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The Effects of Oil Palm Fronds Silage Supplemented with Urea-Calcium Hydroxide on Rumen Fermentation and Nutrient Digestibility of Thai Native-Anglo Nubian Goats

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Abstract: This study aimed to examine the combined effects of urea and calcium hydroxide ensiled oil palm fronds on rumen fermentation and digestibility of Thai native-Anglo Nubian goats. A 4 × 4 Latin square design was used to randomly assign four male crossbred goats (Thai native × Anglo Nubian). The dietary treatments were as follows: ensiled oil palm frond with no additives (EOPF as the control), urea 5% (50 g/kg fresh matter) (E-UOPF 5%), calcium hydroxide ($Ca(OH)_2$) 5% (50 g/kg fresh matter) (E-CaOPF 5%), and combination of urea 2.5% (25 g/kg fresh matter) with $Ca(OH)_2$ (25 g/kg fresh matter) (E-UCOPF 2.5%). The oil palm frond ensiled with different additives did not change the DM intake ($p > 0.05$). The total TMR intakes range from 69.39 to 77.09 g/kg BW0.75. The goats fed with E-UOPF 5.0% consumed significantly more CP than the other groups ($p < 0.05$). The E-UCOPF increased ME intake by 4.8%, compared with the control treatment ($p < 0.05$). E-UOPF 5% and E-UCOPF 2.5% significantly increased the CP digestibility by 19.7% and 17.1%, respectively ($p < 0.05$). Furthermore, E-CaOPF 5.0% and E-UCOPF 2.5% improved the NDF digestibility by about 10.9% and 9.90%, respectively ($p < 0.05$). The urea-containing oil palm frond (E-UOPF 5.0% and E-UCOPF 2.5%) had higher blood urea nitrogen (BUN) than the other groups ($p < 0.05$). The TVFA of goats fed E-UCOPF 2.5% was approximately 15.8% higher than that of goats provide EOPF ($p < 0.05$). The mean concentration of C3 increased by 7.90% and 11.61%, respectively, when E-CaOPF 5.0% and E-UCOPF 2.5% were provided instead of EOPF ($p < 0.05$). The total N intake and absorbed were highest ($p < 0.05$) when goats offered E-UOPF 5.0% ($p < 0.05$). The goats fed oil palm frond without additives had the lowest percentage of N-absorption/N intake ($p < 0.05$). This study clearly shows that the most suitable treatment is E-UCOPF 2.5%, which enhances DMD, nutrient digestibility, TVFAs, and nitrogen balance and has no negative effects on rumen microbes. This indicates that E-UCOPF 2.5% may be utilized as an alternate roughage source in TMR diets, accounting for at least 40% of the OPF. However, several factors still require consideration for urea-$Ca(OH)_2$ treatments to be successful, including other concentrations of urea, moisture content, duration of pre-treatment, and the metabolizable protein system.

Keywords: urea-calcium hydroxide treatment; oil palm frond; rumen fermentation; goat

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1. Introduction

Southeast Asia is the world's biggest producer and exporter of palm oil (*Elaeis guineensis*) [1]. To enter the palm oil industry, palm trees were planted on more than 36 million hectares of land [2]. Therefore, it is not surprising that many unused parts of palm trees, such as fronds, are produced each year. According to Poh et al. [3] one hectare of oil palm-planted area provides approximately 10 tons of oil palm fronds (OPF), implying that there are currently at least 360 million tons of OPF in the world. Therefore, if these agricultural by-products are not dealt with properly, they may lead to environmental issues and lower public confidence in palm oil industries. To optimize the use of resources in a circular and sustainable way within the region, considering the use of palm fronds for animal husbandry is intriguing. Fronds are the low-cost, large, and divided oil palm leaves used for oil palm production. It has recently attracted a lot of attention due to its potential as a roughage source. However, the fiber structure of oil palm frond (OPF) in animal feed is restricted due to its relatively high lignin concentration (14–20% of DM) when compared with other tropical roughage crops [4,5]. Most tropical forages, such as grasses and legumes, have a lignin content of around 6.26% and 8.97% of DM, respectively [6]. The main antiquality role of lignin in forages is in limiting the digestion of animal feed [7]. Astuti et al. [8] found that, by increasing OPF levels to replace elephant grass as a roughage source in animal diets, in vitro dry matter digestibility and in vitro organic matter digestibility were both reduced by 50.7% and 47.7%, respectively. As a result, it has low digestibility, including low contents of energy and crude protein when compared with regular tropical roughage. With this restriction, the OPF's quality must improve, and some techniques, such as the application of urea and $Ca(OH)_2$, may be able to assist because they improve digestibility.

Both chemicals have been found to improve roughage sources significantly, particularly when dealing with high-fiber or low-quality roughage [9]. According to Wanapat [10], adding urea (5%) to rice straw could improve digestibility by allowing the bonds in the carbohydrate structure to be broken down more easily and it can also raise the protein content of rice straw. Under alkaline circumstances, the bond between lignin, hemicellulose, and cellulose can be dissolved, causing structural fibers to swell [11]. In the past, urea may have sufficed to improve the roughage's quality, but currently, that is no longer the case. Urea's price has risen dramatically since it was first introduced [12]. It is important to remember that the way researchers think about urea usage should change and that there should be strategies to maximize it while keeping costs in mind. To maintain the alkalinity bond dissolution efficiency while lowering costs, calcium hydroxide ($Ca(OH)_2$) was used. Calcium hydroxide is inexpensive and widely accessible [13]. The combination of these two compounds allows us to accomplish the same effects as before but at a cheaper cost, making it more appealing to users. Despite the fact that both urea and $Ca(OH)_2$ have been used in low-quality roughage such as rice straw, OPF has yet to be evaluated in contrast with physical and biological techniques, which have both been evaluated [9]. The purpose of this study was to compare the effects of ensiled OPF combined with urea-$Ca(OH)_2$ on feed intake, rumen fermentation, apparent total tract digestibility (ATTD), and nitrogen balance in Thai native-Anglo Nubian goats to a chemical pre-treatment solution.

2. Materials and Methods

2.1. Ensiling Materials and Silage Preparation

The current study was conducted at the Faculty of Natural Resources, Prince of Songkla University, Hat Yai Campus, Songkhla, Thailand. Oil palm fronds (OPF, Elaeis guineensis) were chopped into lengths of 1.0–1.5 cm by a machine (108 Agriculture Machine and Equipment Co., Ltd., Lopburi, Thailand). $Ca(OH)_2$ (Chemiall Co., Ltd., Bangkok, Thailand) and urea were used as additives before ensiling OPF. According to Yitbarek and Tamir [14], to ensure that the fermentation process remains effective, the OPF was sprayed with a solution (3 L of water combined urea and $Ca(OH)_2$ according to a treatment arrangement with 100 kg of fresh OPF (62.7% moisture)) to ensure a homogenous mixture and to control the moisture level to no more than 75%. Therefore, the OPF post-fermentation product contains about 25% of dry matter (DM). The dietary treatments were as follows: ensiled OPF with no additives (EOPF as control), urea 5% (E-UOPF 5%), $Ca(OH)_2$ 5% (E-CaOPF 5%), and combination of urea 2.5% with $Ca(OH)_2$ (E-UCOPF 2.5%).

Plastic bags (size 50 L, Changzhou Treering Plastics Co. Ltd., Jiangsu, China), 18 of them, were filled with the ensiled OPF. Each bag contained about 30–35 kg fresh matter (FM) of ensiled OPF materials. The total ensiled OPF production in this experiment was around 550 kg FM, which would be sufficient to feed the experimental animals for the duration of the study. The bagged samples were completely enclosed by a vacuum machine (Imaflex 1400 W VC-921, Imarflex Industrial Co., Ltd., Bangkok, Thailand) and ensiled for 30 days at an ambient temperature to establish anaerobic conditions.

2.2. Animals, Experimental Design, and Feeding

A 4 × 4 Latin square design was used to randomly assign four male crossbred goats (Thai native × Anglo Nubian), aged 16 months, with a bodyweight of 30.0 ± 1.00 kg. All of the goats were housed in separate well-ventilated shelter cages (0.50 × 1.20 m), where mineral licks and water were accessible at all times. After 30 days of ensiling, the OPF in each treatment was opened and mixed with a concentrate diet to develop the TMR approach as a roughage source. Roughage and concentrates (DM base) were combined in 40:60 ratios with human effort. The TMR diet was provided to goats daily by fed diets (Table 1) *ad libitum* to meet the nutritional requirements for maintenance of matured goats [15]. The experiment was performed in four periods, each period lasting 21 days. All animals were fed individual diets *ad libitum* for the first 14 days in each period. The animals were transferred to metabolic crates for the last 7 days; feed restriction was applied to 90% of the previous voluntary feed intake. Feeds were delivered twice a day in equal quantity at 8:00 a.m. and 4:00 p.m.

Table 1. Ingredients and chemical composition of the total mixed rations (TMR), EOPF, E-UOPF 5.0%, F-COPF 5.0%, and E-UCOPF 2.5% used in the experiment (DM basis).

Items	EOPF	E-UOPF 5.0%	E-CaOPF 5.0%	E-UCOPF 2.5%
Ingredients, %				
EOPF	40	0	0	0
E-UOPF 5.0%	0	40	0	0
E-CaOPF 5.0%	0	0	40	0
E-UCOPF 2.5%	0	0	0	40
Ground corn	35.8	35.8	35.8	35.8
Soybean meal	7.9	7.9	7.9	7.9
Fish meal	0.4	0.4	0.4	0.4
Leucaena leave meal	5.4	5.4	5.4	5.4
Oil palm meal	7.2	7.2	7.2	7.2
Molasses	2.2	2.2	2.2	2.2
Dicalcium phosphate	0.3	0.3	0.3	0.3
Salt	0.2	0.2	0.2	0.2
Mineral and vitamin mix	0.6	0.6	0.6	0.6
Chemical composition				
Dry matter, %	97.7	97.2	97.9	97.6
		% dry matter		
Organic matter	93.8	94.6	91.4	92.6
Crude protein	12.6	17.8	12.0	15.1
Ether extract	2.36	2.45	2.59	2.47
Non-structural carbohydrate	21.0	17.5	18.2	19.1
Neutral detergent fiber	57.8	56.8	58.6	55.9
Acid detergent fiber	31.9	32.7	32.6	31.4
Acid detergent lignin	9.6	10.0	10.2	10.1
Hemicellulose	25.9	24.1	26.1	24.5
Cellulose	22.2	22.7	22.3	21.4
Total digestible nutrient, %	64.5	65.5	63.6	65.9
Gross energy, Mcal/kg DM	4.33	4.3	4.09	4.21

Diets = EOPF = Ensiled oil palm frond, E-UOPF = Ensiled oil palm frond with urea, E-CaOPF = Ensiled oil palm frond with calcium hydroxide, E-UCOPF = Ensiled oil palm frond with urea plus calcium hydroxide. Total digestible nutrient = DCP + DCF + DNFE + (DEE × 2.25).

2.3. Procedures for Collecting Samples and Sampling

The TMR diet intake of each goat was monitored daily by calculating the feed offered minus the feed residue. The trial was split into 4 periods, each lasting 21 days, with 14 days for the adaptation period and 7 days for fecal matter and urine collection. Urine and feces were collected with a urine container in the middle of the crate and a feces tray at the back of the crate. Fecal collections began at 07:00 a.m. on day 17, ending at 07:00 a.m. on day 21 (5 days). All feces were removed from the crate and placed in plastic bags, keeping each goat separate. Total feces were weighed, and 5% of total feces were pooled in each goat throughout the last 5 days of each period. The fecal samples were refrigerated at 4 °C and separated into two sub-samples. The first sample was analyzed for the dry matter (DM) content; the second was kept refrigerated at 4 °C. The ensiled OPF samples were collected after an open silo (3 replications for each sample). The TMR samples (offers and refusals) and fecal samples were collected within the last 7 days. These samples were oven-dried for 72 h at 60 °C; crushed to pass through a 1 mm sieve; blended on an equal weight basis; and evaluated for DM (ID 967.03), ash (ID 492.05), ether extract (EE; ID 455.08), and crude protein (CP; ID 984.13) [16]. The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) content were measured using a detergent analysis method [17]. Gross energy (GE) was determined by using an Isoperibol Bomb Calorimeter (Parr Instrument Co., model 1261, Moline, IL, USA) according to the standard operating instructions (No 242M) [16]. Digestible energy (DE) was calculated by subtracting the gross energy in the feces from the gross energy consumed by the animal. DE was determined as the metabolizable energy (ME) by the equation ME = 0.82 × DE [15]. Total urine was collected by using a plastic container fixed with sulfuric acid (H_2SO_4, 10%) to protect against nitrogen (N) loss and then pooled at the end of each period for total N analysis to determine N utilization. Then, N intake, N excretion, N absorption, and N retention were computed as follows:

$$\text{Nitrogen retention (g/d)} = \text{Total nitrogen intake (g/d)} - (\text{Fecal nitrogen excretion} + \text{Urine nitrogen excretion})\ \text{(g/d)}$$

$$\text{Nitrogen absorption (g/d)} = \text{Total nitrogen intake (g/d)} - \text{Fecal nitrogen excretion (g/d)}$$

Goats were individually weighed before the morning feeding at the beginning and end of the experiment. During the ATTD test, rumen fluid was collected from all goats at the end of each period, before the morning feeding and four hours later, using a stomach tube. The pH was immediately measured using a pH meter (HANNA Instruments HI 98,153 microcomputer pH meter, Singapore) connected to a paired electrode after it was strained through four layers of cheesecloth. To analyze volatile fatty acid (VFA), the ruminal fluid was acidified with 3 mL of 1 M H_2SO_4 added to 30 mL of ruminal fluid. The VFA concentration was determined by high-performance liquid chromatography (HPLC; ETL Testing Laboratory, Inc., Cortland, NY, USA; instruments by controller water model 600 E, Water model 484 UV detector, column Novapak C18, column size 4 150 mm, mobile phase 10 mM H_2PO_4 (pH 2.5)) according to Mathew et al. [18]. The mixture was centrifuged at 16,000 g for 15 min, and the supernatant was kept at −20 °C before micro-Kjeldahl NH_3-N analysis [16]. Blood samples (about 10 mL) were obtained from a jugular vein (at the same time as ruminal fluid sampling) and deposited in tubes containing 12 mg of EDTA (ethylenediamine tetra-acetic acid) for blood urea nitrogen (BUN) assay [19]. Plasma was divided by a centrifuge separator at 2500 g for 15 min at 5 °C and kept at 20 °C before analysis. Commercial kits were used to assess plasma glucose and packed cell volume (PCV) (No. 640, Sigma Chemical Co., St. Louis, MO, USA). Protozoa, bacteria, and fungal zoospores in the rumen were counted using the direct count technique [20].

2.4. Statistical Analyses

The GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA) SAS [21] was used to analyze the data, according to the following general model:

$$Y_{ijk} = \mu + M_i + A_j + P_k + e_{ijk}$$

where Y_{ijk} is the observation from goats j receiving TMR diet i in period time k; μ is the overall mean; M_i is the effect of the different OPF additives (i = 1, 2, 3, 4); A_j is the effect of goats (j = 1, 2, 3, 4); P_k is the effect of the period (k = 1, 2, 3, 4); and e_{ijk} is the overall error (residual). The results are provided as mean values with standard deviations. Differences between treatment means were calculated using Duncan's New Multiple Range Test, and differences among means with $p < 0.05$ are represented as statistically significant differences.

3. Results

3.1. Nutrient Content in Total Mixed Ration Diets

Table 1 presents the TMR ingredients and chemical composition. The TMR diets contained 12.0% to 17.8% CP and 63.6 to 65.9% concentrations of total digestive nutrients (TDN), sufficient for supporting the crossbred goats' requirements [15]. Oil palm frond ensiled with various additives was established as a roughage resource for animals fed with the TMR diet of 40% DM. The TMR diets have a range of OM, EE, hemicellulose, and cellulose content of approximately 91.4 to 94.6 %DM, 2.36 to 2.59 %DM, 24.1 to 26.1 %DM, and 21.4 to 22.7 %DM, respectively.

3.2. Chemical Compositions of Oil Palm Fronds Ensiled with Various Additives

Table 2 shows the chemical compositions of oil palm frond ensiled with various additives. The DM of EOPF was 37.1% and decreased by 15.6%, 7.5%, and 8.4% when supplemented with urea (E-UOPF 5.0%), calcium hydroxide (E-CaOPF 5.0%), and urea plus calcium hydroxide (E-UCOPF 2.5%), respectively (<0.01). When compared with other groups, the E-UOPF 5% had a higher OM (93.1%) than the use of the E-CaOPF 5% and E-UCOPF 2.5% ($p = 0.02$), as well as the highest CP (16.3%) (<0.01). The E-CaOPF 5.0% had the lowest fiber content of NDF 67.3% and ADF 56.6% ($p = 0.03$). While ADL was lowest in the E-CaOPF 5%, it was unchanged in E-UCOPF 2.5% (<0.01). When compared with the control group, the additives did not influence the contents of EE (range 0.78 to 0.89%) and GE (range 3.59 to 4.26 Mcal/kg DM) ($p > 0.05$).

Table 2. Chemical composition of ensiled oil palm fronds supplemented with urea-calcium hydroxide.

Item	Dietary Treatments [1]				SEM [2]	*p*-Value
	EOPF	E-UOPF 5.0%	E-CaOPF 5.0%	E-UCOPF 2.5%		
Dry matter, %	37.1 [a]	31.3 [c]	34.3 [b]	34.0 [b]	0.31	<0.01
		% of DM				
Organic matter	92.6 [a]	93.1 [a]	86.3 [c]	90.1 [b]	0.52	0.02
Crude protein	7.90 [c]	16.3 [a]	5.61 [d]	12.1 [b]	0.26	<0.01
Ether extract	0.78	0.88	0.89	0.88	0.07	0.34
Neutral detergent fiber	82.2 [a]	78.9 [ab]	67.3 [c]	74.3 [b]	1.87	<0.01
Acid detergent fiber	66.5 [a]	65.6 [a]	56.6 [b]	63.5 [a]	1.73	<0.01
Acid detergent lignin	26.5 [a]	24.3 [a]	19.4 [b]	22.8 [ab]	1.28	0.03
Gross energy, Mcal/kg DM	4.14	4.26	3.61	3.59	0.26	0.45

[a–d] Means within the same row not sharing a common superscript are significantly different $p < 0.05$, $p < 0.01$. [1] Diets = EOPF = Ensiled oil palm frond, E-UOPF = Ensiled oil palm frond with urea, E-CaOPF = Ensiled oil palm frond with calcium hydroxide, E-UCOPF = Ensiled oil palm frond with urea plus calcium hydroxide. [2] SEM = Standard error of the mean ($n = 3$).

3.3. Feed and Nutrient Intake

Table 3 presents the DM and nutrient intake of goats that consume TMR. The oil palm frond ensiled with different additives did not change the DM intake ($p > 0.05$). The total TMR intakes range from 69.39 to 77.09 g/kg $BW^{0.75}$. The goats fed with E-UOPF 5.0% consumed significantly more CP than the other groups ($p = 0.04$). Nutrient intake, OM, NDF, ADF, and ADL were comparable across treatments, ranging from 0.965 to 1.035 kg/d, 0.577 to 0.652 kg/d, 0.335 to 0.365 kg/d, and 0.100 to 0.115 kg/d, respectively ($p > 0.05$). The E-UCOPF increased ME intake by 4.8% compared with the control treatment ($p = 0.02$).

Table 3. Effect of ensiled oil palm fronds supplemented with urea-calcium hydroxide on feed and nutrient intake in goats.

Item	Dietary Treatments [1]				SEM [2]	*p*-Value
	EOPF	E-UOPF 5.0%	E-CaOPF 5.0%	E-UCOPF 2.5%		
Total dry matter intake, kg/d	1.10	1.05	1.13	1.11	0.04	0.41
Dry matter intake, %BW	2.98	2.81	3.16	2.99	0.16	0.54
Dry matter intake, g/kg $W^{0.75}$	73.52	69.39	77.09	73.08	3.69	0.50
Nutrients intake (kg/d)						
Organic matter	1.035	0.965	1.015	1.007	0.03	0.64
Crude protein	0.140^{b}	0.182^{a}	0.135^{b}	0.165^{ab}	0.01	0.04
Neutral detergent fiber	0.632	0.577	0.652	0.607	0.03	0.45
Acid detergent fiber	0.350	0.335	0.365	0.342	0.01	0.46
Acid detergent lignin	0.115	0.100	0.112	0.107	0.006	0.41
Energy intake						
Metabolizable energy, Mcal/d	2.50	2.37	2.58	2.58	0.10	0.44
Metabolizable energy, Mcal/kg DM	2.28^{b}	2.33^{ab}	2.32^{ab}	2.39^{a}	0.010	0.02

[a,b] Means within the same row not sharing a common superscript are significantly different $p < 0.05$, $p < 0.01$. [1] Diets = EOPF = Ensiled oil palm frond, E-UOPF = Ensiled oil palm frond with urea, E-CaOPF = Ensiled oil palm frond with calcium hydroxide, E-UCOPF = Ensiled oil palm frond with urea plus calcium hydroxide. [2] SEM = Standard error of the mean ($n = 4$).

3.4. Apparent Total Tract Digestibility of TMR

The ensiled OPF and additives fed to Thai native × Anglo Nubian goats influence the ATTD (Table 4). The DM and OM digestibility was significantly enhanced when goats were fed E-UCOPF 2.5% (<0.01). Furthermore, E-UOPF 5% and E-UCOPF 2.5% increased CP digestibility by about 19.7% and 17.1%, respectively, when compared with EOPF ($p = 0.03$). In addition, E-CaOPF 5.0% and E-UCOPF 2.5% improve the NDF digestibility of goats ($p = 0.04$).

Table 4. Effect of ensiled oil palm fronds supplemented with urea-calcium hydroxide on the apparent total tract digestibility of goats.

Item	Dietary Treatments [1]				SEM [2]	*p*-Value
	EOPF	E-UOPF 5.0%	E-CaOPF 5.0%	E-UCOPF 2.5%		
Apparent total tract digestibility, %						
Dry matter	62.09^{c}	62.89^{bc}	63.60^{b}	65.14^{a}	0.38	<0.01
Organic matter	64.03^{b}	64.78^{b}	67.01^{a}	67.96^{a}	0.35	<0.01
Crude protein	56.04^{b}	67.09^{a}	61.18^{ab}	65.60^{a}	2.11	0.03
Ether extract	55.98	56.51	65.48	63.42	3.19	0.17
Neutral detergent fiber	55.25^{b}	56.24^{ab}	61.31^{a}	60.72^{a}	1.46	0.04
Acid detergent fiber	36.31	38.63	42.41	41.66	2.33	0.22
Acid detergent lignin	23.99	21.19	32.31	25.97	3.37	0.30

[a–c] Means within the same row not sharing a common superscript are significantly different at $p < 0.05$ and $p < 0.01$. [1] Diets = EOPF = Ensiled oil palm frond, E-UOPF = Ensiled oil palm frond with urea, E-CaOPF = Ensiled oil palm frond with calcium hydroxide, E-UCOPF = Ensiled oil palm frond with urea plus calcium hydroxide. [2] SEM = Standard error of the mean ($n = 4$).

3.5. The pH, NH3-N, and Blood Metabolites

Table 5 reveals the influence of oil palm frond ensiled with various treatments fed to crossbred goats on ruminal pH, NH_3-N, and blood metabolites. The pH of the rumen remained the same when treatments were provided. However, the mean pH of the oil palm fronds without treatments was lower than E-UCOPF (<0.01). The NH_3-N at both 0 h and 4 h post-feeding and the mean value were highest (<0.01) when oil palm fronds were ensiled with urea at 17.5, 22.9, and 20.2 mg/dL, respectively. Moreover, E-UOPF 5.0% and E-UCOPF 2.5% had higher BUN levels than the other groups (<0.01). When goats were fed with E-UCOPF 2.5%, the blood glucose level was higher than in the control group at 65.0 mg/dl at 4 h after feeding (p = 0.03). The control group and E-CaOPF 5% had the largest PCV at 0 h after feeding (p = 0.02).

Table 5. Effect of ensiled oil palm fronds supplemented with urea-calcium hydroxide on ruminal pH, NH_3-N, and blood metabolites in goats.

Item	Dietary Treatments [1]				SEM [2]	p-Value
	EOPF	E-UOPF 5.0%	E-CaOPF 5.0%	E-UCOPF 2.5%		
Ruminal pH						
0 h post feeding	6.59^{b}	6.69^{ab}	6.732^{ab}	6.94^{a}	0.08	0.03
4 h post feeding	6.38	6.53	6.51	6.50	0.08	0.54
Mean	6.49^{b}	6.61^{ab}	6.62^{ab}	6.72^{a}	0.04	0.05
Ammonia nitrogen mg/dL						
0 h post feeding	12.14^{b}	17.50^{a}	11.43^{b}	13.57^{b}	0.91	<0.01
4 h post feeding	12.86^{c}	22.86^{a}	13.21^{c}	16.43^{b}	0.64	<0.01
Mean	12.50^{c}	20.18^{a}	12.32^{c}	15.00^{b}	0.60	<0.01
Blood urea nitrogen, mg/dL						
0 h post feeding	14.74^{b}	27.04^{a}	14.00^{b}	24.92^{b}	0.96	<0.01
4 h post feeding	14.81^{b}	28.01^{a}	14.79^{b}	25.31^{a}	0.92	<0.01
Mean	14.78^{b}	27.53^{a}	14.39^{b}	25.12^{a}	0.90	<0.01
Glucose, mg/dL						
0 h post feeding	62.25	64.75	60.00	63.00	1.28	0.07
4 h post feeding	61.50^{b}	64.25^{ab}	62.50^{ab}	65.00^{a}	0.91	0.03
Mean	61.88	64.50	61.25	64.00	0.92	0.09
Packed cell volume, %						
0 h post feeding	29.00^{a}	27.00^{b}	27.75^{ab}	26.50^{b}	0.40	0.02
4 h post feeding	26.00	26.25	27.00	25.75	1.21	0.89
Mean	27.50	26.62	25.37	26.12	0.76	0.58

[a–c] Means within the same row not sharing a common superscript are significantly different at $p < 0.05$ and $p < 0.01$. [1] Diets = EOPF = Ensiled oil palm frond, E-UOPF = Ensiled oil palm frond with urea, E-CaOPF = Ensiled oil palm frond with calcium hydroxide, E-UCOPF = Urea-ensiled oil palm frond with calcium hydroxide. [2] SEM = Standard error of the mean (n = 4).

3.6. Volatile Fatty Acid Profiles

Table 6 shows the amounts of total VFA, acetic acid (C2), propionic acid (C3), and butyric acid (C4) and the acetic acid–propionic acid ratio (C2:C3). The concentrations of TVFAs and the molar proportions of VFA differed significantly (p = 0.02). When compared with the other groups, goats fed ensiled OPF with urea, $Ca(OH)_2$, or their combination showed the greatest TVFA 4 h after feeding (p = 0.02). The C2 concentration was significantly higher when goats were fed 5% E-UOPF (4 h and 8 h, p = 0.02; mean, <0.01). The mean concentration of C3 increased to 28.85% and 29.84% when goats were fed F-COPF 5.0% and E-UCOPF 2.5%, respectively (p = 0.02). As a result, the E-CaOPF 5.0% and E-UCOPF 2.5% had a lower C2:C3 ratio than the other groups (1.94 and 1.91, respectively, p = 0.02). The goats' methane emissions were reduced by E-CaOPF 5.0% and E-UCOPF 2.5%. However, EOPF and E-UOPF 5.0% resulted in the highest methane emissions in the goats (p = 0.02).

Table 6. Effect of ensiled oil palm fronds supplemented with urea-calcium hydroxide on volatile fatty acid profiles in goats.

Item	Dietary Treatments [1]				SEM [2]	*p*-Value
	EOPF	E-UOPF 5.0%	E-CaOPF 5.0%	E-UCOPF 2.5%		
Total VFA, mmol/L						
0 h post feeding	107.56	114.36	116.45	120.53	5.52	0.46
4 h post feeding	101.90 [b]	113.45 [a]	115.04 [a]	122.08 [a]	3.16	0.02
Mean	104.73 [b]	113.90 [ab]	115.74 [a]	121.31 [a]	3.06	0.04
Proportion of individual VFA, %						
Acetate (C_2)						
0 h post feeding	60.74 [a]	61.97 [a]	55.27 [b]	58.52 [ab]	1.38	0.04
4 h post feeding	58.34 [ab]	63.98 [a]	55.87 [b]	54.23 [b]	2.04	0.02
Mean	59.54 [ab]	62.98 [a]	55.57 [b]	56.37 [b]	1.12	<0.01
Propionate (C_3)						
0 h post feeding	24.42	25.80	27.59	28.58	0.51	0.28
4 h-post feeding	29.06	24.88	30.10	31.10	0.58	0.07
Mean	26.74 [bc]	25.34 [c]	28.85 [ab]	29.84 [a]	0.47	0.02
Butyrate (C_4)						
0 h post feeding	14.83	12.22	17.13	12.89	1.95	0.36
4 h post feeding	12.59	11.12	14.02	14.66	1.00	0.16
Mean	13.71	11.67	15.57	13.77	1.40	0.36
Acetate: propionate ratio (C_2:C_3)						
0 h post feeding	2.51	2.43	2.03	2.07	0.14	0.10
4 h post feeding	2.05	2.67	1.86	1.75	0.20	0.07
Mean	2.28 [a]	2.55 [a]	1.94 [b]	1.91 [b]	0.07	0.02
Estimated methane production (CH_4)						
0 h post feeding	26.98	26.14	24.63	24.14	0.97	0.23
4 h post feeding	23.82	26.84	23.01	22.27	1.00	0.07
Mean	25.40 [ab]	26.49 [a]	23.82 [bc]	23.21 [c]	0.48	0.02

[a–c] Means within the same row not sharing a common superscript are significantly different at $p < 0.05$ and $p < 0.01$. [1] Diets = EOPF = Ensiled oil palm frond, E-UOPF = Ensiled oil palm frond with urea, E-CaOPF = Ensiled oil palm frond with calcium hydroxide, E-UCOPF = Urea-ensiled oil palm frond with calcium hydroxide. [2] SEM = Standard error of the mean (n = 4).

3.7. Microorgarnism Count in Rumen Fluid

Regarding ensiled oil palm fronds with various additives, populations of bacteria, protozoa, and fungal zoospores of incubation fluids were not affected by additives (Table 7). The bacteria, protozoa, and fungal zoospores in the rumen were measured, and the mean value ranged from 3.88 to 4.16 $\times$ 10^{10} cell/mL, 3.27 to 5.29 $\times$ 10^6 cell/mL, and 1.78 to 2.09 $\times$ 10^6 cell/mL, respectively.

Table 7. Effect of ensiled oil palm fronds supplemented with urea-calcium hydroxide on rumen microbes in goats.

Item	Dietary Treatments [1]				SEM [2]	*p*-Value
	EOPF	E-UOPF 5.0%	E-CaOPF 5.0%	E-UCOPF 2.5%		
Total direct count						
Bacteria ($\times 10^{10}$ cell/mL)						
0 h post feeding	3.89	4.04	3.01	3.56	0.31	0.19
4 h post feeding	4.07	4.28	4.74	4.76	0.45	0.65
Mean	3.98	4.15	3.88	4.16	0.26	0.85
Fungal zoospores ($\times 10^6$ cell/mL)						
0 h post feeding	5.79	2.54	3.00	3.99	0.68	0.06
4 h post feeding	4.78	3.99	3.79	3.78	0.54	0.55
Mean	5.29	3.27	3.39	3.89	0.47	0.08
Total Protozoa ($\times 10^6$ cell/mL)						
0 h post feeding	1.56	1.31	1.96	1.90	0.39	0.64
4 h post feeding	2.00	2.26	2.22	2.23	0.46	0.97
Mean	1.78	1.79	2.09	2.06	0.35	0.86

[1] Diets = EOPF = Ensiled oil palm frond, E-UOPF = Ensiled oil palm frond with urea, E-CaOPF = Ensiled oil palm frond with calcium hydroxide, E-UCOPF = Ensiled oil palm frond with urea plus calcium hydroxide. [2] SEM = Standard error of the mean.

3.8. Nitrogen Balance

Table 8 presents the influence of oil palm frond ensiled with additives on the N balance of goats. Differences were noted in the total nitrogen intake, urinary N excretion, total N excretion, N absorption, and N-absorption/N intake between the additives of ensiled oil palm frond (p = 0.04). The total N intake and absorbed were high (p = 0.03) when goats were offered E-UOPF and E-UCOPF. The goats fed with the urea-containing oil palm frond (E-UOPF 5.0% and E-UCOPF 2.5%) produced higher urinary N (<0.01) and total N excretion (p = 0.04) than the other groups. The goats fed oil palm fronds without additives had a low percentage of N-absorption/N intake at 55.96% (p = 0.03).

Table 8. Effect of ensiled oil palm fronds supplemented with urea-calcium hydroxide on N balance of goats.

Item	Dietary Treatments [1]				SEM [2]	p-Value
	EOPF	E-UOPF 5.0%	E-CaOPF 5.0%	E-UCOPF 2.5%		
Total N intake, g/d	22.09 ^{b}	29.12 ^{a}	21.26 ^{b}	26.03 ^{ab}	1.73	0.04
N excretion, g/d						
Fecal N	9.74	9.46	8.24	9.10	0.38	0.12
Urinary N	1.24 ^{b}	4.04 ^{a}	1.06 ^{b}	4.15 ^{a}	0.54	<0.01
Total N excretion	10.98 ^{ab}	13.50 ^{a}	9.31 ^{b}	13.25 ^{a}	0.82	0.03
N absorption, g/d	12.35 ^{b}	19.65 ^{a}	13.02 ^{b}	16.93 ^{ab}	1.55	0.04
N retention, g/d	11.11	15.61	11.95	12.78	1.52	0.27
N-absorption/N intake, %	55.96 ^{b}	67.02 ^{a}	61.11 ^{ab}	65.46 ^{a}	2.15	0.03
N-retention/N intake, %	50.52	53.46	56.16	50.31	3.18	0.55

^{a,b} Means within the same row not sharing a common superscript are significantly different at $p < 0.05$ and $p < 0.01$. [1] Diets = EOPF = Ensiled oil palm frond, E-UOPF = ensiled oil palm frond with urea, E-CaOPF = ensiled oil palm frond with calcium hydroxide, E-UCOPF = Ensiled oil palm frond with urea plus calcium hydroxide. [2] SEM = Standard error of the mean (n = 4).

4. Discussion

4.1. Chemical Compositions of Oil Palm Fronds Ensiled with Various Additives

The chemical treatment of agricultural by-products has been extensively researched to improve the nutrient value of low-quality livestock feeds [22,23]. The disruption of lignin structures in agricultural residues and the rise in cellulose and hemicellulose are crucial for ruminant livestock diets [24]. This study compared the addition of Ca $(OH)_2$ during fermentation and lowered the NDF, ADF, and ADL contents by 18%, 15%, and 27%, respectively, with EOPF (Table 1). Gunun et al. [25] discovered that using 2% urea + 2% $Ca(OH)_2$-ensiled sugarcane bagasse reduced the NDF, ADF, and ADL contents by 9.1%, 13.0%, and 33.0%, respectively. In addition, Polyorach and Wanapat [9] revealed that the fiber content of the feed was reduced when the chemical additives contained $Ca(OH)_2$. In our study, the combination of urea and $Ca(OH)_2$ resulted in a small fiber reduction when compared with $Ca(OH)_2$ alone. This may be associated with a difference in the amount of $Ca(OH)_2$. The alkali in the lignocellulosic material causes swelling, thereby increasing the internal surface, lowering the polymerization degree and crystallinity, causing the lignin to rupture [26]. The main cause of the increase in CP content is that urea is a non-protein nitrogen that supplies a large amount of nitrogen to the substrate [27]. Since nitrogen binds OPF during fermentation, it promotes rumen bacteria and produces a greater microbial protein in animals.

4.2. Effects on Feed Intake and Apparent Total Tract Digestibility

In this study, the goats' feed intakes were restricted for the last seven days of the research, and their feed consumptions were evaluated to be similar. The total DM intake (DMI) of all goats (average of BW 30 kg) ranged from 69.39 to 77.09 g/kg $BW^{0.75}$, which was close to the previous experiment and showed that the DMI was within the normal range. Thoh et al. [28] found that goat (average BW 40 kg) feed intake ranged from 67.32 to 79.38 g/kg $BW^{0.75}$. In agreement with Hamchara et al. [29], the DMI in male crossbred (Thai Native Anglo Nubian; average BW 33.5 kg) goats varied from d 74.74 to 82.23 g/kg $BW^{0.75}$

when using OPF ensiled with fungi. The urea-ensiled group had the highest crude protein intake (CPI), which makes it reasonable to estimate that the 5.0% urea content applied to fermentation raised the animal's chances of receiving CP. In this study, the CPI ranged from 0.135 to 0.182 kg/d, which was the amount that the animal should receive based on the standard guidelines. Thus, the daily energy intake met the animal's requirements. Following that NRC [15] requirements, our feed formula offers CP and ME, appropriate to promote goat performance.

OPF has a significant amount of lignin, a large complex structure present in the cell wall that has a direct impact on digestion if the quality is not improved [30]. Previous research has found that including fresh OPF in a goat's diet increases the digestibility of DM, OM, and CP by about 46.9%, 51.7%, and 41.0%, respectively [31], which is lower than ensiled OPF in this study (digestibility of more than 60%). In our study, the combination of 2.5% urea and $Ca(OH)_2$ in treating E-UCOPF increased the digestibility of DM, OM, CP, and NDF by 4.9%, 6.1%, 11.7%, and 9.9%, respectively, when compared with EOPF. The findings support our idea that the usage of alkaline would cause structural alterations in the OPF, as evidenced by changes in the chemical composition. This is consistent with Wanapat et al. [11], who explained that the concentrated alkaline treatments physically swell structural fibers by chemically breaking the ester linkages between lignin, hemicellulose, and cellulose. Likewise, Mason et al. [32] hypothesized that the increase in cell wall degradability after treatment with urea and calcium hydroxide was due to the treatment's influence on lignin or its linkage, correlating to the chemical composition. As a result of the OPF restructuring, rumen microbes may be more accessible, resulting in increased digestibility [33]. Aside from the fact that the E-UOPF achieves the OPF's digestibility performance, some evidence suggests that the type of alkaline, roughage source, and animal species may have different effects on digestion. According to Rusli et al. [34], not all alkaline compounds are beneficial in OPF. This review shows that, since sodium hydroxide (NaOH) was used to improve OPF, it is possible that it is ineffective in decreasing the amount of lignin. As a reason, digestion may not be as efficient as it may be. Differences in roughage source and animal species had a significant impact on digestion when compared with our experiment. Wanapat et al. [11], who discovered that, when urea and $Ca(OH)_2$ were supplemented on rice straw and fed to dairy cows at 70.1 g/kg $BW^{0.75}$, the DMD, OMD, CPD, and NDFD increased by 24.4%, 22.4%, 20.4%, and 31.5 %, respectively. While adding urea and $Ca(OH)_2$, ensiled sugarcane bagasse in the feeds of Thai native beef cattle at 50.0 g/kg $BW^{0.75}$ increased the digestive coefficients of DM, OM, CP, and NDF by 13.6%, 14.5%, 15.0%, and 39.3%, respectively, compared with the control group, according to Gunun et al. [25]. Regardless, the digestibility percentages for OPF in this experiment were lower than those for urea and Ca(OH)2 in other conditions, but this is the first time urea and Ca(OH)2 have been applied to OPF, and the results at least follow the same pattern that has been used to improve the quality of various feedstuffs.

4.3. *Rumen Characteristics, Volatile Fatty Acid Profiles, and Blood Parameters*

Ruminal pH increased slightly (from 6.59 to 6.94) after alkali addition treatment. However, NH_3-N was predominant in the urea group (from 12.14 to 17.50 mg/dL). The findings are consistent with prior research [13]. According to Polyorach and Wanapat [9], the pH of the ensiled rice straw with urea and $Ca(OH)_2$ was higher than that of the control group. Despite the fact that the addition of urea and $Ca(OH)_2$ altered the rumen fermentation parameters in our experiment, both results were within normal ranges.

In agreement with Hamchara et al. [29], a pH of 6.5 to 7.0 is ideal for bacterial adhesion and required for fiber digestion. Moreover, a pH of less than 6.0 can suppress cellulolytic bacteria. Furthermore, a range of 15 to 30 mg/dl NH_3-N was effective for improving rumen fermentation, microbial proliferation, and feed intake in ruminants fed urea-ensiled rice straw [35]. NH_3-N is a well-known primary nitrogen source for protein synthesis and bacterial development [36]. Increasing E-UOPF feeding levels raises NH_3-N levels, thereby encouraging microbial growth. As shown by Hung et al. [37], an increase in CP intake stimulates proteolytic bacteria growth. As a result, our investigation shows greater CP digestibility. Given the higher amounts of NH_3-N in the rumen, the increase in blood BUN following urea-containing treatments was not surprising. This shows that urea treatment increased OPF's nitrogen content, leading to the addition of nitrogenous substrate [38]. Furthermore, the BUN of goats fed with ensiled OPF varied from 14.39 to 27.53 mg/dL, which was within the acceptable physiological accepted range, estimated in the range of 11.2 to 27.7 mg/dL [39].

The TVFAs concentration is the ruminants' major source of energy, and their increase in the rumen indicates animal feeding has improved [40]. According to our results, 2.5% E-UCOPF provided a significant amount of TVFAs, which was correlated with higher digestibility than the other groups. In terms of blood parameter, balance body tissue and systemic function blood profile, i.e., glucose, may be used to assess livestock performance test of complete feed [41]. Glucose is composed of glycogenic compounds that have performed gluconeogenesis. This substance can be produced by a compound that performs a direct net conversion to glucose, or partially metabolized glucose is transported to the liver or kidney to be produced into glucose [42]. In ruminants, glycogenic C3 is absorbed from the rumen into the portal circulation and then transported to the liver to be changed into glucose [43]. Hamdi [41] stated that any feed that is easily digested in the rumen could increase the amount of C3 and glucose in the blood. As a consequence of our findings, it is quite likely that E-UOPF's potential C3 increase is the primary cause of the 5.7% increase in blood glucose compared with EOPF. Packed cell volume (PCV) was slightly lower with E-UOPF, including in the TMR diet. Packed cell volume (PCV) is used as an index of toxicity in the diet; PCV levels that are below the normal range might suggest poor feed or anemia [44]. However, the hematology of this trial showed that the PCV values were within the normal range between 26.0 and 29.0% for clinically healthy goats, as reported by Kraiprom and Jantarat [45]. Based on this study, these data indicate that the inclusion of ensiled OPF had no effect on BUN, blood glucose, and PCV concentrations.

4.4. Nitrogen Utilization

Nitrogen balance was considered to be the most common index of the protein status of ruminants [29]. Our study showed that goats fed with OPF ensiled with urea (E-UOPF 5.0% and E-UCOPF 2.5%) had higher N intake and higher urinary N excretion and total N excretion. However, N-absorption per intake was better in percentage than the other groups (66.24% vs. 55.96%), which indicates that the efficacy of utilization of N per unit intake was better than the control group. Although, the use of urea ensiled OPF should be the main factor in providing additional non-protein N and in improving microbial protein synthesis in the rumen [46], alkaline chemicals may impact the structure of OPF to assist digestion and to generate great VFA content, which might be one reason why both N and energy levels are appropriate for N-absorption per intake. This is in agreement with the case of Chanjula et al. [47], who discovered that animals fed with ensiled OPF with fungus and urea had higher N intake (24.4%) and N absorption per N intake (32.2%) than the control group. The combination of N-rich urea and fiber-digesting fungus, which leads to enhanced water soluble VFA production, may result in optimal N and energy levels [48–50]. Gabriel et al. [51] found that increasing amounts of urea (N-source) and molasses (energy source) in goat feed additives resulted in a linear increase in N intake and N absorption per N intake.

5. Conclusions

This study clearly shows that the most suitable treatment is E-UCOPF 2.5%, since it enhances DMD, nutrient digestibility, TVFAs, and nitrogen balance and has no negative effects on rumen microbes. This indicated that E-UCOPF 2.5% may be utilized as an alternate roughage source in TMR diets, accounting for at least 40% of the OPF. However, several factors still require consideration for the urea-$Ca(OH)_2$ treatment to be successful, including the other concentration of urea, moisture content, duration of pre-treatment, and the metabolizable protein system.

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