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EFFECT OF EMULSIFIED HIGH-PUFA OILS ENRICHED WITH NATURAL ANTIOXIDANTS ON IN VITRO GOAT RUMEN FERMENTATION PARAMETERS AND NUTRIENT DEGRADABILITY

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ABSTRACT: This study aims to evaluate the effect of emulsified high-PUFA oils enriched with natural antioxidants derived from jackfruit leaf extract on in vitro goat rumen fermentability and degradability. The study used a completely randomized design with two treatments and 5 replications. The treatments were supplementation with high-PUFA oils + natural antioxidants (P1, as the control), and supplementation with emulsified high-PUFA oils + natural antioxidants (P2). The parameters observed were rumen pH, total volatile fatty acid (VFA) concentration, NH_3 concentration, bacterial population, protozoa population, dry matter digestibility (DMD), and organic matter digestibility (OMD). The results showed that supplementing goat feed ration with emulsified high-PUFA oils enriched with natural antioxidants did not alter (P>0.05) rumen pH, total VFA, NH_3 concentration, bacterial and protozoal population, DMD, or OMD. Overall, emulsified high-PUFA oils with natural antioxidants did not alter the examined rumen fermentation parameters and degradability. Thus, emulsified high-PUFA oils with natural antioxidants can be potentially used as an effective feed supplement to support goats' performance.

Keywords: Emulsification, Feed supplement, Fermentation characteristics, Jackfruit leaves, Polyunsaturated fatty acid.

INTRODUCTION

Previous studies have shown that polyunsaturated fatty acids (PUFA), particularly n-3 and n-6, have a positive effect on the reproductive function, health, and performance of goats (Almeida et al., 2019; Geng et al., 2020). However, ruminants cannot synthesize n-3 and n-6 PUFAs de novo, so the fatty acids must be supplied through their diet (Roque-Jiménez et al., 2021). Flaxseed and sunflower oils are notable sources of essential n-3 and n-6 PUFAs. Flaxseed oil is rich in n-3 fatty acids, contains about 50% α-linoleic acid (ALA), which can be metabolized into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and has anti-inflammatory and antiatherosclerosis effects (Liao et al., 2021; Romanić et al., 2021). Sunflower seed oil is predominantly composed of n-6 PUFAs, particularly linoleic acid (LA), which reaches 60%-75% (Akkaya, 2018). However, high-PUFA oil supplementation in ruminant diets may negatively affect rumen fermentation by inhibiting microbial activity, particularly fiber-degrading bacteria, thereby reducing fermentation efficiency when supplied at inappropriate levels or in unprotected forms (Mirzaei-Alamouti et al., 2021). A previous study has also reported that dietary lipid supplementation exceeding 4-6% of dry matter may negatively affect rumen fermentation (Ribeiro et al., 2025).

In addition, PUFA metabolism may induce oxidative stress in livestock, leading to increased reactive oxygen species (ROS) and altering PUFAs' biological functions. Exogenous antioxidants can be used to prevent the side effects of PUFA metabolism (Fassah and Khotijah, 2016). A natural antioxidant derived from Jackfruit leaf extract, containing 0.34% antioxidant compounds, has been proposed as a strategy to mitigate oxidative stress (Pujiawati et al., 2023). Ojwang et al. (2017) reported that jackfruit leaves contain various bioactive compounds, including flavonoids (5.02-6.70 mg/g), tannins (1.97-2.56 mg/g), and phenolic compounds (30.92 - 37.39 mg/g). Nevertheless, directly mixing PUFA oils with jackfruit leaf extract may pose problems, as differences in polarity between hydrophilic antioxidants and lipophilic PUFA oils result in poor mixing efficiency, ineffective stabilization, and limited protective effects (Roschel et al., 2019).

Emulsification has emerged as a promising approach to improve the physical stability and dispersion of PUFA oils and antioxidants. Emulsification allows for a more homogeneous mixture of PUFA oils and antioxidants by adding surfactants that reduce interfacial tension between two liquid phases (Saxena and Mandal, 2022). Tween 80 is a non-ionic surfactant commonly used in emulsions that generally do not alter the pH since it does not dissociate into ions that could affect pH levels (López-Martínez and Rocha-Uribe, 2018; Pernin et al., 2019). Therefore, emulsifying high-PUFA oils with natural antioxidants may improve ruminal fermentation efficiency by stabilizing high-PUFA oils and natural

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antioxidants without impairing nutrient degradability. However, studies on the impact of emulsified high-PUFA oils-antioxidants combinations on rumen fermentation remain limited. Therefore, this study aims to evaluate the effect of emulsified high-PUFA oils enriched with natural antioxidants on in vitro rumen fermentation and degradability in goats.

MATERIALS AND METHODS

The research was conducted from October to December 2024 at the Meat and Draught Animal Nutrition Laboratory, IPB University, Indonesia. The in vitro analysis using goat rumen fluid was approved by the Animal Ethics Committee of the Directorate of Research and Innovation Licensing Governance and Scientific Authority (No. 123/KE.02/SK/06/2023).

Antioxidant extraction

The antioxidant compounds were extracted from jackfruit leaves using a maceration method in 70% ethanol (Najini and Wahdaningsih, 2024). The jackfruit leaves were briefly oven-dried at 60°C, then ground into a fine powder. A total of 1 kg of jackfruit leaves powder was macerated in 70% ethanol, then kept at room temperature for 24 hours. The mixture was then filtered using filter paper and concentrated using a rotary evaporator to form a viscous extract.

Feed supplement preparation

The feed supplement was prepared in oil-in-water (O/W) emulsion containing 15% high-PUFA oils (flaxseed oil and sunflower oil), 10% Tween 80, and 75% distilled water with 15 mg of antioxidant compounds derived from jackfruit leaf extracts. The oil phase consisted of 62% sunflower oil and 38% flaxseed oil (w/w), chosen to balance n-6/n-3 PUFA profile. Then, the mixture was homogenized using a sonicator (Lab Ultrasonic Homogenizer, Tefic Biotech Co.,China) at 60 °C, power 100W, and 30 minutes of sonication to ensure a uniform distribution of the oil phase in the water phase (oil-in-water) (Chen et al., 2023). For both treatments, the total amount of high-PUFA oils and antioxidant compounds was standardized to deliver 150 mg of total oil and 15 mg of antioxidant per 100 mL emulsion.

In vitro ruminal fermentation characteristics

A Completely Randomized Design with 2 treatments and 5 replications per treatment was applied in this study. The treatments consisted of adding high-PUFA oil and natural antioxidant compounds separately to rumen fluid (P1; the control), or adding an emulsified high-PUFA oil enriched with natural antioxidant compounds to rumen fluid (P2). The treatments were evaluated using the in vitro two-stage method described by Tilley and Terry (1963). The formula and nutrient composition of the basal diet, as well as the composition of the supplement, are provided in Table 1.

Table 1 - Formula and nutrient compositions of the treatment feed rations

Feedstuff \ Composition (%)	P1	P2
Pennisetum purpureum	30.00	30.00
Cassava waste	15.51	15.51
Copra meal	20.68	20.68
Soybean meal	10.34	10.34
Pollard	17.17	17.17
$CaCO_3$	0.72	0.72
Premix	0.72	0.72
Salt	0.72	0.72
Molasses	4.14	4.14
Total	100	100
Supplement		
High-PUFA oils and natural antioxidants	2.31	-
Emulsified high-PUFA oils and natural antioxidants	-	2.31
Nutrient composition (%DM)		
Dry matter (DM)[1]	89.22	89.22
Ash[1]	7.48	7.48
Crude protein (CP)[1]	15.02	15.02
Ether extract (EE)[1]	2.01	2.01
Crude fiber (CF)[1]	15.13	15.13
Nitrogen-free extract (NFE)[1]	60.38	60.38
Total digestible nutrient (TDN)[2]	69.14	69.14

P1: High-PUFA oils and natural antioxidants supplementation; P2: Emulsified High-PUFA oils and natural antioxidants supplementation; [1]Analyzed using Near infrared spectroscopy (NIRS); [2]TDN = 40.265 – 1.379 (CF) + 1.1903 (EE) + 1.363 (NFE) + 0.1969 (CP) (Wardeh, 1981)

Rumen fluid was collected from three healthy donor goats using a stomach tube method before morning feeding (Shen et al., 2012). The animals were fed a basal diet consisting of 70% concentrate and 30% elephant grass (Crude protein = 9.52%, TDN = 66.45%). Rumen fluid was filtered through six-layer gauze, pooled into a single homogenized sample to reduce individual variation, and placed in an insulated bottle at 39 °C. A total of 0.5 g of the sample was incubated under anaerobic conditions at 39 °C with 10 mL of rumen fluid and 40 mL of McDougall buffer solution. Both supplements were added at a concentration of 23 mg/g sample. After 48 hours of incubation, 2-3 drops of $HgCl_2$ were added, followed by centrifugation at 3000 rpm for 15 minutes. The samples were filtered and separated into residue and liquid samples. The residue sample was collected, dried at 105 °C for 24 hours, and combusted in a muffle furnace for 4 hours (AOAC, 2019) to determine dry matter (DMD) and organic matter degradability (OMD). Meanwhile, the liquid sample was collected to analyze rumen pH, total volatile fatty acid (VFA), ammonia (NH_3), and microbial population. Although the method primarily assessed nutrient degradability, the rumen fermentation parameters were also determined at the end of incubation as endpoint indicators of rumen fermentation conditions.

The liquid samples were tested for rumen pH using a pH meter. The concentration of ammonia (NH_3) was calculated using the method by Chaney and Marbach (1962) using UV-Vis spectrophotometry at 630 nm. The total VFA was measured using a steam distillation method (Despal et al., 2022). The total culturable bacterial population in the rumen was determined using the spread plate method with Brain Heart Infusion (BHI) medium, as described by Oliveira et al. (2021). The rumen protozoa population was analyzed following Ogimoto and Imai (1981). Briefly, 2 drops of the rumen fluid sample were mixed with a trypan blue formalin saline solution in a 1:1 ratio, and the mixture was observed under a microscope.

Data analysis

The data obtained were analyzed using an independent sample t-test in the statistical software SPSS 25.0. Data was considered significantly different between treatments at P<0.05.

RESULTS AND DISCUSSION

Ruminal fermentation characteristics

The results showed that adding emulsified high-PUFA oils enriched with natural antioxidants to the ration did not significantly affect (P>0.05) pH value, total VFA, ammonia concentration, and bacterial and protozoal population (Table 2), indicating the emulsion's lack of adverse effect on rumen fermentation. In this study, the emulsion containing high-PUFA oils and natural antioxidants was stabilized with Tween 80, which has been shown to remain stable during digestion (Lamothe et al., 2019). Therefore, Tween 80 may have contributed to maintaining stable rumen pH. The ruminal pH values in this study were within the normal range of 6-7 (McDonald et al., 2022), which supports the growth and activity of rumen microbes required for optimal feed degradation. Therefore, the emulsion of high-PUFA oils-enriched with antioxidants did not interfere with the rumen's buffering capacity.

Table 2 – Rumen fermentation characteristics due to Emulsified high-PUFA oil with natural antioxidant supplementation

Parameters \ Treatments	P1	P2	P-value
pH	7.10 ± 0.24	6.95 ± 0.30	0.523
Bacteria population (log cfu/mL)	6.25 ± 0.85	6.95 ± 0.20	0.446
Protozoa population (log cell/mL)	6.21 ± 0.12	6.19 ± 0.03	0.767
NH_3 (mM)	8.07 ± 1.91	8.12 ± 0.69	0.971
Total VFA (mM)	100.04 ± 4.23	111.76 ± 6.41	0.057

P1 = high-PUFA oils and natural antioxidants supplementation, P2 = emulsified high-PUFA oils and natural antioxidants supplementation. VFA = volatile fatty acid, NH_3 = ammonia

Furthermore, the emulsified high-PUFA oils with natural antioxidants had no significant effect (P>0.05) on both the total ruminal bacteria and protozoa population. The lack of significant changes in the rumen microbial population may be due to the protective role of emulsifiers, which prevent oils and antioxidants from directly interacting with bacteria and protozoa. Previous study has reported that nano-emulsified oils modulate the proportions of volatile fatty acids in ruminal cultures, but do not affect total bacterial counts (El-Sherbiny et al., 2016). In addition, caffeic acid, a phenolic antioxidant, did not significantly affect bacterial counts when administered in emulsified form (Sørensen et al., 2008). In this study, the bacterial population findings align with those of Suharti et al. (2019), who reported that the total ruminal bacterial population with the addition of canola and flaxseed oil calcium soap ranged from 6.00 to 6.68 log cfu/mL. Meanwhile, the protozoa population in this study aligns with Zahera et al. (2024), who reported that the proportion of unprotected to protected fat supplementation in dairy rations ranged from 5.96 to 6.27 log cells/mL.

Next, the emulsified high-PUFA oils enriched with natural antioxidants did not change (P>0.05) NH_3 concentration. This finding indicates that protein degradation and microbial nitrogen assimilation remained unaffected by the supplementation form. In this study, the overall rumen microbial population remained unchanged with the addition of emulsified high-PUFA oils enriched with natural antioxidants, which may have maintained the ammonia concentration. Rumen-ammonia-producing bacteria, such as *Clostridium aminophilum*, are primarily responsible for producing ammonia from protein degradation (Adeniyi et al., 2023; Chanu et al., 2024). Additionally, protozoa such as *Entodinium caudatum* engulf bacteria and release oligopeptides and amino acids, which are fermented to ammonia by amino acid-fermenting bacteria (Park and Yu, 2023). The NH_3 concentration in this study aligns with that reported by Riestanti et al. (2024), who found that unsaturated fat supplementation in the form of calcium soap and prill fat resulted in NH_3 concentrations ranging from 7.73 to 9.73 mM. Therefore, our research indicates that supplementing goat feed rations with emulsified high-PUFA oils enriched with natural antioxidants can maintain normal NH_3 concentrations, supporting a well-functioning rumen.

Moreover, the total VFA was not altered by the supplementation of high-PUFA oils enriched with natural antioxidants (Table 2). Emulsification reduces the tension between oil and water, allowing better dispersion of oil droplets (Losada - Barreiro et al., 2020). In addition, the antioxidants at the oil-water interface effectively enhance the oxidative stability of emulsions (Li et al., 2022). The absence of significant changes in total VFA concentration suggests that the emulsified high-PUFA oils enriched with natural antioxidants neither stimulated nor suppressed overall fermentation activity. This result also indicates that emulsification may have played a protective role, maintaining PUFA and antioxidant function without affecting microbial fermentation. The total VFA levels in this study were comparable with those reported by Muktiani et al. (2024), ranging from 87.52 to 164.38 mM. Thus, emulsified high-PUFA oils with natural antioxidants may enhance supplementation efficiency in goats. However, further in vivo studies are required to confirm the protective effect of emulsification.

Ruminal in vitro degradability

Adding emulsified high-PUFA oils enriched with natural antioxidants did not influence (P>0.05) DMD and OMD (Table 3). The lack of a significant difference in DMD and OMD levels with supplementation in the emulsified form may be caused by the content of active compounds within the emulsion that did not interfere with the rumen microbial population and rumen pH value. The microbial population and pH conditions in the rumen may facilitate the fermentation and feed degradation process (Mickdam et al., 2016). In our study, the DMD and OMD remain unchanged as the emulsified substrates may not be fully utilized by rumen microbes (Eftaxias et al., 2021). Additionally, the antioxidant components in the emulsion may have created a barrier that limits the nutrient degradability (Obando et al., 2015). The digestibility of DM and OM in this study aligns with those reported by Reproto (2025), which ranged from 42.01% to 76.90% and from 65.1% to 88.36%, respectively. Thus, adding emulsified high-PUFA oils enriched with natural antioxidants to the goat ration results in consistent DMD and OMD levels, reflecting its potency as a feed supplement without altering rumen fermentation or degradability.

Table 3 - Ruminal dry matter and organic matter degradability

Parameters \ Treatments	P1	P2	P-value
Dry matter degradability (DMD)	76.58 ± 4.73	74.97 ± 2.98	0.643
Organic matter degradability (OMD)	77.28 ± 6.26	74.80 ± 2.65	0.562

P1 = high-PUFA oils and natural antioxidants supplementation, P2 = emulsified high-PUFA oils and natural antioxidants supplementation

CONCLUSION

This study found that emulsified high-PUFA oils enriched with natural antioxidants does not alter rumen pH, microbial population, total VFA, ammonia concentration, and the degradability of dry matter and organic matter. Thus, emulsified high-PUFA oils enriched with natural antioxidants may be used as an efficient and safe feed supplement for goats. Nevertheless, further in vivo studies are strongly required to assess the long-term effects of supplementation on rumen fermentation and animal performance.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

Dilla Mareistia Fassah: Conceptualization, methodology, data curation, data analysis, writing – original draft; Manik Nurul Alfiyyah; Methodology, data curation, data analysis; Safiena Maura Azka: Methodology, data curation, data analysis; S P Damayanti: Methodology, data curation, data analysis; Y Pujiawati: Conceptualization, methodology, writing – review and editing; Lilis Khotijah: Conceptualization, methodology, validation, writing – review and editing; N A Sholeha: Conceptualization, methodology.

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Ethical approval

All the experimental procedures were conducted by the Animal Ethics Committee of the Directorate of Research and Innovation Licensing Governance and Scientific Authority (No 123/KE.02/SK/06/2023).

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Consent to publish

All authors agree to the publication of this manuscript.

Competing interests

The authors have not declared any competing interests.

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