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Original Article

Nutrient digestibility, fermentation pattern, blood biochemical level and inflammatory response of nulliparous dairy goats fed with various levels of oil palm fronds

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Abstract

This study was conducted to investigate the effect of oil palm frond (OPF) inclusion in total mixed ration (TMR) diets on nutrient digestibility, fermentation pattern, blood biochemical level and inflammatory response in nulliparous dairy goats. Three nulliparous female dairy goats (Saanen x Thai native, 33.88±1.78 kg) were randomly assigned in a 3x3 Latin square design. The diets consisted of 0% OPF (CON), 20% OPF (LOPF) and 40% OPF (HOPF) on a dry matter (DM) basis. The inclusion of OPF in the diets significantly increased the DM and nutrient intake of the dairy goats (p<0.05). There was no significant difference in the digestibility of DM, organic matter and crude protein of LOPF and HOPF, (p>0.05) but those were significantly higher than CON (p<0.05). Dietary OPF did not affect the digestibility of EE and ADF. No significant difference was observed in rumen pH, NH_3-N, individual VFA proportions, C2:C3 ratio, C2,C4:C3 ratio, and CH_4 production (p>0.05). However, dietary OPF altered the total production of VFA at 0 h before feeding (p<0.05). Total N intake, N absorption, and N retention significantly increased (p<0.05) as the level of OPF inclusion increased. Goats receiving CON diet showed a negative N balance. The results indicate that dietary HOPF lowered the TNF-α level (p<0.05). However, there was no significant difference in the level of IL-6, IL-1β and blood biochemistry (p>0.05). In conclusion, the inclusion of 40% OPF in a TMR-based diet enhanced nutrient intake, nutrient digestibility and lowered the secretion of TNF-α in nulliparous dairy goats.

Keywords: oil palm fronds, dairy goats, nutrient digestibility, ruminal fermentation, blood biochemistry, inflammatory response

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Introduction

Oil palm is the most important economic crop for several countries and the most thriving plant producing vegetable oil. The need for palm oil is projected to be tripled in 2050 and will surpass 250 million tonnes (Masani *et al*., 2018). In 2020, Thailand was among the third-largest palm oil producer in the world (Index Mundi, 2020). Approximately, 24-30% of oil palm fronds (OPF) were obtained from oil palm plantations during harvesting in the field (Dungani *et al*., 2018; Ghani *et al*., 2017). This means that OPF availability will rise continuously as planting rises throughout the year and maybe become an interesting sustainable feed alternative for animals (Buranakari *et al*., 2020; Dungani *et al*., 2018; Ebrahimi *et al*., 2015).

Zahari *et al*., (2003) reported that OPF consists of 4.7% crude protein (CP), 38.5% neutral detergent fiber (NDF), 55.6% acid detergent fiber (ADF) and 2.1% ether extract (EE) on a dry matter (DM) basis. Due to the high fiber and low metabolizable energy (5.65 MJ/kg), the inclusion of OPF has been limited to 30% and 50% in the dairy cow and beef cattle diets, respectively (Ghani *et al*., 2017; Hassim *et al*., 2010). The inclusion of 50% OPF in diet improved the DM and organic matter (OM) digestibility of cattle (Jarmuji *et al*., 2017). A study of meat goats showed that feeding OPF altered the pH and volatile fatty acid (VFA) of rumen fluid and increased *Entodiniomorphs* concentration in the rumen microbial population (Ebrahimi *et al*., 2015). This data indicates the possibility that OPF might improve the nutrient digestibility and fermentation pattern of animals.

Additionally, OPF is one of the sources of polyunsaturated fatty acids (PUFAs), especially linolenic acid (C18:3n-3; 46.6%) and linoleic acid (C18:2n-6; 4.39%) (Hassim *et al*., 2010). Linoleic acid and linolenic acid derive most of the mediators of lipids for inflammatory regulation (Wendell *et al*., 2014; Calder 2018; Hassim *et al*., 2010). Linolenic acid mitigates tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine, in humans and pigs (Bjorgvinsdottir *et al*., 2013; Li *et al*., 2014). The balance of n-6:n-3 fatty acid (FA) ratio also markedly affects animal performance and inflammatory responses through distinct mechanisms. Decreasing the n-6:n-3 FA ratio in the diet to 4:1 lowered the inflammatory response of cows in the lactation period with lipopolysaccharide (LPS) challenge (Greco *et al*., 2015). Dietary n-3 FA lowered serum triglycerides, thus, producing a significant decrease in total cholesterol in humans (Ander *et al*., 2003; Fernandez & West, 2005).

The information concerning dietary OPF on nutrient digestibility, fermentation pattern and blood biochemical levels in nulliparous dairy goats is limited. Furthermore, there has been no report regarding the effects of n-6:n-3 FA from OPF-based diet on the inflammatory response of dairy goats. Therefore, this paper will provide a detailed evaluation of the diverse inclusion levels of OPF in a total mixed ration (TMR) diet to enhance inflammatory response, blood biochemical levels, nutrient digestibility and fermentation pattern in nulliparous dairy goats.

Materials and Methods

Animals, Housing and Experimental Diets: This study was based on the Institutional Animal Care and Use Committee, Prince of Songkla University (U1-01633-2558), under the Ethical Principles and Guidelines for the Use of Animals, National Research Council of Thailand. Three nulliparous female dairy goats (Saanen x Thai native, 9-11 months old) were randomly set according to a 3x3-Latin-square design. The goats (33.88±1.78 kg, mean±s.d.) were placed in individual stainless steel pens in the goat section of the Animal Production Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University. The goats were treated with ivermectin injection (Ivomec) against intestinal parasites. Mineral blocks and water were available at all times. The goats were fed three different diets, consisting of 0% OPF (CON), 20% OPF (LOPF), and 40% OPF (HOPF). All treatment diets were prepared as TMR (concentrate: roughage, 60:40) and based on a DM basis. The OPF used in this experiment was a *Tenera* hybrid breed (*Dura* x *Pisifera*) - over 5 years after plantation.

Fresh OPF was daily harvested from the Faculty of Natural Resources, Prince of Songkla University, Hat Yai Campus, Thailand. Both OPF and rice straw were chopped into 0.5-2 cm lengths using a grass chopper machine. The chopped roughage was then mixed daily with grains following the composition of CON, LOPF and HOPF. The diets were provided *ad libitum.* The experiment was conducted for three periods with 19 days per period. Each period consisted of adaptation time (14 days) and total collection (5 days). The goats were weighed, before morning feeding, on the first and the last day of each total collection.

Data Collection and Laboratory Analysis Procedures

Feed and feces sampling procedures: Dry matter intake (DMI) was calculated by daily weighing offered and refused feed. Roughage samples were collected daily to analyze the DM content. Additional feed samples were taken for further FA analysis. Feed, residual feed and feces samples were individually collected, following the total collection method for the consecutive last 5 days of each period to examine nutrient digestibility (DM, CP, EE, NDF, ADF, and OM). After weighing the 24 h feces of each goat, 10% of the feces were dried in a forced-air oven with a temperature of 60^{o}C for 72 h. Feces samples were then pooled, ground (1 mm screen using HC-300Y2 Stainless Steel Electric High-speed Grain Grinder Mill, Huangcheng, China), and kept before chemical analysis.

DM, EE, CP and ash were determined from the feed, residual feed and feces samples using the standard methods of AOAC (1998). Further analysis of NDF and ADF used a method described by Van Soest *et al*.. (1991) with adapted methods for Fiber Analyzer. Petroleum ether in a Soxtec System based on AOAC (1998) was utilized to analyze EE in the samples. Final DM was used for expressing the results of chemical analysis. Gross energy (GE) of the treatment diets was measured using a calorimeter (LECO AC500).

Urine sampling procedures: At the same time as feces collection, a 5 L plastic bucket containing 50 ml of 10% sulfuric acid (H_2SO_4) was put beneath each cage. The daily output of urine was individually recorded. Approximately 120 ml of the total urine volume of each goat was collected daily. The urinary samples were then stored at -20°C and pooled to determine total nitrogen (N) and calculate N utilization.

Rumen fluid sampling procedures: Rumen fluid, approximately 100 ml, was collected through a stomach tube at 0 h before and 4 h after morning feeding on the last day of each experimental period. This tube was attached to both a vacuum pump (A-C Motor Pump G21DX, GE Motors & Industrial System, USA) and a strainer. Rumen pH was immediately measured using a pH meter (HANNA Instruments HI 98107 pHep pH Tester, Romania). The collected rumen fluid was then sieved by passing through four-layered cheesecloth. The obtained liquid fraction was acidified using 1M H_2SO_4 (ratio 9:1, w/w) and centrifuged for 15 minutes, at 9,000xg (Centrifuge Universal 320R, Hettich Zentrifugen, Germany). The supernatant was stored at -20°C until the analysis of ammonia nitrogen (NH_3-N) (Digestor Auto 2508 TecatorTM Line, FOSS, Sweden; KjeltecTM 8200 TecatorTM Line distillation unit, FOSS, China; EasyPlus Titrator Easy pH 30060041, Mettler Toledo Ltd., Thailand) and VFAs. Gas chromatography (GC) attached to Agilent CP7489 CP-Sil 88 capillary column (100 m x 250 µm and with a thickness of the film 0.2 µm) was used to determine VFAs. The temperature of the column was initially 80°C for 0 min. It was heated up to 220°C at 4°C/min, then was held for 5 mins. Afterward, the temperature of GC was escalated to 240°C at 4°C/min and was restrained for 10 mins. The temperature of the injector was at 250°C, and the detector temperature was at 270°C. The volume of injection was 1 µL. Pure methyl ester standards were applied for identifying each peak.

Blood sampling procedures and determination of inflammatory response and blood biochemical levels: On the first and the last day of each total collection (d 15 and d 19, before morning feeding), blood samples from the jugular venous (8 ml for each goat) were taken and stored in conical tubes. A 4 ml additional blood sample was collected into the tube with a clot activator for blood biochemical level analysis. Blood biochemical analysis consisted of total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides. It was measured by standard methods using Automatic Clinical Chemistry (A15 Analyser).

Blood samples used for inflammatory analysis were stored at room temperature for 2 h and were centrifuged to separate blood serum at 9,000x*g* at 4°C for 25 mins (Centrifuge Universal 320R, Hettich Zentrifugen, Germany). The obtained blood serum of each goat was moved into three Eppendorf tubes and they were stored at -20°C until the day of analysis. TNF-α, interleukin-1β (IL-1β), and interleukin-6 (IL-6) concentrations in the blood serum were identified using commercial ELISA kits from Wuhan Fine Biotech Co., Ltd., China (EG0037 Goat IL1B, EG0032 Goat TNFα, and EG0029 Goat IL-6) according to the following instructions from the manufacturer. Before addition of the sample, standard and control wells, the plates were washed in wash buffer. A 100 µL sample or standard was added to each well and then incubated at 37°C for 90 minutes. After incubation, plate contents were discarded and the plates were washed 2 times. Each well was augmented with a 100 µL biotin-labeled antibody working solution and incubated for 1 h at 37°C. The liquid in the plates was separated and washed 3 times. HRP-streptavidin conjugate (SABC) working solution (100 µL) was pipetted into each well and incubated for 30 mins at 37°C. The content of the plates was removed and washed 5 times. A 100 µL TMB substrate was added into the wells and incubated at 37°C for 30 minutes until the wells turned blue. Finally, stop-solution (50 µL) was pipetted, and the absorbance was promptly read using a 450 nm microplate reader (Thermo ScientificTM MultiskanTM GO Microplate Spectrophotometer, Thermo Fischer Scientific Oy, Finland). The results were interpreted with SkanIt Software 3.2. The standard curve was the respective concentration of the standard solution (X) and the relative 450 nm optical density (OD 450) of each standard solution (Y). The concentrations of TNF-α, IL-6, and IL-1β of the samples were interpolated from the obtained standard curve by the CurveExpert version 1.4 software program, following the manufacturer's instruction. All inflammatory response parameters were as a picogram per milliliter (pg/ml) of sample supernatant.

Statistical Analysis: All data of this research was statistically analyzed using analysis of variance (ANOVA) for 3x3 Latin Square using SPSS Statistics version 23. The parametric design described as $X_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \epsilon_{ijk}$, where X_{ijk} stands for the examination from animal j fed with diet i, in period k. In the model, μ is the overall mean, α_i is the effect of different inclusion levels of OPF (i = 0%, 20%, 40%), β_j is the animal effect (j = 1, 2, 3), γ_k is the effect of the period (k = 1, 2, 3), and ϵ_{ijk} is the result of residual. Significant (p<0.05) differences in treatment means were further analyzed for significance using Duncan's new multiple range test (Steel and Torrie, 1980).

Results

Chemical Compositions and Fatty Acids Profile of Experimental Diets: Table 1 and Table 2 present the chemical compositions and the FA profile of the experimental diets. The DM content of CON was the highest, followed by LOPF, then HOPF. All treatment diets had similar CP and GE contents. HOPF indicated the highest percentage of EE, while CON was the lowest. The content of NDF and ADF increased as the level of OPF inclusion in the diet was increased. LOPF and HOPF had higher concentrations of linolenic acid than the CON diet. Diet with OPF had a higher total n-3 family FA. All treatment diets had a similar PUFA: SFA ratio. As expected, the inclusion of OPF in the diet lowered the n-6:n-3 ratio. The n-6:n-3 ratios in CON, LOPF, and HOPF were 8:1, 5:1, and 4:1, respectively (Table 2).

Table 1 Chemical composition and fatty acids profile of experimental diets

Items	Treatment[1)]		
	CON	LOPF	HOPF
Ingredients (%)			
OPF	0.00	20.00	40.00
Rice straw	40.00	20.00	0.00
Soybean meal	25.15	23.01	21.66
Corn	28.86	30.99	32.34
Molasses	3.00	3.00	3.00
Premix[2)]	3.00	3.00	3.00
Chemical composition			
DM (%)	90.56	70.72	60.62
CP %(DM)	17.86	17.43	17.07
EE %(DM)	1.80	2.42	2.84
Ash %(DM)	10.44	8.65	7.57
NDF %(DM)	31.61	38.07	48.80
ADF %(DM)	19.31	20.26	22.04
Gross energy (Cal/kg DM)	3984.93	4021.32	4087.62

DM :dry matter; CP :crude protein; EE :ether extract; NDF :neutral digestible fiber; ADF :acid detergent fiber.

1) CON :60 %concentrate +40 %rice straw; LOPF :60 %concentrate +20 %rice straw +20 %oil palm fronds; HOPF :60 %concentrate + 40 %oil palm fronds.

2) 1% mineral-vitamin mix, 1% dicalcium phosphate, and 1% salt.

Table 2 Chemical composition and fatty acids profile of experimental diets

Items	Treatment[1)]		
	CON	LOPF	HOPF
Fatty acids profile %(of Total FA)[2)]			
C18:0	1.61	2.48	1.81
C18:1n-9*c*	8.21	10.22	9.82
C18:1n-9*t*	0.20	0.22	0.30
C18:2n-6*c*	16.67	20.78	16.59
C18:2n-6*t*	1.85	1.09	1.35
C18:3n-3	2.08	5.10	5.06
C18:3n-6	7.99	6.68	8.54
C20:0	0.41	0.46	0.22
C20:2	0.32	0.11	0.11
C20:3n-3	0.44	0.11	0.09
C20:4n-6	0.18	0.09	0.09
C22:0	0.33	0.36	0.23
C22:2	0.56	0.12	0.12
C24:0	0.73	0.47	0.44
C24:1	0.60	0.07	0.21
SFA	58.29	54.35	56.07
MUFA	10.55	11.08	11.52
PUFA	31.16	34.56	32.41
Total n-3[3)]	3.43	5.65	5.62
Total n-6[4)]	26.84	28.68	26.56
PUFA:SFA	0.54	0.64	0.58
n-6:n-3	8.67	5.12	4.78

1) CON :60 %concentrate +40 %rice straw; LOPF :60 %concentrate +20 %rice straw +20 %oil palm fronds; HOPF :60 %concentrate + 40 %oil palm fronds.

2) FA :fatty acids; SFA :sum of saturated fatty acids; MUFA :sum of monounsaturated fatty acids; PUFA :sum of polyunsaturated fatty acids.

3) Sum of n-6 fatty acids.

4) Sum of n-3 fatty acids.

Feed Intake and Nutrient Digestibility: Table 3 shows the DMI and nutrient intake of the dairy goats fed with various inclusion levels of OPF. The DMI and nutrient intake of the goats significantly increased when the level of OPF inclusion was increased (p<0.05). The intake of CP, EE, NDF, ADF and OM of the goats fed with HOPF was the highest. Table 4 presents an evaluation of OPF inclusion on the apparent digestibility of dairy goats. The digestibility of DM, OM and CP of LOPF and HOPF was not significantly different (p>0.05) but significantly higher than CON (p<0.05). Goats from the HOPF group performed a higher NDF digestibility than those from LOPF and CON groups. Nevertheless, the inclusion of OPF up to 40% in the TMR-based diet did not influence the digestibility of EE and ADF.

Rumen Fermentation and Volatile Fatty Acids Profiles: Table 5 shows the effects of dietary OPF on rumen fermentation (rumen pH and NH_3-N), VFA production and methane (CH_4) production of dairy goats at 0 h before and 4 h after feeding. The inclusion of different levels of OPF did not affect (p>0.05) rumen pH, NH_3-N, individual VFA proportions or CH_4 production. The ratios of acetic to propionic acid (C2:C3) and acetic plus butyric to propionic acid (C2,C4:C3) were not significantly different among all

treatment diets (p>0.05). Dietary OPF only altered the total production of VFA at 0 h before feeding (p<0.05).

Nitrogen balance: The effects of different dietary levels of OPF on N balance is shown in Table 6. The amount of N excreted through urine was not significantly different for all treatment groups (p>0.05). There was no significant difference (p>0.05) on feces N excretion and total N excretion of dairy goats fed with CON and LOPF. Nevertheless, those values were significantly lower compared to HOPF (p<0.05). Total N intake, N absorption and N retention significantly increased (p<0.05) as the level of OPF inclusion was increased. Goats fed with LOPF and HOPF statistically absorbed and retained similar percentages of N (p>0.05). A negative N retention occurred in the CON group.

Inflammatory response and blood biochemical level: Table 7 shows the effect of dietary OPF on inflammatory responses and blood biochemical levels of nulliparous dairy goats. The concentrations of IL-6 and IL-1β of goats fed with different levels of OPF were not significantly different (p>0.05). IL-6 and IL-1β ranged from 46.2 to 73.0 pg/ml and 53.5 to 65.4 pg/ml, respectively. This study indicates that there was no significant difference in the TNF-α level of the goats fed with CON and LOPF (p>0.05). However, those concentrations were significantly higher than the HOPF group (p<0.05). Goats from the HOPF group produced 22.25% and 19.37% lower TNF-α concentration than the CON and LOPF groups, respectively. The inclusion of OPF in a TMR-based diet did not alter the level of cholesterol, triglycerides, HDL-C, LDL-C and the ratio of LDL:HDL in the blood serum of dairy goats.

Table 3 Dry matter and nutrient intake of dairy goats fed with different levels of oil palm fronds

Item	Treatment[1)]			SEM	P-value
	CON	LOPF	HOPF		
DM Intake					
Total (g/d)	522.8^{c}	728.3^{b}	1173.1^{a}	30.7	0.003
%BW	1.59^{c}	2.14^{b}	3.40^{a}	0.07	0.006
g/kg $BW^{0.75}$	38.1^{c}	51.7^{b}	82.4^{a}	1.75	0.007
Nutrient intake (%BW/day)					
OMI	1.44^{c}	1.99^{b}	3.26^{a}	0.21	0.001
CPI	0.17^{c}	0.23^{b}	0.44^{a}	0.01	0.004
EEI	0.03^{c}	0.05^{b}	0.10^{a}	0.03	0.002
NDFI	0.52^{c}	0.68^{b}	1.72^{a}	0.03	0.001
ADFI	0.31^{c}	0.44^{b}	0.78^{a}	0.01	0.002

SEM: standard error of the means (n = 3); DMI: dry matter intake; BW: body weight; CPI: crude protein intake; EEI: ether extract intake; NDFI: neutral digestible fiber intake; ADFI: acid detergent fiber intake; OMI: organic matter intake.
[1)] CON: 60% concentrate + 40% rice straw; LOPF: 60% concentrate + 20% rice straw + 20% oil palm fronds; HOPF: 60% concentrate + 40% oil palm fronds.
[a,b,c] Means in the same row with different superscript are statistically different (p<0.05).

Table 4 Nutrient digestibility of dairy goats fed with different levels of oil palm fronds

Item	Treatment[1)]			SEM	P-value
	CON	LOPF	HOPF		
Digestibility (%)					
DM	49.2^{b}	72.6^{a}	72.2^{a}	3.14	0.023
OM	51.7^{b}	75.8^{a}	75.4^{a}	4.80	0.015
CP	37.2^{b}	60.6^{a}	62.4^{a}	2.56	0.008
EE	49.1	63.1	62.5	5.24	0.128
NDF	55.5^{b}	62.1^{b}	71.8^{a}	2.39	0.014
ADF	48.8	48.9	49.2	3.49	0.996

SEM: standard error of the means (n = 3); DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral digestible fiber; ADF: acid detergent fiber; OM: organic matter.
[1)] CON: 60% concentrate + 40% rice straw; LOPF: 60% concentrate + 20% rice straw + 20% oil palm fronds; HOPF: 60% concentrate + 40% oil palm fronds.
[a,b] Means in the same row with different superscript are statistically different (p<0.05).

Table 5 Rumen fermentation and volatile fatty acids profile at 0 h before feeding and 4 h after the feeding of dairy goats fed with different levels of oil palm fronds

Item	Treatment[1)]			SEM	P-value
	CON	LOPF	HOPF		
Rumen pH					
0 h	6.80	6.77	6.80	0.09	0.956
4 h	6.67	6.53	6.37	0.08	0.147
NH_3-N (mg/dL)					
0 h	26.9	25.9	24.1	3.96	0.857
4 h	25.7	21.6	24.9	3.45	0.697
Total VFA (mmol/L)					
0 h	22.5[b]	26.3[b]	44.4[a]	4.11	0.039
4 h	47.7	32.5	40.9	10.6	0.630
Individual VFA proportions %(total VFA)					
Acetic acid(C_2)					
0 h	43.0	51.0	58.2	5.40	0.252
4 h	64.8	55.5	57.7	7.50	0.684
Propionic acid (C_3)					
0 h	12.8	12.8	18.1	1.84	0.174
4 h	10.1	12.0	17.7	2.03	0.118
Butyric acid (C_4)					
0 h	11.9	10.9	8.86	2.27	0.657
4 h	10.1	11.1	10.4	2.73	0.965
C_2:C_3 ratio					
0 h	3.56	4.19	3.24	0.75	0.687
4 h	6.45	4.60	3.27	1.57	0.310
C_2,C_4:C_3 ratio					
0 h	4.52	4.88	3.74	0.83	0.571
4 h	7.41	5.56	3.95	1.53	0.244
Methane (CH_4(production)mol/100 mol)[2)]					
0 h	20.6	23.8	24.8	2.70	0.567
4 h	30.4	26.1	25.3	2.99	0.487

SEM :standard error of the means (n = 3); NH_3-N :ammonia nitrogen; VFA :volatile fatty acids.

[1)] CON :60 %concentrate +40 %rice straw; LOPF :60 %concentrate +20 %rice straw +20 %oil palm fronds; HOPF :60 %concentrate + 40 %oil palm fronds.

[2)] Methane (CH_4) = (0.40 x butyric acid) - (0.275 x propionic acid) + (0.45 x acetic acid) (Moss et al., 2000).

[a,b] Means in the same row with different superscript are statistically different (p<0.05).

Table 6 Nitrogen (N) balance of dairy goats fed with different levels of oil palm frond

Item	Treatment[1)]			SEM	P-value
	CON	LOPF	HOPF		
N balance (g/d)					
Total N intake	9.20[c]	12.4[b]	23.1[a]	0.47	0.017
N excretion (g/d)					
Feces N	4.66[b]	4.90[b]	8.41[a]	0.20	0.006
Urine N	6.48	5.72	7.67	0.70	0.156
Total N excretion	11.1[b]	10.6[b]	16.1[a]	0.72	0.028
N absorption	4.55[c]	7.48[b]	14.7[a]	0.55	0.003
N retention	-1.93[c]	1.77[b]	7.03[a]	1.20	0.001
N absorption (% of N intake)	37.2[b]	60.5[a]	62.4[a]	3.18	0.014
N retention (% of N intake)	-45.7[b]	12.5[a]	28.4[a]	11.5	0.025

SEM: standard error of the means (n = 3); N: nitrogen.

[1)] CON: 60% concentrate + 40% rice straw; LOPF: 60% concentrate + 20% rice straw + 20% oil palm fronds; HOPF: 60% concentrate + 40% oil palm fronds.

[a,b,c] Means in the same row with different superscript are statistically different (p<0.05).

Table 7 Inflammatory response and blood biochemical level detected in blood serum of dairy goats fed with different levels of oil palm fronds.

Item	Treatment[1)]			SEM	P-value
	CON	LOPF	HOPF		
Inflammatory response (pg/mL)					
IL-6	46.2	57.3	73.0	8.15	0.138
IL-1β	53.5	65.4	56.2	6.87	0.323
TNF-α	353.0[a]	340.4[a]	274.5[b]	17.6	0.007
Blood biochemical level (mg/dL)					
Cholesterol	76.8	79.7	85.3	7.34	0.712
Triglyceride	33.3	29.5	27.5	4.91	0.701
HDL-C	46.0	43.0	45.7	4.04	0.849
LDL-C	23.5	28.7	32.2	4.44	0.406
LDL:HDL ratio	0.51	0.69	0.73	0.11	0.373

SEM: standard error of the means (n = 3); IL-6: interleukin-6; IL-1β: interleukin-1β; TNF-α: tumor necrosis factor-α; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.
[1)] CON: 60% concentrate + 40% rice straw; LOsPF: 60% concentrate + 20% rice straw + 20% oil palm fronds; HOPF: 60% concentrate + 40% oil palm fronds.
[a,b] Means in the same row with different superscript are statistically different (p<0.05).

Discussion

Chemical Composition and Fatty Acid Profile of Experimental Diets: The nutrition of TMR feed was designed as iso-caloric and iso-nitrogenous, thus, it showed an unequal amount of corn and soybean meal in each treatment diet. HOPF (60.6%) became the feed with the lowest DM content, compared to LOPF (70.7%) and CON (90.6%) because the OPF used in this experiment was in fresh form and was chopped daily. HOPF was the most fibrous feed amongst all treatment diets This is reflected by the proportion of NDF and ADF. Based on Beauchemin (1996), the determination of NDF and ADF can be used to ensure adequate fiber in the diet. NDF content of LOPF and HOPF was similar to a study by Ebrahimi *et al*., (2015) who reported that diet with 25% and 50% OPF inclusion contained 39.7% and 43.8% NDF, respectively. However, those values were lower than NDF in the feed supplemented with fungal treated OPF which ranged from 50.4% to 56.7% (Hamchara *et al*., 2018). Crop species, maturity, environment, techniques of harvesting and storage, variety and soil fertility are the major factors affecting forage quality (Fulgueira *et al*., 2007).

The decreased n-6:n-3 ratio in the diets with higher OPF inclusion could be beneficial for inflammation biomarkers. Increasing n-6 FA intake might alter the expression of genes and synthesis of eicosanoids related to a pro-inflammatory state. Arachidonic acid (AA), a PUFA from the n-6 family, can generate eicosanoids to promote inflammatory processes. On the other hand, increasing n-3 FA intake might alleviate inflammation processes by producing specialized pro-resolving mediators (SPMs) (Calder, 2018). In this study, the n-6:n-3 ratio of HOPF corresponded to the suggestion of Greco *et al*., (2015) who reported that dietary n-6:n-3 to lactating Holstein cows with a ratio of roughly 4:1 yielded a greater DMI and a better inflammatory response.

Feed Intake and Nutrient Digestibility: The DMI of goats fed with CON, LOPF, and HOPF were 1.59%, 2.14%, and 3.40% of BW, respectively. It means that 40% OPF inclusion in a TMR-based diet could fulfill the DMI of goats in tropical regions (3.05-3.66% of BW) as suggested by Ashok and Wadhwani (1992). Increasing the inclusion level of OPF in the TMR diet of dairy goats significantly increased total DMI, thus elevated the intake of all nutrients. These results may have been due to the higher moisture content of the diets with OPF inclusion. Complete diets with an adequate amount of moisture content may dilute undesirable flavors and improve texture, thus increasing palatability and DMI (Lahr *et al*., 1983). OPF inclusion in a TMR-based diet also reduced dust production, compared to the CON diet, which can lower the risk of nose and eyes irritation of the animals, thereby increased feed intake. Zereu (2016) reported that one of the factors that can affect feed intake is the dusty effect of feed.

The inclusion of OPF up to 40% in the dairy goat diet improved fiber digestion and finally increased OM digestibility (Table 4), whereas, the lowest nutrient digestibility was indicated in the diet without OPF inclusion (CON). These findings suggested that an increase in nutrient intake, especially CP intake, of goats fed with OPF inclusion may provide a better environment for rumen bacterial activity. Previous studies have reported that dietary CP is important to promote nutrient digestibility and ruminal fermentation. An Increase in CP intake tends to improve apparent digestibility. Animals receiving a high CP level diet produce a significantly higher bacterial population and microbial protein synthesis in rumen fluid, thus resulting in an improved ruminal fermentation (Xia *et al*., 2018; Kang *et al*., 2015; Norrapoke *et al*., 2012; Suharti *et al*., 2011).

Fat digestion and absorption in farm animals are affected by the degree of saturation of fats, the length of FA chains, the structure of fats and the amount of fat supplementation. In digestive tracks, saturated fatty acids (SFAs) will be more difficult to digest and absorb than unsaturated fatty acids (UFAs) (Cetingul and Yardimci, 2008). All treatment diets in this study contained a similar amount of SFA, UFA and a ratio of PUFA:SFA. This led to a similar fat digestibility among the treatment diets.

Rumen Fermentation and Volatile Fatty Acid Profile: The values of rumen pH resulting from all experimental diets were within the optimum range (5.8-7) for the digestion of protein and the activity of

cellulolytic bacteria as suggested by de Veth and Kolver (2001). Increasing the inclusion of OPF and lowering the ratio of n-6:n-3 in the TMR diet did not influence the rumen pH of nulliparous dairy goats. A similar result was reported by Hamchara *et al*., (2018), who noted that dietary fungal-treated OPF did not affect the rumen pH of goats. Factors affecting rumen pH are salivary buffer secretion, the endogenous buffering capacity of feeds or digestion and volatile fatty acid (VFA) synthesis and absorption rates (Allen, 1997).

The NH_3-N levels of LOPF and HOPF groups in this study were in the normal range of the optimum ammonia level for growth and microbial activity (5-25 mg/dL), according to Preston and Leng (1987). On the other hand, the CON group at 0 h before feeding had ruminal NH_3-N concentration above the normal range. Ruminal NH_3-N concentration was lower if measured 4 h after feeding, compared to 0 h before feeding. Short synchrony between protein and fermentable energy results in a higher amount of NH_3-N before feeding (Beauchemin *et al*., 2000).

The inclusion of OPF in TMR-based diet and the n-6:n-3 ratio were not connected to the concentration of NH_3-N in rumen fluid. This was linear to the previous study conducted by Chanjula *et al*. (2018), Ebrahimi *et al*. (2017), Kim *et al*. (2007), and Toral *et al*., (2010) who reported that the n-6:n-3 ratio in diets did not affect NH_3-N in the rumen fluid. Atikah *et al*., (2018) mentioned that the presence of the longer chain UFA tended to increase rumen ammonia concentration. This was due to the reduction of the ruminal bacteria protein cycle. The treatment diets in this present study contained a similar amount of SFA and PUFA, thus the treatment diets could not alter the ruminal NH_3-N concentration of nulliparous dairy goats.

The total VFA levels of this study were similar to the study by Paengkoum *et al*., (2006) who reported that total VFA production of dairy goats fed with a steamed OPF based diet and supplemented with varying levels of urea ranged from 32.5 to 49.1 mM. The higher OM intake and better NDF digestibility of HOPF could be the possible reasons for the higher total VFA of HOPF at 0 h before feeding, although it had the highest NDF content. According to Nikkhah (2014), the content of NDF in diet performs a significant role in rumen fill. Higher NDF intake reduces the rate of rumen clearance. However, elevated rumen NDF digestibility and thus increased VFA production can weaken the suppressing effect of NDF.

OPF inclusion in a TMR-based diet did not affect individual VFAs, CH_4 production and the ratio of C2:C3 and C2,C4:C3 in the rumen. This finding is contradictory to Kulivand and Kafilzadeh (2015) who reported that higher NDF raises CH_4 production by changing the proportion of short-chain FA to acetate which yields more hydrogen for CH_4 production. Considering individual FAs used to predict CH_4 production in this study were not significantly different, therefore similar CH_4 productions among all treatment diets were observed.

Nitrogen Balance : As indicated in Table 6, the total N intake and N retention were higher in the HOPF group compared with the other groups due to the higher DMI of goats fed with HOPF. The positive N balance discovered in the LOPF and HOPF groups reflected that dietary OPF on TMR basis provided adequate N to nulliparous dairy goats. This could be the result of the increase in N intake of the goats and the lower n-6:n-3 ratio in the feed. Since the goats fed with OPF ingested a higher N, consequently, they retained more N than the CON group. Similarly, Hamchara *et al*., (2018) found that feeding goats with fungal-treated OPF in TMR could maintain a positive N balance. Moreover, Hăbeanu *et al*., (2019) stated that a n-3 FA enriched diet had a significant effect on nitrogen metabolism of barrows. Barrows fed with a lower n-6:n-3 ratio by the addition of extruded linseed and walnut meal resulted in a higher N retention and a higher efficiency of nitrogen utilization. In contrast, goats from the CON group had a lower DMI and N intake but excreted a higher amount of N than their N intake. Therefore, the CON diet could not help dairy goats balance N levels in their bodies and resulted in a negative N balance. Comparable to this study, Nurfeta *et al*., (2009) reported that feeding untreated straw to sheep generated a negative N balance because of the low intake of DM, thus N excretion was higher compared to N intake.

Inflammatory Response and Blood Biochemical Levels: TNF-α is one of the major inflammation mediators during the early stage of infection which is produced by macrophages, neutrophils and mammary gland epithelium. Ovines infected by either E. coli or its endotoxin express an increase in leucocyte and pro-inflammatory cytokines (PICs), especially IL-1β and TNF-α (Alnakip *et al*., 2014). Hence, reducing the concentration of TNF-α may be beneficial because it is a prominent inflammatory cytokine that has been involved in several systemic disorders (Popa *et al*., 2007). It also has been proven that inflammatory response severity has a positive correlation with the concentration of IL-6 in plasma (Trevisi *et al*., 2015).

The findings of this study show that lowering the n-6:n-3 ratio to approximately 4:1 by adding 40% OPF in a TMR-based diet was effective in decreasing TNF-α in nulliparous dairy goats. PUFAs from the n-3 family can weaken the pathway of nuclear factor kappa B (NF-κB), which induces the expression of TNF-α. Contradictorily, PUFAs from the n-6 family such as linoleic acid will be transformed into arachidonic acid, the precursor of the PICs (including TNF-α, IL-1 β, and IL-6) by the induction of 15-lipoxygenase (Xu, 2017; Lessard *et al*., 2004; Kang and Weylandt, 2008). There may also be competition between n-3 and n-6 PUFAs since n-3 PUFAs play a role as a competitor for the substrate in n-6 PUFAs metabolism (Scaioli *et al*., 2017). Similar to the present study, Li *et al*. (2014) also found that dietary n-3 PUFA-rich diet could decrease TNF-α concentration in the blood plasma of weaning piglets from d 0 to d 28. Lowering the ratio of n-6:n-3 from 6:1 to 4:1 can attenuate the acute phase response after inflammatory defiance in lactating Holstein cows (Greco *et al*., 2015).

The concentrations of IL-6 and IL-1β were not affected by OPF inclusion. A similar result was reported by Caroprese *et al*., (2009) that dietary fatty acids from fish oil and flaxseed resulted in non-

significant differences in the secretion of IL-1β and IL-6 in cows. Blood biochemical levels amongst all treatment diets were not significantly different. The diets used in this study contained a similar ratio of SFA:PUFA and slightly different concentrations of fat. These probably could not drastically alter the rumen biohydrogenation pattern, thus could not change the production of blood IL-6, IL-1β, and biochemical levels. Meng *et al*., (2018) also reported that OPF supplementation did not influence the levels of blood plasma total cholesterol and triglycerides of crossbred male sheep at different months of feeding.

In conclusion, the DMI and nutrient intake of nulliparous dairy goats were increased by the inclusion of OPF in TMR-based diets. Dietary OPF also significantly increased nitrogen retention. However, it did not alter blood IL-6, IL-1β, and biochemical levels. The inclusion of 40% OPF in TMR-based diet had the highest nutrient digestibility and the lowest TNF-α secretion. A feeding trial regarding the inclusion of OPF in the TMR diet on inflammation responses of pre-parturition and lactating dairy goats might be considered in a future study since they have a higher risk of inflammation.

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