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Micro-encapsulated Vegetable Oils Supplementation in Dairy Cattle Ration: In Vitro Fermentation and Digestibility Study

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ABSTRACT

Supplementing oils in dairy ration containing polyunsaturated fatty acid (PUFA) increased fatty acid profiles in ration and such an energy source for dairy cow. The high level of supplementing might increase the conjugated linoleic acid (CLA) contained in dairy milk but reduce rumen fermentation and digestion characteristics. This study aimed to identify the effect of supplementing vegetable oil micro-encapsulation on rumen fermentability and digestibility. The experiment used a 6x5 factorial randomized block design with three replications. Rumen liquor was obtained from fistulated dairy cow Frisien Holstein. The first factor was the vegetable-based oil (corn, palm, sunflower, soybean, sesame, and canola), and the second factor was the percentage level of micro-encapsulated vegetable oils supplementation (0, 4, 5, 6, and 7). The results showed that supplementing micro-encapsulation vegetable oil significantly decreased ($P<0.05$) the pH score, protozoal and bacteria population, NH_3 concentration, and ration digestibility. The variation of oil used significantly influenced pH score, total VFA concentration, and digestibility, except for OMD. Interaction effects between two factors were found in total protozoa population and NH_3 concentration. Micro-encapsulation vegetable oil should be considered with fatty acid content and availability of oil, but the supplement levels should be given under 4% to reach the optimal results and not interfere with rumen microbial.

Key words: Digestibility, Fermentability, Micro-encapsulation, Vegetable Oil

INTRODUCTION

Milk fatty acids profile has taken the increasing attention of consumers nowadays due to the previous adverse image of fat (Anzhany et al. 2022). Not all the fatty acids are harmful for human consumption. Many of fatty acids in milk have beneficial effects on human health. The short chain fatty acids such as butyric acid (C4:0) have been reported to affect cancer cell growth inhibition, while C6-C10 has been used to reduce body fat (González-Martín et al. 2020). The long chain fatty acids, especially polyunsaturated fatty acids (PUFA), also have been reported to have beneficial effects on human health (Chen and Liu 2020). Even trans fatty acids such as conjugated linoleic acids (CLA) in milk have been reported to have a good impact on human health (Despal et al. 2021). Milk rich in conjugated linoleic acid (CLA) and PUFA was used as a healthy milk indicator (Despal et al. 2021). CLA in milk has been reported to have properties as anti-diabetic (Martha et al. 2019), anti-hypertension (Koba and Yanagita 2014), anti-atherosclerosis, anti-carcinogenic, and anti-obesity (McGuire and McGuire 2000). Milk with healthy fatty acid profiles has been suggested to be valued as milk prices bonus system (Despal et al. 2021; Anzhany et al. 2022).

The PUFA and CLA in dairy milk appeared in many profiles that resulted from isomerization and biohydrogenation process in the rumen, and were assisted by rumen microbes (McCrorie et al. 2011). CLA is also formed by the desaturation process in the mammary gland assisted by Δ9 desaturase enzyme that changed C18:1 cis9 to C18:2 cis9 trans11 (Prandini et al. 2009). CLA levels in milk were around 1.3-1.5% of the total fatty acids. PUFA and CLA in milk are influenced by altitude and ration (Anzhany et al. 2022), forage quality (Despal et al. 2021), milking time (Oktavianti et al. 2022) and fat supplementation (Riestanti et al. 2021).

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Supplementation of vegetable oil groups have been reported to improve PUFA and CLA content in milk (Jokic et al. 2013) and increase energy in the dairy ration (Riestanti et al. 2021). However, the level of unprotected oil supplementation has been suggested not to exceed 1%. Unprotected oil supplementation was not only harmful to rumen microbes and reduced fiber digestibility but reduced milk fat content. Therefore, supplementation of oil in dairy ration should be in a protected form (Despal et al. 2022).

There were many methods to protect vegetable oil that had undergone biohydrogenation in the rumen. Ca-soap, Zn-soap, encapsulation, micro-encapsulation, and prilling (Faizah et al. 2019; Riestanti et al. 2021) were some examples. Micro-encapsulation of vegetable oils has the beneficial effect of avoiding rumen biohydrogenation and bypassing rumen fermentation, thus hindering metabolic disorders (Jenkins et al. 2008). Feeding micro-encapsulated vegetable oil enhanced the intake and energy ration without interfering rumen microbial population and activities (Riestanti et al. 2021). Supplementing micro-encapsulated vegetable oil also benefitted dairy cows experiencing heat stress, a typical case in the tropical environment. To our limited knowledge, the level of diversity of micro-encapsulated vegetable oil supplementation for dairy cattle in a tropical country that did not hinder rumen fermentation has not been studied intensively. Therefore, this study aimed to compare the effect of different types and levels of protected oil supplementation on fermentability and dairy ration digestibility using in vitro study.

MATERIALS AND METHODS

Treatment ration

Dairy ration composition contained a local concentrate and Napier grass (35th days of defoliation) with 59.73%:40.27% (w/w DM) ratio. The ration is based on the local provision given in South Bandung Cooperative (KPBS) by traditional dairy farmers that produced the best milk fatty acid profiles (Anzhany et al. 2022). The nutrient content of feed ingredients has been shown in Table 1.

In vitro procedure

Study of *in vitro* procedure in this study followed the Tilley and Terry (1963) method. The rumen fluid was drawn from one fistulated dairy cows for *in vitro* analysis. The animal used was housed and cared according to the Animal Ethics Committee, IPB University guidelines number 113/KEH/SKE/IX/2023. Samples of the ration were weighted for 0.5g, put in 100mL tube fermentor, and added with 40mL prewarmed McDougall buffer solution and 10mL rumen liquid. The tube fermentor was then aerated with CO_2 for 30s and closed with a ventilated rubber. The fermenters were put into a 39°C shaker water bath. The fermentation lasted for four h to measure the fermentability condition (pH, bacteria, and protozoa population) and product (NH_3 and VFA productions). The pH value was analyzed using a digital pH meter. Ammonia concentration (NH_3) was analyzed using Conway micro diffusion method and the total VFA concentration was analyzed using the steam distillation method. For digestibility measurement, the set of fermentor tubes lasted 48h before the fermentation was cancelled. After removing the supernatant from fermenter tube, 50mL of HCl-pepsin was added to imitate the enzymatic process of digestion. The fermentors were put back into the water bath and incubated for another 48h aerobically. The digestion residues were collected, dried, and weighed. The *in vitro* residue was analyzed according to Van Soest et al. (1991) method using ANKOM 200 fiber tech analyzer for measuring the NDF and ADF composition.

Research design and data analysis

The experiment diets of *in vitro* used analysis of factorial randomized block design in 6 x 5 with three replications. The first factor was the vegetable oil (corn, palm, sunflower, soybean, sesame, and canola). The second factor was the percentage supplementation level of micro-encapsulated vegetable oils (0, 4, 5, 6 and 7). Data were analyzed using ANOVA, and the significant differences among treatments were further tested by Tukey's test using SPSS version 25.

RESULTS

Effect of supplementation on dairy ration fermentability

The effect of micro-encapsulated vegetable oil addition on the fermentability of dairy ration is shown in Table 2. Types of oil and their level of supplementation influenced rumen pH independently without interaction effect. All rumen pH found in this study were within the normal range for the optimum fermentation activity process (6.5-6.8) (McDonald et al. 2010). The pH value in soybean oil supplemented ration was significantly higher than sunflower, palm oil, and corn oil but did not significantly different from sesame and canola oil. The effect of micro-encapsulated palm oil supplementation in this study has the highest VFA concentration which indicated that this supplement is considered to be a beneficial energy source.

Effect of supplementation on dairy ration digestibility

Based on the data obtained, the type of oil and supplementation level was shown in the DMD and ADFD variables without showing an interaction between the two factors. In contrast, supplementation level affected OMD variables, and the type of oil showed an effect on the NDFD value. Micro-encapsulated vegetable oil supplementation showed a pattern of decreasing the DMD variable compared to the control. This result is similar to the OMD variable, which also shows a decrease as the level of supplementation increases. The types of oil used, such as corn, soybean, sesame, and canola, showed the best results for the resulting DMD value.

DISCUSSION

Effect of supplementation on dairy ration fermentability

The treatment diets changed rumen pH. Therefore, the ruminal pH in this study was in the normal condition (6.00-7.00) to support microbial activity and growth (McDonald et al. 2010). Different proportions of fatty acid saturation in the oils produced different pH values (Harvatine and Allen 2006). Reducing rumen pH after supplementation of

Table 1: Composition and nutrient content of dairy feed ingredients

Feed Ingredients	Dry Matter	Ash	Crude Protein	Ether Extract	Crude Fiber	NFE	TDN
			---------- % ----------				
Napier grass[a]	24.94	17.78	13.05	1.70	36.36	31.11	47.510
Local concentrate[a]	90.16	12.25	9.28	3.43	17.85	57.19	55.347

TDN: total digestible nutrient; calculation results (Wardeh, 1981), NFE: Nitrogen Free Extract; [a] Near Infrared Reflectance Spectroscopy (NIRS) analysis

Table 2: Effect of vegetable oil micro-encapsulation supplementation on ruminal fermentability

Variables	Types of oil	Level (%)					Mean±SD
		0	4	5	6	7	
pH	Corn	6.73±0.02	6.46±0.13	6.51±0.14	6.48±0.20	6.59±0.15	6.55±0.11^{c}
	Sunflower	6.74±0.02	6.55±0.11	6.64±0.04	6.77±0.09	6.73±0.15	6.68±0.09^{ab}
	Soybean	6.75±0.03	6.82±0.12	6.71±0.13	6.77±0.12	6.82±0.20	6.77±0.04^{a}
	Palm	6.78±0.02	6.77±0.23	6.61±0.16	6.59±0.13	6.74±0.08	6.69±0.08^{ab}
	Sesame	6.84±0.02	6.50±0.02	6.60±0.15	6.67±0.18	6.57±0.29	6.63±0.12^{bc}
	Canola	6.87±0.03	6.70±0.08	6.63±0.21	6.67±0.20	6.51±0.10	6.67±0.12^{bc}
	Mean±SD	6.75±0.06^{a}	6.63±0.15^{b}	6.64±0.06^{ab}	6.66±0.11^{ab}	6.66±0.12^{ab}	
Protozoa (log cell ml$^{-1)}$	Corn	6.60±0.09^{bcd}	6.47±0.02^{efg}	6.51±0.07^{def}	6.38±0.06^{fghi}	6.26±0.04^{i}	6.43±0.13
	Sunflower	6.76±0.09^{a}	6.35±0.04^{efgh}	6.40±0.01^{hi}	6.48±0.10^{hi}	6.39±0.06^{fghi}	6.46±0.20
	Soybean	6.78±0.06^{a}	6.41±0.01^{efghi}	6.17±0.11^{fghi}	6.25±0.02^{fghi}	6.26±0.01^{fghi}	6.36±0.19
	Palm	6.52±0.07^{cde}	6.32±0.08^{efghi}	6.33±0.01^{ghi}	6.42±0.07^{ghi}	6.34±0.07^{hi}	6.38±0.09
	Sesame	6.70±0.04^{ab}	6.32±0.02^{efghi}	6.22±0.05^{efghi}	6.30±0.01^{efghi}	6.28±0.02^{ghi}	6.35±0.15
	Canola	6.63±0.13^{bc}	6.44±0.02^{efghi}	6.27±0.05^{fghi}	6.41±0.05^{fghi}	6.30±0.05^{ghi}	6.39±0.13
	Mean±SD	6.61±0.10	6.39±0.06	6.32±0.12	6.37±0.08	6.31±0.05	
Total Bacteria (log CFU ml$^{-1)}$	Corn	11.03±0.03	11.06±0.06	10.96±0.04	10.86±0.10	10.70±0.03	10.92±0.13
	Sunflower	10.95±0.04	10.89±0.02	10.68±0.15	10.79±0.03	10.65±0.08	10.79±0.27
	Soybean	11.06±0.04	11.03±0.05	10.99±0.06	10.47±0.66	10.67±0.01	10.84±0.22
	Palm	11.03±0.05	10.90±0.04	10.88±0.11	10.70±0.05	10.65±0.07	10.83±0.14
	Sesame	10.98±0.13	10.93±0.07	10.76±0.14	10.84±0.04	10.71±0.08	10.84±0.17
	Canola	11.05±0.03	10.99±0.11	10.57±0.47	10.72±0.09	10.67±0.05	10.80±0.17
	Mean±SD	11.02±0.05^{a}	10.97±0.07^{a}	10.81±0.18^{b}	10.73±0.14^{b}	10.67±0.04^{b}	
NH_3 (mM)	Corn	8.71±0.02^{ab}	7.02±0.30^{def}	7.84±0.21^{abcd}	7.07±0.33^{def}	7.41±0.55^{cde}	7.61±0.71
	Sunflower	8.74±0.08^{ab}	6.85±0.48^{def}	7.41±0.34^{cde}	7.20±0.53^{def}	7.82±0.61^{abcd}	7.60±0.74
	Soybean	8.41±0.24^{abc}	7.81±0.52^{abcd}	7.20±0.63^{def}	7.27±0.35^{def}	7.01±0.79^{def}	7.54±0.58
	Palm	8.40±0.0.24^{abc}	6.86±0.58^{def}	7.33±0.59^{de}	6.86±0.32^{def}	7.18±0.41^{def}	7.33±0.65
	Sesame	8.85±0.24^{a}	6.51±0.64^{ef}	6.46±0.56^{ef}	6.22±0.55^{ef}	7.71±0.55^{def}	7.15±1.09
	Canola	8.61±0.32^{ab}	7.74±0.13^{bcd}	7.91±0.75^{abcd}	7.28±0.21^{def}	7.10±0.14^{def}	7.71±0.57
	Mean±SD	8.61±0.19	7.13±0.52	7.36±0.52	6.98±0.41	7.28±0.29	
VFA (mM)	Corn	112.60±8.82	137.02±26.78	135.70±10.71	139.13±17.85	127.68±5.74	130.43±5.00^{a}
	Sunflower	115.52±6.12	116.52±15.18	141.82±21.75	131.90±24.95	113.22±26.68	123.80±13.40^{ab}
	Soybean	114.40±6.12	129.59±9.31	118.00±12.40	134.56±2.00	113.73±16.57	122.06±9.73^{ab}
	Palm	118.96±6.50	138.27±21.34	142.47±22.93	139.55±3.56	137.72±6.63	135.39±2.12^{a}
	Sesame	112.10±1.46	140.39±9.49	121.24±14.92	113.86±18.86	115.08±21.52	120.53±12.26^{ab}
	Canola	116.72±4.19	116.88±17.63	127.98±9.09	130.96±7.51	103.08±25.39	119.12±12.64^{b}
	Mean±SD	115.05±3.07	129.78±10.76	131.20±10.43	131.66±9.42	118.42±12.28	

VFA: volatile fatty acid; NH_3: ammonia; mM: millimolar. Different superscripts on the same row show significant differences (P<0.05).

protected oil high in PUFA has been reported by Riestanti et al. (2023). However, this finding differed from previous researchers who did not find any impact of oil rich in PUFA on rumen pH (Eburu and Anya 2020; Riestanti et al. 2020). Although microbes cannot absorb fatty acids in a protected form, such as encapsulated vegetable oil and their effect on microbial fermentation was low, however, as ruminal pH decreased, the complex dissociates and allowing microbial uptake and biohydrogenation (Harvatine and Allen 2006). In this study, the supplementation level significantly influenced rumen pH. Addition of encapsulated oil of 4% reduced pH from 6.75 to 6.63, but increasing the level of the oil did not significantly reduce the pH further.

There was an interaction effect of oil and their supplementation percentage level on the protozoa population. Protozoa population declined as the supplementation level increased. However, the value was still in the normal range for the rumen protozoa population (6 log cell ml^{-1}) (McDonald et al. 2010). The decline was due to the inability of protozoa to digest fat for its absent lipolytic activity. Protozoa have also been reported for their unlikely role in PUFA biohydrogenation (Francisco et al. 2019). The decline of protozoal number might be caused by the defaunation effect of oil supplementation that resulted in protozoa lysis (Ibrahim et al. 2021). Protozoa play a role in stabilizing rumen pH by engulfing starch granules and predating amylolytic bacteria to slow down starch fermentation. Oil supplementation rich in PUFA declined the protozoa population, which might reduce its capacity to stabilize rumen pH and result in quivering the ecosystem equilibria and microbial populations balance (Francisco et al. 2019).

The micro-encapsulated oil supplementation level significantly influenced the bacterial population, but types of oil had no significant effect. The addition of more than 4% of the oil reduced the bacteria population. Declining in the bacteria population was caused by the oil inhibition on the feed particle and prevented bacteria from contacting and digesting the feed (Pantoja et al. 1994). The association of oil to the feed particle surface inhibited the digestion and

Table 3: Effect of vegetable oil micro-encapsulation supplementation on nutrient digestibility

Variables (%)	Types of oil	Level (%)					Mean ± SD
		0	4	5	6	7	
DMD	Corn	55.70±0.31	51.69±1.00	46.40±6.38	49.10±2.07	47.33±2.76	50.03±3.74^{a}
	Sunflower	53.23±0.41	48.81±0.57	49.83±4.07	47.63±2.27	45.81±2.44	49.28±2.76^{ab}
	Soybean	55.32±0.89	51.51±3.09	48.49±4.36	49.11±0.21	47.09±5.35	50.30±3.16^{a}
	Palm	53.37±0.30	50.59±1.12	47.93±2.32	48.08±1.54	45.75±2.61	49.22±2.91^{ab}
	Sesame	53.09±0.11	49.21±3.99	50.59±3.23	50.08±1.96	47.73±2.05	50.11±1.97^{a}
	Canola	53.35±0.74	51.20±2.66	50.63±2.07	50.83±2.29	48.17±2.17	50.87±1.84^{a}
	Mean±SD	54.24±1.17^{a}	50.33±1.12^{b}	48.98±1.67^{bc}	49.14±1.19^{bc}	46.98±0.99^{c}	
OMD	Corn	55.98±0.82	50.81±1.67	42.78±6.77	47.22±2.96	44.67±2.95	48.27±5.25
	Sunflower	53.32±0.18	46.96±1.83	47.75±4.59	45.81±2.40	44.15±2.92	47.64±3.47
	Soybean	56.66±1.08	48.89±3.80	46.43±4.69	47.09±0.34	47.25±7.69	49.25±4.22
	Palm	54.13±0.02	46.84±1.87	46.25±3.00	45.75±2.29	47.03±4.52	48.02±3.46
	Sesame	53.98±0.20	46.88±5.81	50.83±3.82	47.73±2.03	46.00±3.06	49.07±3.28
	Canola	54.52±0.80	49.41±3.88	48.94±2.11	48.17±3.30	46.31±3.20	49.50±3.05
	Mean±SD	54.80±1.28^{a}	48.29±1.66^{b}	47.17±2.73^{bc}	46.96±0.99^{bc}	45.91±1.25^{c}	
NDFD	Corn	45.93±1.48	43.33±3.26	37.61±5.68	40.86±4.05	39.91±5.49	41.51±3.19^{b}
	Sunflower	43.36±1.29	42.28±4.29	44.17±7.45	43.45±4.60	41.10±5.93	42.88±1.19^{b}
	Soybean	43.31±1.85	40.16±0.65	41.76±14.92	38.99±3.32	40.46±9.90	40.95±1.65^{b}
	Palm	44.64±1.30	50.39±9.36	48.14±8.22	46.77±8.99	48.34±11.34	47.64±2.12^{a}
	Sesame	43.33±1.34	36.27±5.68	41.20±6.82	40.14±1.26	34.45±6.56	39.06±3.63^{b}
	Canola	44.62±1.28	42.14±2.78	43.55±2.71	42.46±6.60	38.07±2.85	42.14±2.48^{b}
	Mean±SD	44.15±1.06	42.43±4.63	42.73±3.50	42.11±2.78	40.39±4.57	
ADFD	Corn	33.78±0.58	32.24±3.07	24.70±6.89	27.23±8.66	28.05±7.47	29.20±3.73^{abc}
	Sunflower	34.75±1.32	32.18±5.94	35.01±8.47	34.78±5.58	31.71±7.03	39.69±5.64^{a}
	Soybean	31.98±1.27	26.97±0.42	27.38±2.33	25.62±7.39	26.50±12.11	27.69±6.43^{bc}
	Palm	34.20±1.39	34.97±2.64	30.25±2.88	28.82±0.48	30.29±7.78	31.71±9.04^{ab}
	Sesame	33.35±1.27	20.05±6.34	27.31±1.18	25.39±1.35	20.43±9.19	25.31±8.34^{c}
	Canola	32.98±1.21	30.33±6.02	31.25±3.75	31.52±9.26	25.47±4.56	30.31±7.88^{abc}
	Mean±SD	33.51±1.01^{a}	29.46±5.31^{ab}	29.32±3.64^{b}	28.89±3.67^{ab}	27.08±4.00^{b}	

DMD: dry matter digestibility; OMD: organic matter digestibility; NDFD: neutral detergent fiber digestibility; ADFD: acid detergent fiber digestibility. Different superscripts on the same row show significant differences ($P<0.05$).

metabolism process of the feed by rumen bacteria. The use of coating materials also affects the protective power of vegetable oils. According to Balasubramani et al. (2015), maltodextrin has low emulsifying ability so that the protective layer of the core material is not thick which causes the release of the core material may not be on the target and undergone biohydrogenation. Maltodextrin has a low viscosity at high solubility. The corn oil addition has the highest value in total bacterial population. This result was in line by Zain et al. (2008) stated that the use of corn oil as a defaunation agent increased the population of rumen bacteria from 8.80×10^{10} CFU mL to 11.40×10^{10} CFU mL.

Alteration of a bacterial community after PUFA supplementation was shown by Francisco et al. (2019). It was shown that both EPA and DHA altered rumen bacteria from cattle and sheep, which caused changes in ruminal bacteria (*Prevotella*, *Butyrivibrio*, *Ruminococcus*, or *Ruminobacter*). Riestanti et al. (2021) showed a significant difference in total bacteria, amylolytic, and proteolytic bacteria after supplementation of prill fat. However, the cellulolytic bacteria were significantly reduced with the prill fat supplementation. Reducing pH as an impact of protozoa defaunation inhibited cellulolytic bacterial activity. The growth of fibrolytic bacteria are severely inhibited when pH falls below 6.2 (Crawford et al. 1980). The changes in bacterial populations as an impact of PUFA supplementation may not be necessarily linked to the biohydrogenation process in the rumen but are more likely to the toxic effect of fatty acids (Carreño et al. 2019).

Ammonia (NH_3) concentration in rumen was influenced by the oil interaction and their supplementation level. In general, adding encapsulated oils to dairy ration reduced NH_3 concentration significantly. Adding encapsulated soybean oil up to 4% did not significantly lower NH_3 concentration. Ammonia is a vital substrate for microbial protein synthesis (Rosmalia et al. 2022). Ammonia results from the degradation of protein in rumen by rumen microbes (Sahroni et al. 2021). An insufficient amount of ammonia available in rumen, reduced microbial protein synthesis, consequently reducing the digestion process of nutrients in the rumen (Rosmalia et al. 2022). The decline in NH_3 concentration after supplementation of the encapsulated oil might be caused by the decline in the fermentation process. The inhibition of bacteria access to the feed reduced the possibility of the bacteria digesting the feed (Pantoja et al. 1994). The ammonia concentration in this study (6.98-8.61 mM) was within the normal range for normal microbial growth, 5-17.65 mM (McDonald et al. 2010). Reducing NH_3 concentration as an impact of encapsulated fat supplementation was also reported in a previous study conducted by Riestanti et al. (2021). However, Riestanti et al. (2020) did not find an effect in NH_3 due to the encapsulated fat supplementation. Moreover, the NH_3 concentration reported in the later study was higher (9.44-9.90 mM). Different oil used might cause different findings.

The addition of micro-encapsulated vegetable oils significantly produced different rumen VFA concentrations. Micro-encapsulated corn and palm oil produced higher VFA than the other encapsulated oil and significantly different from others. The degree of saturation and fatty acid length influence the different VFA results. Saturated and unsaturated fatty acids provide different

energy density for ruminants. According to Harvatine and Allen (2006), saturated fatty acid provided higher energy for dairy cattle than unsaturated fatty acid due to its higher energy efficiency of conversion. Palm oil which contained the most saturated fatty acids, produced the highest VFA and significantly higher than canola oil which contained the most unsaturated fatty acids. The higher VFA produced after supplementation of palm oil compared to canola was also reported by Despal et al. (2022). The higher VFA value after protected palm oil supplementation indicated an excellent indicator for higher energy availability for ruminants. The slightly higher VFA in the protected oil-supplemented ration indicated that not all the oils were protected. Some parts of the protected oil were undergone hydrolysis in the rumen to form glycerol and fatty acids. The glycerol was further fermented into VFA and used as an energy source by rumen microbes (Jenkins and Palmquist 1984).

Effect of supplementation on dairy ration digestibility

According to Lestari et al. (2015), the dry matter digestibility in this research is lower than usual. The normal condition of dry matter digestibility should above 60%. There was a depression in the ration digestibility, along with the increasing level of micro-encapsulated addition. Factors that affect the low digestibility value of the ration include the quality of the local concentrate used and the presence of particles of oil that are not perfectly protected so that the surface of the bacteria is covered with free oil, which interferes the activity of the bacteria in degrading feed. It can also be predicted due to the methodology used in this research. The oil protection method in the form of micro-encapsulation aims to provide a bypass effect on the rumen. It will be digested optimally after rumen digestion, but the *in vitro* methodology in this study has deficiencies in carrying out post-digestion of fat sources. It can be assumed that the micro-encapsulation of supplemented vegetable oils tends not to be well digestible.

The use of vegetable oil rich in PUFA composition can stimulate PUFA, which is formed in the feed ration, thereby affecting the content of the resulting product. However, the supplementation of micro-encapsulation oil still needs to be balanced with the quality of the ration used to obtain optimal results, and this result was linear with Li et al. (2009). A decrease in ration digestibility values can be avoided by paying attention to the oil supplementation limitation, even though the administration is carried out in a protected form.

The type of oil and the administration level of micro-encapsulated vegetable oil supplementation had a significant effect ($P<0.05$) on the OMD value and no interaction was observed between parameters. McDonald et al. (2010), stated that the digestibility value was influenced by the composition of feed ingredients and feed ratio, feed treatment, supplementation in feed and the feeding level.

Supplementation of protected vegetable oil at the 4% level significantly reduced the DMD value. A decrease in the DMD value also occurred in administrating the calcium soap-protected vegetable oil with a supplementation level of 4-7% (Riestanti et al. 2023). According to Wina and Susana (2013), the decrease in the DMD value in the administration of unprotected fat was not as well as protected fat. DMD value is influenced by the amount of fat addition, the type of feed, and the type of fat. Protected fats are generally not easily hydrolyzed by rumen bacteria and the decrease in DMD value is also affected by interaction between levels and oil types on ammonia in the above-mentioned. Changes and lower of the ammonia value in this study indicates low protein degradation in feed ingredients by rumen microbes, which impacts DMD values (Wijayanti et al. 2012).

Rumen bacteria participate directly in fiber degradation in the rumen (Chesson and Forsberg 1997). Rumen protozoa mechanism on fiber degradation are poorly characterized, but it is assumed that protozoa activities in cellulose digestion might affect the rumen fibrolytic bacteria (Ushida et al. 1991). Otherwise, the role of protozoa is not well-established and Firkins et al. (2020) found that some other rumen protozoa are beneficial in enhancing fibrolytic activity. The activity of rumen microbes also affect the NDF and ADF digestibility. It can also be predicted due to the physically intact by fat and the bacteria have no capability to maintain the rumen ecosystem. The NDF digestibility decreases along with the high level of microencapsulated addition. The palm oil-microencapsulated treatment increase NDF digestibility and it predicted to enhance ruminant's performance. The increased energy supply from fibrous feeds that humans do not consume is a second reason to increase fiber digestion. Consequently, it was a paramount effect to augment forage utility as a fiber digestion to enhance dairy cow productivity and farming environment.

Conclusion

Differences in micro-encapsulation vegetable oil cause an augmentation and depression in ruminal fermentability and digestibility. Micro-encapsulation vegetable oil should be considered with fatty acid content and availability of oil, but the supplement levels should be given under 4% to reach the optimal results and not interfere with rumen microbial.

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Author's Contribution

Despal designed the conceptual work, interpreted the data, and proofread the manuscript. Yuli Retnani and Nuri Andarwulan supervised the experimental work and interpreted the data. Lolita Udin Riestanti and Berliana Oktavianti conducted the laboratory work, interpreted data, proceed the data and wrote the manuscript.

Conflict of Interest

The authors have no conflict of interest to declare.

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