

Original Research Paper

Supplementation of Prill Fat Derived from Palm Oil on Nutrient Digestibility and Dairy Cow Performance

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Abstract: Prill fat is a hydrogenated vegetable oil which contains more than 85% palmitic acid with a high melting point. Due to this reason, it does not melt at rumen temperature and bypass rumen degradation. This study was aimed to determine the optimum level of prill fat (86% palmitic acid) supplementation in dairy ration on fermentation characteristics, feed digestibility, milk production, milk components and milk fatty acid profiles. *In vitro* analysis used Randomized Block Design with 4 treatments and 4 replications and the *in vivo* analysis using T-Test. The *in vitro* result showed prill fat supplementation was significantly affected the total Volatile Fatty Acids (VFA) concentration ($p < 0.05$). The addition of prill fat significantly decreased dry matter and organic matter digestibility ($p < 0.01$), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) digestibility ($p < 0.05$), total protozoa biomass ($p < 0.01$) and population of cellulolytic bacteria ($p < 0.05$). Furthermore, supplementation of prill fat in the level of 2% had no effect on pH, ammonia (NH_3)N concentration and total rumen bacteria activity. The prill fat treatment at the level of 2% had no effect on dry matter intake, milk component and milk yield on *in vivo* analysis. In addition, milk yield, milk component and milk fatty acids were elevated after the prill fat treatment. This research concluded, the optimum level of prill fat addition in dairy ration was at the level of 2%. Supplementation of prill fat had no effect on rumen fermentation and fiber digestibility. Prill fat supplementation can be used to augment the milk yield, milk component, fatty acid composition in milk and had no effect on Atherogenicity Index (AI) and Hypocholesterolemic/Hypercholesterolemic (HH).

Keywords: Milk, Fatty Acid, Palmitic Acid, Rumen Fermentation

Introduction

Dairy cow production is likely to be adversely affected by feed nutrients and adequate nutrient requirements. The insufficiency of feed availability and quality for its production cause low productivity of dairy cows (Hasanah *et al.*, 2017). Dairy cows in Indonesia mostly fed with Napier grass and concentrate which produced by cooperative (Zahera and Permana, 2015). Napier grass is a productive forage (250 ton/ha/year) (Despal *et al.*, 2019) but it has a low quality (55% Total Digestible Nutrient (TDN), 8-12% Crude Protein (CP)) (Riestanti *et al.*, 2020) and is influenced by seasons (Sajimin and Purwantari, 2019). To fulfil the requirement of dairy cattle average production (13.5 L/head/day) that require 60.9% TDN and 12.20% CP (NRC, 2001), dairy farmers often added concentrate. Concentrate that used by a traditional dairy farmer contained <60% TDN. Lack of nutrient fulfilment has become bigger during the dry

season when forage quality rapidly decreased (Retnani *et al.*, 2014) as an increased of livestock requirement which driven by the higher maintenance requirement of dairy cattle (Sutarno and Setyawan, 2016). Supplementation with the other energy sources should be considered to fulfil the lack of energy in dairy ration.

Fat can be used as an alternative for low energy ration supplementation (Riestanti *et al.*, 2020). Fat supplementation in dairy ration can be used as a high energy density source for ruminants with a low heat increment (Santos *et al.*, 2017). Fat can be used as a post-rumen energy source which could overcome the low quality of forages and increase milk production (Naik, 2013). However, in fat supplementation, level and techniques should be considered to avoid rumen metabolic disturbance. The addition of fat in dairy rations often causes termination in the rumen fermentation due to the nature of fat that surrounds protozoa (Firkins *et al.*, 2007), so that protozoa immobilization will be agitated. The problems

occur mainly due to the high utilization level of fat in form of oil (more than 6-7% total fat in ration) (NRC, 2001).

The technique of fat supplementation was aimed at protecting fat (rumen bypass). Ca-soap, hydrogenated partially process, formaldehyde, are among the popular bypass fat protection technique. Currently, prill fat has become popular that used for temperate dairy cattle especially, during the summer season to increase energy ration and decrease heat increment.

Prill fat is a hydrogenated vegetable oil which contain more than 85% palmitic fatty acid with high melting point. Due to this reason, prill fat does not melt at rumen temperature and bypass rumen degradation so that these fatty acids are absorbed directly in the small intestine and digested in small intestine by lipase enzyme (Kundu *et al.*, 2014).

Prill fat supplementation was reported to increase energy consumption and known to have a significant effect on increasing milk production. It has been found that hydrogenated palm oil provides a better energy supply for lactating dairy cows than calcium soap of palm oil fatty acid (Karcagi *et al.*, 2010). Prill fat is more effective to work as a source of energy to maintain the energy balance in lactating ruminants during the summer conditions (Somagond *et al.*, 2020). Accordingly, many studies have been conducted with the aim of fat as an energy source using prill fat high in palmitic fatty acid was reported by Kundu *et al.* (2014), de Souza *et al.* (2017) and Mathews *et al.* (2016), which stated that there was an increase in milk production and milk fat in dairy cow, but had no effect on body weight gain and dry matter intake of dairy cows.

Utilizations of prill fat in Indonesia are still limited. Our previous research showed that utilization of 2% prill fat contained 95% palmitic acid improved the total VFA concentration without interfering the rumen fermentation and digestibility (Riestanti *et al.*, 2020). Study on utilization of 2% prill fat in dairy ration with different palmitic acid content showed that prill fat with 86% palmitic acid has improved milk production and quality. So far, there is no information on the optimum level of 86% palmitic acid prill fat supplementation in the dairy ration.

Therefore, this study was aimed to determine the optimum level of prill fat (86% palmitic acid) supplementation in dairy ration on fermentation characteristics, feed digestibility, milk production, milk components and milk fatty acid profiles.

Materials and Methods

Ration Preparation

The study was conducted from February 2020 to August 2020 at the Laboratory of Dairy Nutrition, IPB University and KUNAK, Cibungbulang, Dairy Farm. Dairy cows ration was constructed using elephant grass, concentrate and soybean curd with 58.28% : 33.62% : 8.10% ratio of DM. The ratio of feed was made based on the provision which made at KUNAK dairy farm, Cibungbulang, Bogor.

Table 1 showed the composition and nutrient content of feed ingredients for dairy cow.

The dietary nutrient requirement and the maintenance needs of the dairy cows were calculated in accordance with NRC (2001), where the needs of dairy cow with an average body weight of 417 kg and the 12 L of milk production need of 12.20% crude protein and 61.30% TDN. Based on the calculations, crude protein and TDN were not fulfilled if we provided with the local feed only. Supplementing with prill fat is expected to improve the energy. Prill fat is needed to fulfill the energy of the dairy cow which has a low energy in their ration. The ration composition and prill fat composition of palm oil by-product was presented in Table 2 and 3. respectively.

In Vitro Trial

In vitro analysis in this study was carried out following Tilley and Terry (1963) method. The rumen fluid was drawn from two fistulated dairy cows for *in vitro* analysis, following standard laboratory procedures. Sample of the ration (0.5 g) and McDougall solution (40 mL) for each treatment was poured into fermenter tubes. Then, the rumen fluid from different cows (10 mL) added to the tube followed by the addition of CO₂ (anaerobic condition). Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) measurements were conducted by incubating the fermenter tubes filled with the sample of ration at 39°C for 48 h in the shaker water bath. pH, ammonia (NH₃)N concentration and total VFA were carried out in 4 h after the incubation.

Research variables observed consisted of fermentation characteristics (pH, microbe's activity, ammonia (NH₃)N concentration and total VFA production), nutrient digestibility (dry matter and organic matter), NDF and ADF digestibility.

Collection and Measurement Samples of In Vitro Analysis

Characteristics of rumen fermentation observed in this study were the concentration of ammonia which analyzed through Conway micro diffusion method (Conway, 1962), pH (analyzed by pH meter) and steam distillation method used to analyze the total VFA concentration. Measurement of the total population of protozoa and bacterial was carried out using the Ogimoto and Imai (1981) method. Nutrient digestibility (dry matter digestibility and organic matter digestibility) were measured after 48 h of incubation. The 2 drops of HgCl₂ were added to the substrate after 48 h incubation period (to stop microbe's activity), then it was centrifuged at 3000 rpm in 15 min to separate the supernatant and solid component. Supernatants were eliminating and 50 mL of pepsin solution were added to the solid component in each tube. Tubes were incubated in the shaker water bath at 39°C for 48 h. Then, supernatants were eliminated and the solids were washed with hot water and filtered using vacuum pump and Whatman filter paper. Substrate placed in the porcelain cup and dried at the oven

(105°C in 24 h) for dry matter measurements. Then, substrates were incinerated in the furnace at 6 h (600°C) for the organic matter measurements.

Neutral Detergent Fiber Digestibility (NDFD) and Acid Detergent Fiber Digestibility (ADFD)

The residue from *in vitro* was analyzed according to the method described by Van Soest *et al.* (1991) using ANKOM 200 fiber analyzer. The *in vitro* residue was put into F57 fiber bag and then heated to a temperature of 100°C in neutral detergent solution for 75 min and acid detergent solution for 60 min to get the residual weight after the extraction process. NDF and ADF values are calculated from the residual weight after extraction divided by the initial sample weight.

In Vivo Trial

The study was conducted for 49 days, located in KUNAK, Cibungbulang dairy farm. A total of 20 multiparous Holstein cows in early and mid-lactation were managed under the intensive condition and adjusted to the breeder preference, researchers only added feed supplements in the morning. The feed consumption calculated every day. The forage and concentrate used are weighed using a hanging scale with a of 100 kg capacity. Milking is done 2 times a day, at 06.30-08.00 WIB in the morning and at 15.30-17.00 WIB in the afternoon. The milk production is measured using a 3000 mL measuring cup. Dairy cow milk production is collected in the morning and afternoon during milking. Analysis of the quality of milk is carried out using Lactoscan.

Analysis of the Fatty Acid Milk Profile

The milk fatty acid profile was analyzed on the last day of observation using Near-Infrared Spectroscopy (NIRS),

Animal Logistic Indonesia Netherland (ALIN) Laboratory. Milk samples were put into a petri dish, then placed on a device for further spectrum analysis using NIRS.

Research Design and Data Analysis

In Vitro Trial

The experimental diets of *in vitro* were arranged using Randomized Block Design with 4 treatments and 4 replications, as follows:

- P₁ = Control ration in the form of elephant grass 58.28+33.62% soybean curd +8.10% concentrate
- P₂ = P₁+2% palm oil prill fat
- P₃ = P₁+4% palm oil prill fat
- P₄ = P₁+6% palm oil prill fat

In Vivo Trial

The *in vivo* trials were designed by T-Test using the SASUniversity edition. This study was carried out with 2 treatments and 5 replications with each treatment consisting of 2 lactating dairy cows, as follows:

- T₀ = Control ration in the form of forage + concentrate + soybean curd
- T₁ = T₀ +2% palm oil prill fat (optimum results in *in vitro* tests)

The data were analyzed using ANOVA and the significant different among treatments were further tested by Duncan multiple range test using the SAS University edition. Differences among treatments were considered significant at *p*<0.01 and *p*<0.05.

Table 1: Composition and nutrient content of feed ingredients for Holstein cows

Feedstuffs	Nutrient content (%)						
	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	NFE ^b	TDN ^b
Elephant grass ^a	24.94	11.80	9.05	2.13	31.75	45.27	51.459
Concentrate ^a	93.62	7.46	15.60	3.60	8.41	64.93	65.212
Soybean curd ^a	13.60	7.36	18.67	1.15	14.10	58.72	64.901

^aResult of Dairy Laboratory, using NIRS

^bNitrogen free extract (NFE)

^bTotal digestible nutrient (TDN) = -14.8356+1.3310(%CP: Crude Protein) + 0.7923(%NFE: Nitrogen Free Extract) + 0.9787(%EE: Ether Extract) + 0.5133(%CF: Crude Fiber) (Wardeh, 1981)

Table 2: Composition and nutrient content of dairy ration for Holstein cows

Item	Percentage (% DM)
Composition	
Elephant grass	58.280
Soybean curd	33.620
Concentrate	8.100
Nutrient Content	
Total digestible nutrient	57.170
Crude protein	12.030
Ether extract	2.540
Crude fiber	22.470
Calcium	0.667
Phosphorus	0.481

Table 3: Prill fat composition of palm oil by-product

Analysis	Content
Iodine Value (g I ₂ /100g)	10.03
Free Fatty Acid (% as Palmitic)	32.89
Acid Value (mg KOH/g)	65.78
Slip Melting Point (°C)	59.40
Fatty Acid Composition (%)	
C16:0	86.24
C16:1	0.17
C18:0	3.49
C18:1 <i>cis</i>	7.52
C18:2 <i>cis</i>	1.59
C18:3 <i>cis</i>	0.03

Gas chromatography analysis

Results

Characteristics of Ruminal Fermentation and Ruminal Microbial Activity (In Vitro Trial)

Prill fat supplementation had no effect on pH, ammonia concentration, and total rumen bacteria. However, there was a significant effect of prill fat supplementation on total Volatile Fatty Acid (VFA) concentration ($p < 0.01$) along with the high level of prill fat addition in the ration. The addition of prill fat at different levels had a significant effect ($p < 0.05$) on the digestibility of Dry Matter (DM), organic matter (OM), Neutral Detergent Fiber Digestibility (NDFD), Acid Detergent Fiber Digestibility (ADFD), Hemicellulose Digestibility (HSD), and cellulolytic bacteria population. The fermentation characteristics of the ration supplemented with prill fat showed in Table 4 and the effects of prill fat supplementation on nutrient digestibility values showed in the Table 5.

Based on the results of the polynomial test, the optimum level of prill fat supplementation of palm oil by-product in dairy cattle rations was 2% with a linear equation. This value indicates that the optimum level of prill fat addition did not impair the ruminal fermentability. Regression equation were used to examine the effect of prill fat addition on the ruminal fermentability. The regression equation of prill fat addition on the fermentability characteristics were shown in Fig. 1 to 3 respectively. The resulting prediction were shown below.

The protozoa prediction equation:

$$Y = 6.5969 - 0.0337(x), \text{ (Y was the protozoa value and x was the prill fat level).}$$

The total bacteria prediction equation:

$$Y = 9.983 - 0.0422(x), \text{ (Y was the total bacteria value and x was the prill fat level)}$$

The cellulolytic bacteria prediction equation:

$$Y = 9.783 + 0.004(x), \text{ (Y was the cellulolytic bacteria value and x was the prill fat level)}$$

The amylolytic bacteria prediction equation:

$$Y = 9.7403 - 0.0936(x), \text{ (Y was the amylolytic bacteria value and x was the prill fat level)}$$

The proteolytic bacteria prediction equation:

$$Y = 9.0955 - 0.0604(x), \text{ (Y was the proteolytic bacteria value and x was the prill fat level)}$$

The Volatile Fatty Acid (VFA) prediction equation:

$$Y = 105.62 - 3.0246(x), \text{ (Y was the VFA value and x was the prill fat level)}$$

The NH₃(ammonia) prediction equation:

$$Y = 9.8211 - 0.2403(x) \text{ (Y is the NH}_3\text{-N value and x was the prill fat level)}$$

Similar with the rumen fermentability, the results of the polynomial test showed that the optimum level of prill fat supplementation of palm oil by-product in dairy cattle rations was 2% with a linear equation. Regression equation of prill fat addition on the Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) were shown in Fig. 4. The resulting prediction were shown below.

The Dry Matter Digestibility (DMD) prediction equation:

$$Y = 63.495 - 0.897(x), \text{ (Y was the DMD value and x was the prill fat level)}$$

The Organic Matter Digestibility (OMD) prediction equation:

$$Y = 63.746 - 0.916(x) \text{ (Y is the OMD value and x was the prill level fat)}$$

In Vivo Trial

Dry Matter Intake

Feed intake is a description of the amount of feed that can be consumed by cattle for maintenance and production (NRC, 2001). The results showed that dairy cows consumed 16 kg of DM head/day. There was no significant effect of prill fat addition in dairy ration. However, milk yield of dairy cows was affected by the addition of prill fat in the ration ($p < 0.05$). Table 6 showed the dry matter intake of dairy cows in KUNAK, Cibungbulang.

Dairy Cow's Milk Yield

The supplementation of 2% prill fat in the ration between delta (Δ) showed significant effect ($p < 0.05$) on the enhancement of milk production. This study showed that a decrease of milk yield in control (T0) was from 12.10 to 10.75 L during the trial period. However, there was an enhancement of milk yield in T2 treatment, from 8.90 to 10.45 L. The milk yield of dairy cows in KUNAK, Cibungbulang shown in Table 7.

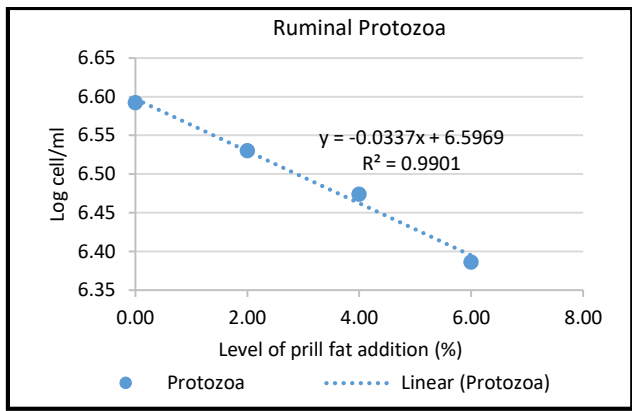


Fig. 1: The effect of prill fat addition on ruminal protozoa

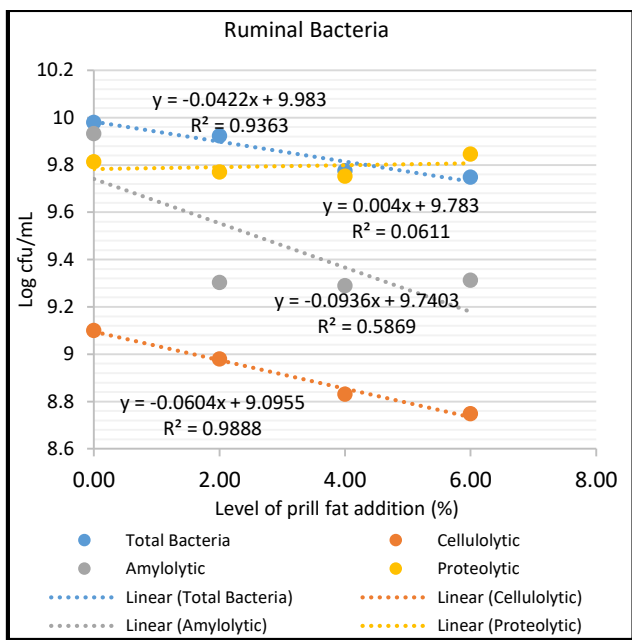


Fig. 2: Effects of prill fat addition on ruminal bacteria

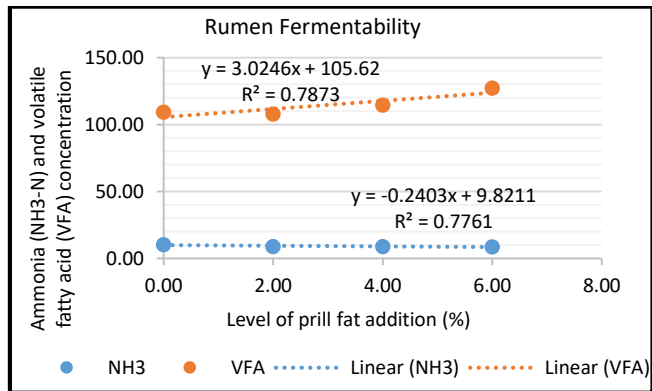


Fig. 3: Effects of prill fat addition on rumen fermentability

Dairy Cow's Milk Components

The addition of prill fat in the ration during maintenance between delta (Δ) did affect ($p < 0.05$) the milk fat component. Supplementation of 2% prill fat had no effect on the other milk component (solid non fat, lactose, protein and total solid). The milk components in KUNAK before prill fat supplementation did not fulfill the National Standards Organization (BSN, 2011), especially in the fat component and total solid. Table 8 showed the milk component of dairy cows in KUNAK, Cibungbulang during the supplementation.

Dairy Cow's Milk Fatty Acid Profile

The addition of prill fat in the rations affected ($p < 0.05$) three types of milk fatty acids from 19 fatty acids analyzed using NIRS. The result shows that caproic acid, palmitoleate and conjugated linoleic acid (CLA) are fatty acids that have significant results on the effect of prill fat addition in dairy cow rations ($p < 0.05$). Table 9. showed the result of the milk fatty acids profile supplemented with prill fat in dairy cow ration.

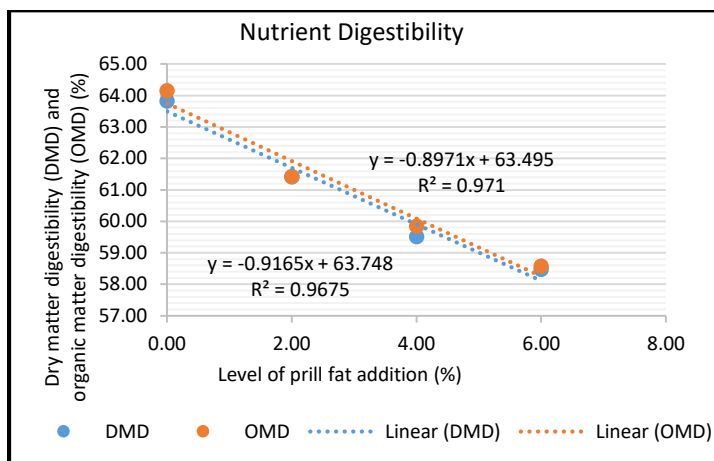


Fig. 4: Effects of prill fat addition on nutrient digestibility

Table 4: Effect of prill fat supplementation on *in vitro* fermentation products

Variables	Treatments				p
	P1	P2	P3	P4	
pH	6.86±0.11	6.88±0.10	6.88±0.17	6.93±0.16	0.312
NH ₃ -N (mM)	10.14±0.99	8.89±0.71	8.81±0.75	8.56±1.54	0.123
Total VFA (mM)	109.25±3.00 ^a	107.78±10.54 ^a	114.59±8.17 ^a	127.14±7.64 ^b	0.007
Total protozoa (log cell/mL)	6.59±0.12 ^a	6.53±0.12 ^{ab}	6.47±0.10 ^b	6.39±0.12 ^c	0.001
Total bacteria (log cfu/mL)	9.98±0.05	9.92±0.19	9.78±0.08	9.75±0.11	0.081
Cellulolytic bacteria (log cfu/mL)	9.10±0.97 ^a	8.98±1.10 ^{ab}	8.83±1.04 ^{bc}	8.75±1.07 ^c	0.014
Amylolytic bacteria (log cfu/mL)	9.93±0.19	9.30±0.82	9.29±0.81	9.31±0.77	0.151
Proteolytic bacteria (log cfu/mL)	9.81±0.12	9.77±0.07	9.75±0.09	9.85±0.11	0.516

NH₃ = ammonia; VFA = volatile fatty acid; P1 = control rations (58.28% : 8.10% : 33.62% DM, TDN 57.17%, CP 12.03%), P2 = P1+2% prill fat, P3 = P1+4% prill fat, P4 = P1+6% prill fat

^{a, b, c} Different superscripts in the same row with various letters show significant differences ($p < 0.01$)

Table 5: Effect of prill fat supplementation on nutrient digestibility values

Variables (%)	Treatments				p
	P1	P2	P3	P4	
DMD	63.82±2.10 ^a	61.41±1.90 ^b	59.51±1.66 ^{bc}	58.47±1.60 ^c	0.002
OMD	64.15±2.23 ^a	61.43±1.75 ^{ab}	59.84±1.78 ^b	58.57±2.17 ^b	0.008
NDFD	59.03±2.27 ^a	55.10±3.28 ^{ab}	53.55±3.90 ^b	51.00±2.75 ^b	0.010
ADFD	45.71±2.75 ^a	39.55±5.16 ^b	39.06±3.67 ^b	37.08±2.34 ^b	0.032
HSD	70.24±2.60 ^a	68.18±5.02 ^{ab}	65.75±5.72 ^{bc}	62.71±3.42 ^c	0.014

P1 = Control rations (58.28% : 8.10% : 33.62% DM, TDN 57.17%, CP 12.03%), P2 = P1 + 2% prill fat, P3 = P1+4% prill fat, P4 = P1 +6% prill fat; DMD = Dry Matter Digestibility; OMD = Organic Matter Digestibility; NDFD = Neutral Detergent Fiber Digestibility; ADFD = Acid Detergent Fiber Digestibility, HSD = Hemicellulose Digestibility

^{a, b, c} Different superscripts in the same row with various letters show significant differences ($p < 0.05$)

Table 6: Effect of prill fat supplementation on Dry Matter (DM) intake of dairy cows in KUNAK, Cibungbulang (n = 20)

Treatments	n	Dry matter intake (kg)			Total
		Forage	Concentrate	Soybean Curd	
T0	10	7.75±1.97	4.51±1.63	3.80±1.84	16.06
T1	10	7.70±1.98	4.51±1.64	3.80±1.85	16.02
T-Test		0.977	0.997	0.997	

T0 = control (without prill fat addition); T1 = addition of 2% prill fat in the ration

Table 7: Effect of prill fat supplementation on milk yield (L) from dairy cows in KUNAK, Cibungbulang (n = 20)

Treatments	n	Milk yield (L)			T-Test
		Before	After	Delta (Δ)	
T0	10	12.10±4.61	10.75±3.67	-1.35 ^a	0.025
T1	10	8.90±3.71	10.45±3.08	1.55 ^b	

T0 = control (without prill fat addition); T1 = addition of 2% prill fat

^{a, b} Different superscripts between delta at the same column show significant differences ($p < 0.05$)

Table 8: Milk component of dairy cows in KUNAK, Cibungbulang (n = 20)

Milk component (%)	T0			T1			T-Test
	Before	After	Delta (Δ)	Before	After	Delta (Δ)	
Fat	3.09±2.13	3.16±2.07	0.07 ^a	2.67±1.15	3.51±1.26	0.84 ^b	0.042
Solid Non Fat (SNF)	7.67±0.59	7.68±0.58	0.01	7.62±0.50	7.54±0.46	-0.08	0.497
Lactose	4.21±0.33	4.22±0.32	0.01	4.19±0.27	4.15±0.28	-0.04	0.074
Protein	2.81±0.22	2.82±0.18	0.01	2.79±0.18	2.77±0.18	-0.02	0.117
Total solid	10.75±1.77	10.84±1.89	0.09	10.45±1.36	11.02±1.02	0.57	0.194

T0 = control (without prill fat addition); T1 = addition of 2% prill fat

^{a, b} Different superscripts between delta on the same row show significant differences ($p < 0.05$)

Table 9: Milk fatty acid profile of dairy cows in KUNAK, Cibungbulang (n = 20)

Fatty acid profile (% milk fat)	Treatments		T-Test
	T0	T1	
Saturated Fatty Acid (SFA)			
Caproic acid (C6:0)	1.54±0.51 ^a	1.61±0.34 ^b	0.030
Caprylic acid (C8:0)	0.72±0.22	0.79±0.16	0.074
Capric acid (C10:0)	0.88±0.21	1.06±0.09	0.166
Lauric acid (C12:0)	1.95±0.45	2.01±0.61	0.324
Myristic acid (C14:0)	3.33±1.13	4.18±0.92	0.074
Pentadecanoic acid (C15:0)	0.42±0.09	0.46±0.11	0.264
Palmitic acid (C16:0)	17.35±4.66	18.38±3.86	0.109
Heptadecanoic acid (C17:0)	0.60 ±0.36	0.72±0.32	0.443
Octadecanoic acid (C:18)	19.30±0.15	19.90±0.31	0.337
Arachidic acid (C20:0)	0.21±0.11	0.21±0.07	0.595
<i>cis</i> -10 pentadecanoic acid (C15:0, <i>cis</i> -10)	1.16±0.59	0.91±0.47	0.337
Mono Unsaturated Fatty Acid (MUFA)			
Myristoleic acid (C14:1)	1.99±1.29 ^a	1.19±0.69 ^b	0.018
Palmitoleic acid (C16:1)	0.64±0.23	0.73±0.17	0.193
<i>cis</i> -10 heptadecanoic (C17:1, <i>cis</i> -10)	1.02±0.12	0.90±0.28	1.000
<i>cis</i> -9 oleic acid metil ester (C18:1, <i>cis</i> -9)	0.04±0.50	0.13±0.25	0.708
<i>trans</i> 9 elaidis acid metil ester (C18:1, <i>trans</i> 9)	36.49±0.21	33.35±0.18	0.984
Poly Unsaturated Fatty Acid (PUFA)			
Linolenic acid (C18:3)	3.40±0.29	3.23±0.86	0.595
Linoleic Acid (C18:2)	6.15±2.62	7.03±2.02	0.178
Conjugated Linoleic Acid (CLA)	1.32±0.42 ^a	1.55±0.58 ^b	0.043
Saturated fatty acid (SFA)	47.46±3.05	50.23±3.52	0.201
Mono unsaturated fatty acid (MUFA)	40.18±3.42	36.30±3.74	0.164
Poly unsaturated fatty acid (PUFA)	10.87±1.46	11.81±1.50	0.346
Short chain fatty acids (C4-C12)	5.09±0.63	5.46±0.53	0.351
Medium chain fatty acids (C14-C16:1)	26.51±2.49	27.48±2.41	0.490
Long chain fatty acids (C18-C20)	66.91±3.14	65.40±3.89	0.518
Hypocholesterolemic/ Hypercholesterolemic (HH)	0.46±0.03	0.46±0.08	0.996
Atherogenicity Index (AI)	2.41±0.52	2.82±0.42	0.217

T0 = control (without prill fat addition); T1 = addition of 2% prill fat

AI: $[(C12:0 + (4 \times C14:0) + C16:0) / \sum \text{UFA}]$; Unsaturated Fatty Acid (Ulbricht and Southgate, 1991); HH: $[(\text{cis}, C18:1 + \sum \text{PUFA}) / (C12:0 + C14:0 + C16:0)]$ (Santos-Silva *et al.*, 2002)

^{a, b} Different superscripts on the same row show significant differences ($p < 0.05$)

Discussion

Characteristics of Ruminal Fermentation and Ruminal Microbial Activity (In Vitro Trial)

The *in vitro* fermentation process can be optimal if the rumen fluid is in a pH condition that is suitable with the rumen microbial environment. The pH value in all treatments was in the range of 6.8 which indicates that this value was in the normal conditions to support fermentation activity in the rumen (McDonald *et al.*, 2011). The protozoa population from the study decreased along with the increase in the percentage of prill fat addition. This condition can be predicted that protozoa had no lipolytic activity as bacteria in entangled conditions with fat particles, so that the metabolic activity of protozoa tend to decrease when higher prill fat levels are added in dairy ration. The result was consistent with Behan *et al.* (2019) which reported that the decrease of total ruminal protozoa population in ruminant is caused by the addition of prill fat in the ration.

The rumen bacteria population decreased along the increasing level of prill fat added to the ration that is in the normal population range according to McDonald *et al.* (2011), was 9-10 log cfu/mL. These results were consistent with Pantoja *et al.* (1994) that fat in the rumen was associated with ration particles with a physical surface covering and inhibiting rumen microbial metabolism.

Similar with the total rumen bacteria count, the effect of prill fat addition in the dairy ration had a significant effect ($p < 0.05$) on the cellulolytic bacteria population. This can be caused by the surface covering of prill fat, so that cellulolytic bacteria unable to digest fiber when the ration has a high-fat content. Cellulolytic bacteria are bacteria that break down cellulose into glucose which is used for the synthesis of macromolecules and microbial cells (Samsu *et al.*, 2010). Cellulolytic bacteria had a slow growth than amylolytic bacteria (Samsu *et al.*, 2010) which caused a decrease in the ability of bacteria to maintain their biomass in digesting rations with high-fat content.

The use of prill fat in different levels did not significantly affect the amylolytic and proteolytic bacteria population. It may be due to the ration that had an equal protein content in each treatment which did not affect the biomass of proteolytic bacteria. Amylolytic bacteria had a faster growth phase to support rumen fermentation than cellulolytic bacteria which can be reflected in a balanced population after the addition level of prill fat.

Proteolytic bacteria are bacteria that break down proteins, amino acids and other peptides into ammonia (Orskov, 1982) and produce intermediate compounds and other end products that vary widely. According to Czerkawski (2013), the type of feed that consumed by livestock will affect the bacterial population and the proportion of each microbial species.

The average ammonia concentration of the rumen fluid with prill fat supplementation in this study ranged from 8.56-10.14 mM. McDonald *et al.* (1995) stated that ammonia (NH_3N) concentration in the rumen was around 5-17.65 mM. In this study, prill fat supplementation had no effect on ammonia (NH_3N) concentrations. This was due to the absence of protein content in prill fat. This study was in accordance with Riestanti *et al.*, (2020) that prill fat supplementation did not affect ammonia (NH_3N) concentrations. Study of Montgomery *et al.* (2008) reported that lipid supplementation did not affect N metabolism in the rumen. Furthermore, the activity of proteolytic bacteria is not affected by the addition of prill fat in term of the non-existence of protein content in prill fat.

VFA roles as an energy source for livestock and a carbon source for microbial protein synthesis (Rodríguez *et al.*, 2007). The average value of total VFA concentration with prill fat supplementation ranged from 107.78-127.14 mM. This value was still in the range of normal VFA concentrations that found in ruminants. According to McDonald *et al.* (1995), VFA concentrations that support microbial growth ranged from 80-160 mM. Addition of 6% prill fat (P4) treatment resulted in the highest level of total VFA concentration. This condition can be predicted that the addition of fat will increase the total VFA due to the enhancement of feed degradation processes by rumen microorganisms. Jenkins *et al.* (2008) stated that lipids which enter the rumen will undergo lipolysis which causes fat to be degraded into fatty acids and glycerol, then glycerol will be converted into VFA.

Recent study conducted by Riestanti *et al.*, (2020) reported that the concentration of total Volatile Fatty Acids (VFA) was influenced by the level of prill fat addition at different levels of palmitic acid. Prill fat that contained 96% Palmitic Acid (PA) produced the highest VFA value compared to the control and the other treatments (76 and 86% PA content), but the total VFA concentration of all treatments was still in the normal range of VFA concentrations in the rumen, so the prill fat addition at the level of 2% was not interfering the rumen fermentation activity.

Research by Naik *et al.* (2010) showed that the addition of bypass fat in the form of Calcium salts of Long Chain Fatty Acids (Ca-LCFA) 300 g/day with a ratio roughage: Concentrate 65:35 using *in vivo* did not affect the ammonia concentration in the rumen. Chalupa *et al.* (1986) also reported that the addition of 5-15% bypass fat had no effect on the rumen fermentation characteristics of dairy cows. Naik *et al.* (2009) reported that the addition of protected fat in the form of Ca-LCFA did not affect Dry Matter Digestibility (DMD), ammonia concentration and pH. The addition of protected fat in the ration showed an effect on the total VFA concentration due to an increase in energy by fat.

Nutrient Digestibility the Dairy Ration Supplemented of Prill Fat

The dairy ration digestibility produced in this study was in the normal digestibility according to Lestari and Abdullah (2015), it was above 60%, although there was a depression in the dry matter digestibility and organic matter digestibility along with the increasing level of prill fat addition. This condition caused by an interfere of palm oil prill fat with the microbial activity of the rumen due to the nature of the fat that can surrounded the feed particles. This result can be seen in the cellulolytic bacterial population (Table 4) which decreased significantly along with the enhancement of prill fat addition in the ration.

The highest NDF digestibility was in control treatment with 59.03% and the digestibility decreased along with the increase in the level of prill fat addition in the ration. This can be caused by a decrease of rumen bacteria activity, especially the cellulolytic bacterial population, which has decreased significantly to the addition of prill fat levels. It can also be predicted due to the physically closure of the feed particles by fat, so that the bacteria cannot maintain the ecosystem conditions in the rumen. Adesogan *et al.* (2019) stated that lignocellulose bonds also become a limitation in the use of feed ingredients in dairy rations because they could reduce the digestibility level in the nutritional value of the feed.

In addition, there were drawbacks of the *in vitro* method of Tilley and Terry (1963), which was unable to measure the post-rumen digestive system. Protected fat will pass through the rumen without changing the post-rumen digestive system and resulting the absorption of fatty acids in the jejunum area of the small intestine (Lock *et al.*, 2006). Measurement of post-rumen digestion of fat can be carried out using the *in vivo* method or the addition of lipase enzymes separately in the *in vitro* method. Therefore, for further research, the addition of lipase enzyme should be done to measure post-rumen fat digestion.

Dairy Cow Performance in KUNAK, Dairy Farm, Cibungbulang (In Vivo Trial)

Dry Matter Intake

Dry matter intake is the amount of feed that can be consumed by cattle for cattle production. Dry matter intake in this study was lower than the study by Despal *et al.* (2013) which was 23 kg DM. In this study, the addition of prill fat to the ration had no effect on the amount of Dry Matter (DM) intake. This can be predicted that prill fat is a hydrogenated fatty acid. Prill fat contains fewer double bonds (6%), so their addition did not affect feed consumption. Prill fat contains high levels of saturated fatty acids (palmitic) so that it does not affect gastrointestinal motility. The degree of saturation of a

fatty acid that reaches the small intestine in dairy cattle, will cause disturbances to gastrointestinal motility and will cause a decrease in dry matter intake (Drackley *et al.*, 1992). Relling and Reynolds (2007) reported that the decrease in dry matter consumption is caused by the fat supplemented into the ration, which will cause an association with an increase in the concentration of peptide-1 in plasma, i.e., glucagon (a peptide hormone in the small intestine with a hypophagic effect).

Dairy Cow's Milk Yield

The enhancement of milk production after prill fat supplementation can be caused by an increase in de novo lipogenesis and pre-formed uptake of fatty acids (Mathews *et al.*, 2016).

The increase in milk production can also be caused by the substitution of starch by protected fat so that there was a positive energy balance in dairy cattle, thereby changing the metabolism in the mammary glands (Wina and Susana., 2013). The energy that used by the dairy cow is obtained from their rations and the mobilization of fat in their bodies which can be maintained for production.

The depression of milk yield in Control (T0) was a normal condition for dairy cows, where the normal persistence in dairy cows is 89-92% which indicates that milk production has decreased by 8% every 30 days (Lowry, 1990). The persistence of milk production in the control treatment was 89.8%. This result showed that there was a decrease of 11% every 30 days. The enhancement of milk yield in Treatment (T1) with a persistence value of 110.6% after the peak was not normal, this can be caused by a nutrient improvements or recovery from disease.

Dairy Cow's Milk Components

Milk fat is one of the most important components in milk and the addition of prill fat in the ration can enhance the total of milk fat. This can be caused by the precursors for the synthesis of milk fat is fatty acids which derived from the rumen fermentation, mobilization of body fat and feed (Despal *et al.*, 2019). According to Maheswari (2004), milk fat content was influenced by feed because most of the milk components were synthesized in udders from simple substrates.

The enhance of the fatty acids availability in feed will increase the rate of milk fatty acid synthesis which can be done by provide the sufficient of energy. It can be reflected in the enhancement of milk fat levels in lactating dairy cows which are given the prill fat in the ration.

Bypass fat supplementation reported to increase energy consumption and known to have a significant effect on increasing milk production. Accordingly, many studies have been conducted with the aim of fat as an energy source using prill fat was reported by Kundu *et al.* (2014), Piantoni *et al.* (2015), Chamberlain and DePeters (2017), de Souza *et al.*

(2017) and Mathews *et al.* (2016), which stated that there was an increase in milk production and milk fat in dairy cow, but had no effect on body weight gain and dry matter intake of dairy cows.

Dairy Cow's Milk Fatty Acid Profile

The enhancement of fatty acid content in milk is influenced by the presence of sufficient energy supply for fatty acid synthesis in the udder glands, in addition to milk production. The addition of Saturated Fatty Acids (SFA) to the ration was reported to increase the circulation of Non-Esterified Fatty Acid (NEFA) on dairy cattle (Piantoni *et al.*, 2013). The ability of saturated fatty acids to increase NEFA synthesis is due to the high supplementation of palmitic acid (C:16) in the ration (Mathews *et al.*, 2016). Long chain fatty acids (C >12) are converted to CoA and the triacylglycerol (TAG) has re-esterification during the absorption process. TAG then broken down into chylomicrons with the support of lipoprotein lipase in the tissues which then contributes to the circulation of NEFA in ruminants and it depends on lipase activity in certain tissues (Bickerton *et al.*, 2007).

Atherogenicity Index (AI) was in the part of milk fatty acid profile which became an important value in functional food. Based on this study, the AI of milk for dairy cows in KUNAK Cibungbulang was in the low AI value according to Sharma *et al.* (2018) with AI values for normal dairy cows ranging from 1.6 to 3.79. AI in the control were 2.41 and 2.82 in the 2% prill fat treatment. Nantapo *et al.* (2014) examined the fatty acid composition during lactation at different periods and found that the lowest AI was resulted a value range from 4.08 to 5.13. The ratio of Hypocholesterolemic/Hypercholesterolemic (HH) is more accurate in reflecting the effect of fatty acid composition on Cardiovascular Disease (CVD) than the PUFA or SFA ratio. The value of HH ratio in dairy cow's milk according to Salles *et al.* (2019) ranging from 0.406 to 0.573. In this study, the value of HH ratio in dairy cow's milk in KUNAK Cibungbulang was 0.46 in control and treatment. These results indicate that 2% prill fat supplementation into the ration did not have a negative effect on the AI index and HH ratio of dairy cow.

Conclusion

Prill fat supplementation of 86% palmitic fatty acid at 2% level in dairy cow's ration did not interfere on rumen ferment ability and *in vitro* digestibility. Supplementation of 2% prill fat on *in vivo* enhanced the milk yield, milk persistence and improve milk fat profile as indicated by the alteration of PUFA and CLA.

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Author's Contributions

Lolita Udin Riestanti: Participated in all experiments, analyzed, interpreted the data and wrote the manuscript.

Despal: Designed, supervised the laboratory and in vivo work, interpreted the data and proofread the manuscript.

Yuli Retnani: Designed and supervised the experiment work and wrote the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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