM, and NDF from oil palm frond-based silages treated with cellulase. Silages treated with cellulase enzyme at leves of 4 ml/kg and 6 ml/kg in both the 40% and 50% oil palm frond groups were higher (P<0.05) in IVDMD than those without cellulase (B, C vs A and E, F vs D). Application of cellulase at a dose of 6 ml/kg in both silage groups caused a higher IVDMD than 4 ml/kg. The effective degradation of NDF by cellulase is also reflected by the improvement of DM digestibility. Ebrahimi *et al.* (2014) reported that oil palm frond silage treated with cellulase at a dose of 2 g/kg increased *in vitro* DM digestibility compared to silage without celulase.

Silage containing 40% oil palm frond treated with cellulase (B and C) had higher IVOMD (P<0.01) than those without cellulase treatment. In addition, increasing the cellulase dose from 4 ml/kg to 6 ml/kg caused a rise in IVOMD to 13.72%. Application of cellulase to both silage groups also increased (P<0.01) IVNDF compared to those without cellulase.

Table 6: *In vitro* nutrient digestibility of oil palm frond-based complete feed silage.

	Complete feed silages						SEM	Orthogonal contrasts				
	A	B	C	D	E	F		1	2	3	4	5
IVDMD (%)	45.7	51.7	57.7	44.7	45.8	50.4	1.31	**	*	**	*	**
IVOMD (%)	44.3	48.1	54.7	43.7	45.3	49.4	1.57	**	NS	*	NS	NS
IVNDFD (%)	26.2	34.4	35.7	24.8	27.3	33.0	1.40	**	**	NS	*	**

SEM: standard error of the mean; 1: A vs B, C; 2: D vs E, F; 3: B vs C; 4: E vs F; 5: A, B, C vs D, E, F; NS: not significant; *(P<0.05); ** (P<0.01).

The results were in line with Khota *et al*. (2017) which observed an increase in IVOMD of sorghum silage after the addition of cellulase. Santoso *et al*. (2020) also concluded that there was a close relationship between increasing cellulase doses and improved *in vitro* nutrient digestibility of DM, OM, as well as NDF. Higher IVNDFD readings seem to be associated with the forage's physical properties, particularly its ADL, ADF, and NDF contents. In the study by Santoso *et al*. (2019), the low concentration of NDF and ADF in feed resulted in a significant increase in the digestibility of DM, OM, and NDF. The findings demonstrated that cellulose might boost the *in vitro* nutritional digestibility of IVDMD, IVOMD, and IVNDF in oil palm frond-based silage.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the results showed that adding cellulase enzyme to oil palm frond-based complete silage at doses of 4 and 6 milliliters per kilogram successfully reduced crude fiber fractions such as ADF, NDF, and ADL and raised nutritional digestibility values. Silage treated with cellulase enzyme showed better quality, indicated by an increase in lactic acid concentration and Fleigh point, as well as a decrease in NH_3-N concentration, the ratio of total VFA/total acids, and CH_4 production. The use of 40% and 50% oil palm fronds in complete feed silage produced good quality. This study is still limited to *in vitro* experiments, therefore further *in vivo* studies are needed to generalize the conclusions. The results of the current study implicate that oil palm frond can be well preserved by ensiling and thereby represents a continuous source of roughage for ruminant diets.

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NOVELTY STATEMENT

In this research, oil palm fronds which are abundantly available in oil palm plantation areas were used in making complete feed silage with a composition of 40% and 50%. The addition of cellulase enzymes to oil palm frond-based complete feed silage reduced the crude fiber fraction and improved the quality of silage fermentation as well as *in vitro* nutrient digestibility.

AUTHOR'S CONTRIBUTION

All authors contributed equally by reading and approving the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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