Research Article

Influence of Wafer Complete Feed from Palm Kernel Replanting on *In vitro* Ruminal Fermentation Characteristics

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Abstract | Feed provision is essential in livestock farming, requiring high-quality feed that meets the animals' nutritional needs. Pith from oil palm replanting offers potential as a feed source, with the pith has variations in moisture content and density depending on the processing method. The addition of turmeric and ginger to ruminant feed from palm kernel replanting is expected to improve feed quality through optimizing rumen fermentation, increasing digestive efficiency, and has the potential to reduce methane production, thereby positively impacting the environment. The aim of this study was to explore the use of wafer feed made from silage of oil palm trunk pith (OPT), with the addition of turmeric, ginger, or their combination as additives, on *in vitro* ruminal fermentation products, ruminal microbiota, and feed degradability. The *in vitro* effects of different levels of complete wafer feed from oil palm replanting on rumen fermentation characteristics were assessed in A Completely Randomized Design Experiment with four treatments and four replicates. The treatments consisted of a basal ration, namely: P0 (Control); P1 (+5% Turmeric); P2 (+5% Ginger); P3 (+2.5% Turmeric + 2.5% Ginger). These treatments were subjected to *in vitro* incubation with buffered rumen fluid for 48 hours. Results showed the combination of turmeric and ginger maintained stable rumen pH and fermentation parameters, indicating no risk of acidosis. These additives may contribute to improved rumen fermentation efficiency. The addition of turmeric and ginger maintained rumen pH within a normal range, indicating stable rumen fermentation. The 5% ginger treatment increased total gas and methane production, while the combination of turmeric and ginger reduced ammonia concentration and improved nutrient degradability. Both ingredients reduced ammonia concentrations, particularly in combination, and improved the degradability of dry matter and organic matter. However, this addition tends to lower NDF and ADF degradation, which may help prevent rumen acidosis. This combination has the potential to enhance rumen fermentation efficiency and meet the nutritional needs of livestock. In conclusion, the use of wafer feed made from silage of oil palm trunk pith, with the addition of turmeric, ginger, or their combination as additives had different effects on ruminal fermentation characteristics. However, both have the potential to enhance rumen fermentation efficiency.

Keywords | Degradability, Fermentation, *In vitro*, Rumen, Palm kernel replanting, Wafer feed complete

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INTRODUCTION

Feed provision is a fundamental requirement in livestock farming. The feed must be of good quality and meet their nutritional needs (Nizzy and Ogwu, 2024). Efforts to develop suitable feed for livestock continue, one of which is utilizing the results of oil palm replanting, called pith (Febrina *et al*., 2024). Oil palm trunk pith (OPT), a by-product of oil palm replanting, contains high levels of lignocellulosic fiber and moisture, which limit its digestibility and nutrient availability in ruminants. Oil palm trunk pith (OPT) contains 42.4% dry matter and 1.48% crude protein, with high levels of neutral detergent fiber (50.71% DM) and acid detergent fiber (30.35% DM) (Chuchai *et al*., 2023). The lignin content of lignocellulosic materials such as OPT ranges from 17% to 20%, which substantially reduces digestibility. For optimal utilization in bovine feed, the lignin concentration should ideally not exceed 7% (Kusmiati *et al*., 2024). The administration of pith can be done in various ways, including fresh feeding, preserved feeding in the form of silage (Sadarman *et al*., 2024), and in the form of wafers. Wafer feed is a solid feed processed through heating and compaction (Somagond *et al*., 2024). This type of feed, made from a mixture of fiber and concentrate, is adjusted to meet the nutritional needs of livestock. There are three types of wafers: Wafer feed as an alternative to concentrate, energy and protein-rich supplement wafers, and complete feed wafers that provide a complete combination of energy, protein, fiber, and minerals to meet the daily needs of livestock. Overall, although various processing methods can improve the quality of palm trunk pith as feed, it has significant limitations, including: Palm trunk pith has low protein and energy content, so it needs to be mixed with additional nutritional sources, palm trunk pith is difficult to digest, which requires special, often costly, processing. This requires special processing, which often comes at a high cost. Some processing methods, such as ammonia or the use of enzymes, require close monitoring and can be expensive, which can reduce their competitiveness as an economical feed. Further research is still needed to find more effective, efficient and cheap processing methods to utilize pith trunk palm oil as a reliable feed source for ruminants. One of them is by adding turmeric and ginger. The addition of turmeric (turmeric) and ginger (ginger) in complete feed wafers resulting from palm kernel replanting has several important functions in the context of in vitro rumen fermentation in ruminants. Both have bioactive compounds that have the potential to improve rumen fermentation performance, animal health, and the nutritional quality of feed. Supplementing with turmeric raises the synthesis of volatile fatty acids (VFAs), which are essential for energy metabolism, by stabilizing rumen pH and improving fermentation patterns (Tian *et al*., 2023). Supplementing with ginger increases the digestibility of crude protein and dry matter, increasing the feed's total energy yield (Ali *et al*., 2024). This study aims to evaluate the in vitro effects of adding turmeric, ginger, and their combination to wafer feed made from oil palm trunk pith silage on ruminal fermentation characteristics, microbial activity, and feed degradability.

MATERIALS AND METHODS

Feed formulation, nutrient analysis and wafer processing

The four experimental diets were formulated to be comparable in dry matter and target nutrient composition while accommodating the inclusion of turmeric and/or ginger. To include 5% (w/w) of turmeric or ginger, selected basal ingredients (Oil palm trunk pith, Zanzibar Grass, Palm kernel meal, Coconut Meal, Fine rice bran, salt, molasses) were proportionally reduced so that the total ration weight remained constant across treatments. Thus, the treatments were not simple top-dressings of additives to P0, but were reformulated diets in which the additive replaced portions of other ingredients. Nutrient composition (DM, CP, NDF, ADF, EE, and energy) of each final formulation was analysed (or calculated from ingredient composition) and is presented in Table 1. The chemical composition of the experimental wafer feed (Table 1) was determined through the calculation results. The probiotic starter (FM) used for silage preparation was a mixed microbial inoculant (*Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus plantarum* and *Pediococcus pentasaceus*), with a guaranteed minimum total viable count of ($1.3 x 10^{11}$ cfu/ml).

The wafer-making process began with the preparation of oil palm trunk pith (OPT), which was chopped and ensiled with 2% dry matter of probiotic starter FM for 21 days at room temperature. After the ensiling process was completed, the oil palm trunk pith silage was harvested, dried, and ground into a powder. The silage powder (15%) was then mixed with concentrate ingredients, including zanzibar grass (45%), palm kernel meal (15%), coconut meal (7%), molasses (2%), and salt (1%), the composition and nutrient content can be seen in Table 1. Turmeric powder and ginger powder were added to the wafer mixture according to the specified treatments. The feed ingredients were carefully mixed and weighed in accordance with the specification. To guarantee uniform dispersion, molasses was added to the mixture after being dissolved in a 1:1 water solution. After ten minutes of steaming, the uniform mixture was formed into flat, round wafers that weighed 150 grams each unit and measured 3.5 cm in height by 10 cm in diameter. In accordance with Nasution *et al*. (2021), the mixture was compacted under a pressure of 200 kg/cm^2 (approximately 19.6 MPa) for 20 minutes without the use of heat. To get a moisture level of 14–15%, the wafers were oven-dried at 55 °C.

Table 1: Composition and nutrient content of experimental wafer feeds based on oil palm trunk pith silage.

Component (% of DM)	P0 (Control)	P1 (+5% Turmeric)	P2 (+5% Ginger)	P3 (+2.5%T + 2.5% G)
Oil palm trunk pith	15	15	15	15
Zanzibar Grass	45	45	45	45
Palm kernel meal	15	10	10	10
Coconut Meal	7	10	10	10
Fine rice bran	15	12	12	12
Salt	1	1	1	1
Molasses	2	2	2	2
Ginger	0	0	5	2.50
Turmeric	0	5	0	2.50
Total	100	100	100	100
Nutrient composition (%DM)				
Dry matter	84.5	84.5	84.2	84.4
Ash	5.52	5.42	5.66	5.52
Crude protein	15.0	14.9	14.9	14.9
Ether extract	3.80	4.01	3.55	3.78
Crude fiber	25.5	24.8	25.2	20.1
Nitrogen-free extract	50.18	50.87	50.69	55.7

The inclusion of turmeric and/or ginger (5% total) was achieved by substituting portions of the basal ingredients.

Feed formulation and nutrient analysis

Rumen fluid collection

The rumen fluid used in this study was collected immediately after slaughter from healthy cattle at a local abattoir. The animals had been fed a mixed forage-concentrate diet similar to that of typical ruminant production systems in the region. To preserve microbial viability, rumen contents were collected within 10–15 minutes post-slaughter into pre-warmed (39°C) insulated containers flushed with CO_2 to maintain anaerobic conditions. The fluid was then filtered through four layers of cheesecloth and transported to the laboratory within 30 minutes for use in the in vitro fermentation assays.

In vitro incubation procedure

The sample underwent evaluation for in vitro ruminal fermentation and degradability following the procedure outlined by Theodorou et al. (1994). Incubations were carried out in four replicates over different weeks. Approximately 1000 mg of sample was placed in 100 mL serum bottles, combined with 25 mL of ruminal fluid and 50 mL of bicarbonate buffer. Each bottle was flushed with CO_2 for 30 seconds before being sealed with butyl rubber stoppers and crimped with aluminum caps, then incubated in a water bath at 39°C. After 36 hours, four tubes were removed, while the rest continued incubation for 48 hours. Gas measurements were taken at intervals of 0, 3, 6, 12, 18, 24, and 36 hours using a 100 mL syringe. Manual shaking was performed at each sampling point. The gas production values were standardized to 200 mg of dry matter (DM) to allow comparison among treatments. After 36 hours, the tubes were centrifuged at 3500 rpm for 15 minutes, and the fermentation fluid was collected for pH and ammonia analysis. Ammonia levels were determined using the Conway microdiffusion technique (General Laboratory Procedure, 1966). Gas production kinetics were estimated using the exponential equation (Orskov and McDonald, 1979). The residue was then analyzed for dry matter (DM) and organic matter (OM) to determine *in vitro* ruminal dry matter degradability (IVRDMD) and organic matter degradability (IVROMD) values (Tilley and Terry, 1963), as well as neutral detergent fiber (IVRNDFD) and acid detergent fiber degradability (IVRADFD) using the Van Soest method.

Experimental design, variables measurements and data analysis

Design A completely randomized design with four treatments and four replications was used in this study. The treatments involved the addition of turmeric and ginger powder to the complete feed-based wafer. The treatments were: P0 (Control); P1 (+5% Turmeric); P2 (+5% Ginger); P3 (+2.5% Turmeric + 2.5% Ginger). The following variables were measured: pH, CH_4, $N\text{-}NH_3$, *in vitro* ruminal dry matter degradability (IVRDMD), *in vitro* ruminal organic matter degradability (IVROMD), *in vitro* ruminal crude protein degradability (IVRCPD), ammonia ($N\text{-}NH_3$), in vitro ruminal neutral detergent fibre degradability (IVRNDFD), and in vitro ruminal acid detergent fibre degradability (IVRADFD).

Statistical analysis

Statistical analysis of the data was conducted using the one-way ANOVA procedure in SPSS v27.0. For the analysis, experimental animals were included as a random effect, and treatment was included as a fixed effect. Duncan's test was used to determine differences between means. Least square means were expressed in tables, and significance was declared for P-values < 0.05 and trends at $0.05 < P < 0.10$ (Adams and McGuire, 2018).

RESULTS AND DISCUSSION

Rumen fermentation pH

The diets for P1 and P2 were reformulated such that 5% turmeric or 5% ginger replaced portions of the basal ingredients (see Table 1). Because the final formulations differed slightly in ingredient proportions (despite our efforts to maintain similar nutrient content), treatment effects may reflect both additive-specific effects and effects arising from the altered basal diet composition. We therefore report the observed differences while interpreting causality with caution. The analysis of variance showed that the addition of turmeric and ginger in oil palm pith wafers had a highly significant effect ($P < 0.01$) on post-incubation rumen fluid pH. Treatment P0 (control) showed an average post-incubation rumen fluid pH of 6.40, close to the normal range of 6.70-7.00 (McDonald *et al*., 2022). The pH levels ranged from 6.10 to 6.27, which is higher than the minimum pH of 5.60 required for the growth and proper function of fibrolytic rumen bacteria (Ørskov and Ryle, 1990). Treatment P1, with the addition of 5% turmeric, showed a decrease in pH to 6.03, significantly lower than P0. This decrease in pH was due to the chemical properties contained in turmeric (Tunkala *et al*., 2022). Turmeric, which contains curcumin, can have antimicrobial and antioxidant effects; the interaction of these two effects can affect rumen microorganisms and the overall fermentation process (Raheem *et al*., 2021).

A limitation of the present feeding trial is that the additive inclusion was implemented via substitution of basal ingredients to maintain constant total ration mass. Therefore, the treatments represent reformulated diets rather than strict "additive-on-top" comparisons. Although nutrient composition was measured/estimated and diets were formulated to be as comparable as possible, changes in the proportions of palm kernel meal, coconut meal, and fine rice bran could themselves influence rumen fermentation and degradability. Consequently, observed effects cannot be unequivocally attributed to turmeric or ginger alone. Future work should include (a) true top-dressing treatments (additive added without substitution), (b) matched iso-nutrient controls, or (c) factorial designs that independently vary additive presence and basal composition to disentangle these effects.

The addition of 5% ginger (P2) and the combination of both additives (P3) showed an increase in pH to 6.49 and 6.50, respectively, but was relatively similar to P0. This indicates that the opposing effects of turmeric and ginger on pH in P2 and P3 may have counterbalanced each other, resulting in a stable pH that was not different from the control. The addition of turmeric and ginger overall can affect the acidity of post-incubation rumen fluid of oil palm trunk pith wafers (Tunkala *et al*., 2022). According to McDonald *et al*. (2022), changes in rumen fluid pH can affect the activity of rumen microorganisms, the fermentation process, and animal health.

Rumen fluid pH, an indicator of acidity in the ruminant digestive system, is influenced by a number of factors, including the nutrient and fiber composition of the feed, feed dose, and feeding time, which play a role in regulating acid production by microorganisms during the fermentation process (Tunkala *et al*., 2022). The addition of additives such as turmeric and ginger can play a role in changing the acid-base balance (Rasmi *et al*., 2024). Rumen microorganisms and the availability of drinking water also affect pH, with saliva production acting as a natural buffer (Ding *et al*., 2023). In addition, animal health and genetic factors can affect the overall pH response (Zhou *et al*., 2024). A deep understanding of these factors helps in designing appropriate feed formulations and livestock management practices to maintain rumen fluid pH balance, which is very important for supporting the health and productivity of ruminant livestock. Significant interactions between rumen microbes and turmeric, especially its active component curcumin, affect fermentation processes and pH. The antimicrobial qualities of curcumin have the ability to change microbial populations, which is important for rumen function and nutrient absorption. Curcumin also tends to suppress the activity of several groups of methanogenic bacteria, which are responsible for the production of methane gas in the rumen. By reducing methane production, some of the energy can be diverted back to the fermentation process, which increases digestive efficiency. Ruminant productivity and the environment may be impacted by changes in metabolic hydrogen fluxes caused by methanogenesis suppression in rumen fermentation (Ungerfeld, 2020). Hydrogen can be diverted to other sinks such propionate, dihydrogen, and possibly reductive acetogenesis when methanogenesis is suppressed (Ungerfeld, 2013, 2015a, b). Turmeric (*Curcuma longa*) has a chemical called curcumin, which may help ruminants produce less methane. According to Jikah and Edo (2025), turmeric contains flavonoids, saponins, tannins, essential oils, and curcuminoids, all of which can enhance digestion and boost cattle productivity.

Total gas and methane production

The rate of gas production from feed *in vitro* reflects the fermentation performance and microbial activity in degrading the feed under simulated laboratory conditions. This is an indicator of rumen microbial fermentation activity and the feed's ability to provide energy for livestock. Evaluating the rate of gas production is important to understand the nutritional potential and digestibility of feed in the animal's digestive system. The gas production curve (Figure 1) shows that the 5% ginger treatment (P2) generated the highest total gas throughout incubation, consistent with the data in Table 2. In contrast, the combination treatment (P3) exhibited a moderate and gradual increase, closely resembling the control (P0) during early fermentation but surpassing it at later stages. This pattern suggests that the inclusion of both turmeric and ginger may modulate fermentation kinetics toward a more stable, controlled gas release, potentially indicating balanced microbial activity rather than excessive fermentation.

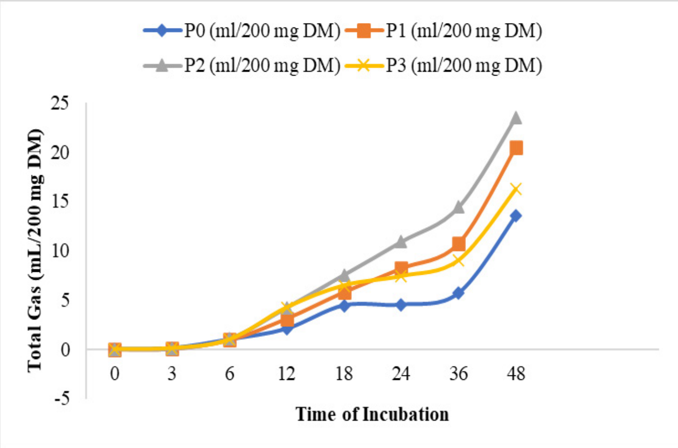

Figure 1: Total gas production (mL/200 mg DM) of complete wafer feeds supplemented with turmeric and ginger P0 (Control); P1 (+5% Turmeric); P2 (+5% Ginger); P3 (+2.5% T + 2.5% G).

Based on the graph, treatment P2 exhibited the highest gas production rate compared to other treatments. It should clearly state that P2 (ginger) led to the highest gas production. As evidenced by the gas production at the 3-hour mark, P2 produced 0.07 mL/200 mg DM, comparable to P1 but slightly lower than P0 and P3. However, over the incubation period, gas production in P2 consistently exceeded that of other treatments. By the 48-hour mark, complete wafer diets in P2 produced 23.5 mL/200 mg DM, significantly higher than all other treatments. The linear responses observed suggest that the phytochemical constituents present at all tested levels were within the acceptable ranges for secondary metabolites and did not adversely affect the functionality of rumen microbes. Optimal levels of phytochemicals content in the turmeric powder were found to positively correlate with the total gas production (Salem *et al*., 2014). The addition of turmeric and ginger powder significantly affected gas production during a 48-hour fermentation experiment (Table 2). Treatments with ginger powder (P2) or turmeric powder (P1) produced significantly more gas than the control (P0) or the combination (P3). This suggests that ginger can improve the fermentability and thus energy availability of animal feed. The active ingredients in ginger, such as gingerols, help break down complex carbohydrates in feed. They also stimulate the growth of beneficial bacteria in the rumen, which leads to more gas production. The feed's ability to be broken down by rumen microorganisms was assessed by measuring methane production. This information helps us understand the feed's energy value and its potential environmental impact, particularly in terms of greenhouse gas emissions. Evaluating these factors is essential for developing more sustainable and nutritious livestock feed. The data on methane production is shown in Table 2. In the present experiment, the methane production of P0 and P3 were lower than P1 and P2. Based on that data indicated that the supplementation of turmeric and ginger powder on complete wafer diet did not reduce the methane production. This finding is inconsistent with a previous study that the inclusion of turmeric into the diet lowered in vitro methane production (Aderinboye and Olanipekun, 2021). Methane (CH_4) production from ruminants can be influenced by phytogenic feed additives. Phytochemicals within these additives often have inhibitory effects on methanogens, leading to a reduction in CH_4 emissions (Ku-Vera *et al*., 2020). Additionally, phenolic compounds can decrease CH_4 production by inhibiting methanogen growth and reducing the transfer of hydrogen between microorganisms (Hodjatpanah *et al*., 2014; Ku-Vera *et al*., 2020). Hydrogen that is not utilized for CH_4 production can be redirected towards the synthesis of short-chain fatty acids (SCFAs) (Ungerfeld, 2020). Phenolic compounds have an important role in reducing methane production in the rumen through several mechanisms: Inhibiting methanogenic microbial activity, reducing hydrogen availability, modulating rumen microbial composition, and increasing digestive efficiency. By reducing methane, phenolic compounds help increase the efficiency of feed energy use, which contributes to better livestock performance and reduced greenhouse gas emissions (Nørskov *et al*., 2023; Battelli *et al*., 2023).

Table 2: *In vitro* total gas production (mL/200 mg DM), methane, and ammonia concentration of wafer complete feed based on oil palm trunk pith silage.

Parameter	P0 (Control)	P1 (+5% Turmeric)	P2 (+5% Ginger)	P3 (+2.5%T + 2.5% G)	P value
Total gas production (ml)	5.69±1.82a	10.8±1.82c	14.4±1.82d	9.02±1.82b	0.010
Methane (ml)	13.28±2. 55a	29.98±2. 55b	36.83±2. 55c	16.90±2. 55a	0.000
NH_3 (mM)	0.75±0. 12a	0.60±0. 12a	0.60±0. 12a	0.28±0. 12b	0.002
Maximum potential gas production (a+b, mL)	13.56±2. 21a	20.51±2. 21c	23.50±2. 21d	16.23±2. 21b	0.003
Gas production rate (c, mL/h)	13.56±1. 13a	20.51±1. 13b	23.50±1. 13b	16.23±1. 13a	0.000

Curcumin tends to suppress the activity of several groups of methanogenic bacteria, which are responsible for the production of methane gas in the rumen. By reducing methane production, some of the energy can be diverted back to the fermentation process, which increases digestive efficiency. Because methanogenesis consumes hydrogen, its reduction can affect hydrogen flow and may slightly decrease the acid produced, thereby maintaining a more stable rumen pH (Tian *et al.*, 2023). Contrary to previous reports that phenolic and curcumin compounds suppress methane formation, the present study showed that the inclusion of 5% ginger increased methane production. This may reflect a dose-dependent response, as higher levels of phytogenic compounds can alter microbial equilibrium by inhibiting certain fiber-degrading or propionate-producing bacteria while allowing hydrogen-producing populations to proliferate. Furthermore, the use of whole ginger powder rather than concentrated extracts could provide additional fermentable substrates that enhance total gas and methane output. These findings suggest that the antimethanogenic effect of phytochemicals is not universal and may depend on additive type, concentration, and microbial adaptation.

Ammonia (N-NH_3) concentration

The post-incubation rumen fluid ammonia concentration refers to the amount of ammonia dissolved in the rumen fluid after the wafer incubation process in a simulated laboratory environment. This ammonia is a by-product of protein degradation in feed by rumen microorganisms. The ammonia concentration values in this study can be seen in Table 2. The research results showed that the addition of 5% turmeric powder (P1) resulted in a decrease in rumen fluid ammonia concentration after in vitro incubation of the complete ration wafer, compared to the control group (P0), with a value of around 0.60 mM. Similarly, the addition of the same level of ginger powder (P2) also resulted in a similar decrease in ammonia concentration, reaching around 0.60 mM. However, when both ingredients were combined at a level of 2.50% each (P3), a more significant decrease in ammonia concentration occurred, which was around 0.28 mM.

The decrease in rumen fluid ammonia concentration after the addition of turmeric and ginger powder was due to the antimicrobial potential of the active compounds contained in both ingredients (Sadarman *et al.*, 2021). Compounds such as curcuminoids have been shown to have antimicrobial activity that can inhibit the growth of certain microorganisms, including bacteria responsible for the degradation of protein into ammonia in the rumen (Ramos *et al.*, 2022). Certain herbal bioactive compounds, particularly essential oils and tannins, exhibit antimicrobial effects that selectively inhibit hyperammonia-producing bacteria in the rumen. For example, studies have shown that a mixture of tannins and essential oils can significantly reduce rumen ammonia formation by suppressing specific bacterial populations responsible for excessive amino acid deamination (Foggi *et al.*, 2024). Furthermore, in vitro and in vivo studies have documented how herbal supplements reduce proteolytic bacterial and protozoan populations, which correlates with lower rumen ammonia concentrations (Wanapat *et al.*, 2013).

Regarding the lower ammonia concentration at P3, both herbal plants can complement each other in inhibiting the growth of ammonia-producing bacteria in the rumen. This combination can synergistically enhance the antimicrobial effect, resulting in a more significant decrease in rumen fluid ammonia concentration compared to the addition of each ingredient individually. Numerous studies have demonstrated that the plant polyphenols in turmeric can significantly enhance nitrogen utilisation (Jafari et al. 2018) and influence the activity and abundance of rumen microorganisms (Cheong *et al.*, 2023).

Dry matter and organic matter degradability

The degradation of dry matter in oil palm trunk pith wafers refers to the process by which the dry components in these wafers are broken down by rumen microorganisms. The value of dry matter digestibility is evaluated to determine the effectiveness of the wafers as a nutrient source for livestock. The goal is to understand to what extent these wafers can be digested by the animal's digestive system and provide the necessary nutrients. The values for the degradation of dry matter in oil palm trunk pith wafers can be found in Table 2.

Based on the results of variance analysis, the addition of turmeric and ginger in the production of wafers made from oil palm trunk pith has a significant effect ($P<0.05$) on *in vitro* ruminal dry matter degradability (IVRDMD). The production of wafers without the addition of any additives (P0) had an acceptable IVRDMD value, but the addition of 5% turmeric (P1) resulted in a significant decrease in IVRDMD, while the addition of 5% ginger (P2) caused a further decrease. The combination of 2.50% turmeric and 2.50% ginger (P3) resulted in a higher IVRDMD value compared to the addition of turmeric or ginger individually. This indicates an interaction between turmeric and ginger that can affect dry matter degradability. The observed effects may be attributed to the ability of plant phytochemicals, such as those found in turmeric rhizomes, to stimulate the activity and growth of ruminal fibrolytic microbes (Singla *et al.*, 2021), leading to accelerated substrate degradation (Kholif and Olafadehan, 2021).

Bioactive compounds in turmeric and ginger, such as curcuminoids (including curcumin, have anti-inflammatory properties and increase the activity of digestive enzymes such as amylase, lipase, and protease in the animal's digestive system (Sadarman *et al*., 2021). The mechanism of action involves the stimulation of the production of these enzymes, which then accelerates the breakdown of dry matter (Raheem *et al*., 2021) in oil palm trunk pith wafers by rumen microorganisms, increasing the overall dry matter digestibility.

When the process of dry matter degradability occurs faster in the rumen, nutrients in the feed can be digested more efficiently and quickly by rumen microorganisms (Rahimi *et al*., 2024). This results in a faster increase in nutrient availability for livestock, so they can consume and use these nutrients for growth, milk or meat production, and maintaining overall body health (Simon *et al*., 2024). Thus, the benefits are to increase the performance and productivity of livestock efficiently, as well as saving time and costs in feeding.

The high content of dry matter degradability in the feed consumed by livestock can indicate better feed quality in supporting the nutritional needs of livestock (Yu *et al*., 2024). Efficient and rapid degradation processes produce more nutrients that can be absorbed and utilized by livestock, increasing the availability of energy, protein, and other nutrients (Ferrari *et al*., 2021). Thus, feed with a high dry matter degradability value tends to have a positive impact on livestock. However, it is important to remember that dry matter degradability is only one of many factors that affect overall feed quality, and other aspects such as nutrient composition, feed diversity, and water availability also need to be considered to assess overall feed quality (Rahimi *et al*., 2024).

The process of organic matter degradability involves the breakdown or decomposition of organic components in the wafer by rumen microorganisms into simpler forms so that they can be absorbed by the animal's body. The value of organic matter degradation of oil palm trunk pith wafer can be seen in Table 3. The addition of turmeric powder at a level of 5% (P1) showed a decrease in the value of organic matter degradability of the complete ration wafer compared to the control treatment (P0), with a value of around 78.7%. Meanwhile, the addition of ginger at the same level (P2) caused a more significant decrease in organic matter degradability, with a value of around 72.6%. However, when a combination of 2.50% turmeric and 2.50% ginger (P3) was added, there was an increase in the value of organic matter degradability, reaching around 81.8%. These results indicate that the addition of turmeric and ginger affects the process of organic matter degradation (Raheem *et al*., 2021) in the wafer, with the combination of the two producing different effects compared to the use of each material individually. However, statistically, the effect of adding both herbal plants did not have a significant effect (P>0.05) on the organic matter degradability of complete diet wafer based on oil palm trunk pith.

The tendency of increasing and decreasing organic matter degradability in this study is related to the active substances contained in turmeric and ginger powder, such as curcuminoids (Greenland *et al*., 2023). These active substances are believed to have bioactive properties that can affect the activity of microorganisms in the animal's digestive system, by stimulating the production of digestive enzymes needed to digest organic matter in oil palm trunk pith wafers (Raheem *et al*., 2021). Although there are numerical differences in the values of organic matter degradation between the control and treatments that added turmeric and ginger, the same statistical results show that these differences are not statistically significant. This is due to natural variations in the data or the influence of other factors that were not considered in the statistical analysis, so that the numerical differences are not large enough to be considered statistically significant.

Several factors that can affect the rate of organic matter degradability in ruminant rumen include feed nutrient composition, rumen microbial activity, rumen environmental conditions (such as pH and temperature), feed retention time in the rumen, and interactions between various feed components consumed by the animal (Belanche *et al*., 2023). In addition, external factors such as the type and quality of feed, feed processing, and animal

Table 3: *In vitro ruminal* degradability of wafer complete feed from palm kernel replanting.

Parameter	Component (% of DM)	P0 (Control)	P1 (+5% Turmeric)	P2 (+5% Ginger)	P-value
IVRDMD	69.0±4.49^{a}	61.7±7.49^{ab}	57.7±5.44^{b}	68.0±5.26^{a}	0.030
IVROMD	83.8±12.8	78.7±24.4	72.6±21.4	81.8±12.0	0.840
IVRCPD	4.60±0.48^{c}	5.52±0.42^{b}	11.7±0.47^{a}	3.87±0.36^{d}	0.000
IVRNDFD	60.3±0.00^{b}	60.9±0.50^{ab}	61.9±0.43^{a}	61.4±1.46^{ab}	0.000
IVRADFD	39.0±0.22^{a}	31.5±0.22^{c}	33.5±0.50^{b}	39.0±0.22^{a}	0.001

a-d: means in the same column with varying superscript differ significantly (P<0.05); IVRDMD: *in vitro* ruminal dry matter degradability; IVROMD: *in vitro* ruminal organic matter degradability; IVRCPD: *in vitro* ruminal crude protein degradability; IVRNDFD: *in vitro* ruminal neutral detergent fiber degradability; IVRADFD: *in vitro* ruminal acid detergent fiber degradability.

health conditions can also contribute to the rate of organic matter degradability in the rumen (McDonald *et al.*, 2022). Protein in the rumen is degraded by microbes into peptides and amino acids, which are then converted into ammonia (NH_3) through the process of deamination. This ammonia serves as an important nitrogen source for the synthesis of rumen microbial protein, which is subsequently absorbed by the animal in the small intestine as high-quality protein. Synchronization between protein degradation and the availability of fermentable energy is crucial for metabolic efficiency; if not synchronized, nitrogen waste occurs through ammonia loss. Protein that is not degraded in the rumen (rumen undegradable protein, RUP) is also important as a direct source of essential amino acids for the animal. The main metabolic pathways involved include proteolysis, deamination, the urea cycle to regulate excess ammonia, and the production of volatile fatty acids (VFAs) from the carbon skeleton of amino acids, which serve as an energy source for the animal.

Crude protein degradability

Crude protein degradability in complete diet wafer containing oil palm trunk pith silage with the addition of turmeric and ginger powder refers to the process of breaking down or decreasing the crude protein content in the wafer by rumen microorganisms, which is influenced by the presence of turmeric and ginger as feed additives. The value of crude protein degradability of complete diet wafer based on oil palm trunk pith silage can be seen in Table 2. Based on our study results, the addition of turmeric and ginger powder had a significant effect ($P<0.05$) on the crude protein degradability of complete diet wafer. Degradability is the process of breaking down nutrients such as crude protein that occurs in the rumen. This process is initiated by enzymes and microbes, which break down proteins into simpler peptides and amino acids (Estevão-Rodrigues *et al.*, 2024). The crude protein degradability of wafers in P0 to P3 represents the percentage of crude protein degraded in the rumen.

The addition of 5% turmeric powder (P1) resulted in an increase in the value of crude protein degradability in complete diet wafer compared to the control treatment (P0), with a value of around 5.52%. Meanwhile, the addition of the same level of ginger powder (P2) resulted in a more significant increase in the rate of crude protein degradability, reaching around 11.7%. However, when a combination of 2.50% turmeric and 2.50% ginger (P3) was added, there was a decrease in the rate of crude protein degradability, reaching around 3.87%. A crude protein degradability level below 50% indicates that the protein is relatively resistant to rumen microbial degradation and is more likely to be digested in the small intestine as bypass protein, whereas a value above 70% suggests that the protein is highly degradable in the rumen (McDonald *et al.*, 2022). This indicates that the extent of crude protein degradability in the rumen is lower in P3 and P0 so that more true protein will reach the small intestine, thus increasing the availability of amino acids needed by livestock for growth, reproduction, and for producing milk in dairy cows and meat in beef cattle (Ivanova *et al.*, 2024).

Higher crude protein degradation in P2 and P1 indicates that rumen microbes break down more protein for their growth and reproduction. This activity basically benefits livestock, especially beef cattle and working cattle, because more feed energy is available due to the high level of feed protein degradation by rumen microbes (Zhang *et al.*, 2024). Another advantage is that livestock can utilize the by-products of protein degradation in the form of amino acids which can be used by livestock for growth and other body functions (Zeng *et al.*, 2023).

These findings confirm that the effects of turmeric and ginger on crude protein degradability in wafers can vary depending on the dose and combination of the two herbal ingredients. However, both high and low levels of feed protein degradation in the rumen both provide benefits to livestock, depending on the purpose of husbandry. According to Rivera-Chacon *et al.* (2024), the rate of protein degradation can be influenced by factors such as rumen pH, oxygen concentration, synchronization of nitrogen and energy release, age and type of livestock, and stress.

The combination of turmeric and ginger (P3) showed relatively high dry matter degradability but low crude protein degradability. This suggests that the increased DM degradation may have originated primarily from carbohydrate fractions, as supported by the higher NDF and ADF degradability values in the same treatment. The low protein degradability could be attributed to phenolic–protein interactions from turmeric and ginger bioactives, which can form complexes that are resistant to microbial proteolysis. While this reduces ruminal protein degradation, it may increase the fraction of rumen bypass protein available for digestion in the lower tract. Thus, the combined supplementation appeared to enhance fiber fermentation efficiency while protecting part of the dietary protein from ruminal degradation.

Neutral and acid detergent fibre degradability

Neutral detergent fiber degradability in complete diet wafer is the process of breaking down neutral detergent fiber, which is insoluble in detergent, by rumen microorganisms, indicating the availability of fiber nutrients for livestock. The NDF degradability values of complete diet wafer made from oil palm trunk silage can be seen in Table 3.

The research results showed that the addition of 5% turmeric powder (P1) resulted in a decrease in NDF degradability in complete diet wafer compared to the control group (P0), with a value of around 60.9%. Meanwhile, the addition of the same level of ginger powder (P2) and the combination of 2.50% turmeric and 2.50% ginger (P3) also caused a decrease in NDF degradability, reaching around 61.9% and 61.4%, respectively. These findings confirm that the addition of turmeric and ginger tends to reduce NDF degradability in wafers, although the difference is not statistically significant.

NDF degradability, which occurs through fermentation in the rumen or in vitro, reflects the reduction in NDF content and directly impacts the availability of nutrients for livestock. The NDF degradability is a critical factor in forage quality, as NDF degradability in the rumen can exhibit substantial variation (Allen and Oba, 1996; Nocek *et al*., 1988). Moreover, the digestibility of NDF has a direct influence on animal performance. Enhanced NDF hydrolysis can facilitate a more rapid rate of NDF disappearance from the rumen. This, in turn, can lead to a reduction in the physical fill effect of NDF within the rumen, thereby creating a greater capacity for voluntary feed intake. The decreased physical fill can stimulate increased appetite and ultimately result in higher levels of feed consumption (Allen and Oba, 1996).

Referring to this study, the high level of NDF degradation in P1 indicates that more NDF was degraded during in vitro incubation, which can increase energy availability. According to McDonald *et al*. (2022), fiber degraded during the fermentation process in the rumen will produce volatile fatty acids (VFAs) which can be used by ruminant animals as an energy source. With the addition of turmeric powder, ginger, and their combination, NDF degradation can be lower than the control, which means that NDF fiber will stay longer in the rumen, indirectly helping to optimize microbial fermentation and reduce the risk of rumen acidosis.

Regarding the high and low NDF degradability, McDonald *et al*. (2022) stated that it all depends on the purpose of livestock farming. For livestock raised for production purposes, a higher level of NDF degradability is more desirable, while for fattening, a lower level of NDF is more desirable to help maintain nutrient balance in the animal's body. The addition of turmeric and ginger to ruminant feed exerts complex effects on NDF degradability, depending on dose, preparation form, and rumen microbial conditions. Their antimicrobial properties can reduce fiber degradation by suppressing certain microbes, but under suitable conditions, they may also improve rumen microbial balance and fiber utilization. Understanding these dynamics is essential to optimizing herbal supplements for improved fiber fermentation and animal health.

The main components of ADF, such as cellulose and lignin, are significant parts of feed fiber that are difficult for livestock to digest. Therefore, evaluating ADF degradation is highly relevant for assessing the nutritional quality and digestibility of feed. The ADF degradability values for complete diet wafer can be found in Table 2. The addition of turmeric powder at a level of 5% (P1) resulted in a significant decrease in ADF degradability of the complete ration wafer compared to the control group (P0), with a value of around 31.5%. Meanwhile, the addition of ginger powder at the same level (P2) also resulted in a decrease in ADF degradation, although not as large as the decrease in P1, with a value of around 33.5%. However, when a combination of 2.50% turmeric and 2.50% ginger (P3) was added, the ADF degradability value returned closer to the control level, which was around 39.0%. These findings indicate that the addition of turmeric at a certain level can be more effective in reducing ADF degradability compared to the addition of ginger or a combination of both.

The addition of turmeric powder, ginger, and a combination of both can increase and decrease ADF degradability in wafers due to the active compounds contained in turmeric and ginger. These compounds stimulate the activity of rumen microorganisms, which can accelerate the breakdown of crude fiber in wafers, including cellulose and lignin, as reflected in the increase in ADF degradability values observed in the study.

The mechanism of action of the active substances in both herbal plants involves a complex interaction between the compounds and rumen microorganisms, through the mechanism of stimulating the activity of digestive enzymes in the rumen responsible for breaking down crude fiber, increasing the population of rumen microbes that are effective in digesting fiber, or changing the rumen environment to be more conducive to the growth and activity of fiber-degrading microorganisms. In addition, the positive effect on ADF degradability can also be influenced by the synergistic interaction between the active compounds in turmeric and ginger, which work more effectively together than when used separately.

Similar to NDF, ADF is also a fiber component that only contains cellulose and lignin, which can affect the digestibility and energy availability for livestock. ADF degradability refers to the decrease in ADF content in feed after undergoing fermentation in the rumen, so the level of degradation can affect the availability of fiber and energy. Higher ADF degradation in P0 and P3 indicates that more ADF fiber is degraded in the rumen, this condition can increase the availability of energy because the degraded fiber can produce volatile fatty acids (especially acetate and propionate) that can be used by livestock as an energy source. Meanwhile, the lower level of ADF degradation (P1 and P2) indicates that most of the ADF fiber stays longer in the rumen, they can help optimize microbial fermentation and reduce the risk of rumen acidosis. Regardless of this, high and low ADF degradability during fermentation in the rumen is equally important when viewed from the nutritional context. Livestock raised with high energy needs require higher ADF degradation, while in livestock raised for fattening, lower levels of ADF degradation are more needed because they can help balance the nutrients needed by livestock. ADF degradation is a key factor supporting the production of essential energy substrates, maintaining rumen health, and influencing livestock performance. Its role varies but remains central across different production systems, ensuring metabolic efficiency and animal well-being. bioactive herbal compounds act as natural modulators of the rumen ecosystem by selectively affecting microbial populations and enzyme activities involved in fiber digestion. This modulation improves fiber utilization, stabilizes rumen fermentation, and enhances animal health and productivity.

Although lower NDF and ADF degradability were observed in the turmeric- and ginger-supplemented treatments, the rumen pH values remained above 6.0 in all cases, indicating stable fermentation conditions rather than acidosis prevention. The reduction in fiber degradability may reflect a moderated fermentation rate that maintains rumen stability without significantly affecting total VFA production. This suggests that turmeric and ginger may influence the balance of microbial populations and fermentation kinetics rather than reducing overall fermentative efficiency.

CONCLUSION

The addition of turmeric and ginger, either individually or in combination, influenced *in vitro* rumen fermentation characteristics and nutrient degradability in oil palm trunk pith silage-based wafer feed. The combination treatment (P3) maintained stable rumen pH and fermentation profiles without significantly altering methane production compared to the control. These results suggest that turmeric and ginger can be incorporated as natural feed additives to support rumen fermentation stability, although further *in vivo* and microbial-level studies are needed to confirm their potential role in modulating methanogenesis.

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NOVELTY STATEMENT

The novelty of this study lies in the use of oil palm trunk pith silage, a by-product of oil palm replanting, as a base material for wafer feed supplemented with natural additives such as turmeric and ginger. Furthermore, this research provides new insights into how these phytoadditives, individually or in combination, affect rumen fermentation efficiency, nutrient degradability, and potential methane reduction under in vitro conditions.

AUTHOR'S CONTRIBUTION

SN, JA, FM and FF: Conducted the experiment, performed data analysis, and wrote the article draft. RPH, MNA, SS, EE: checked data analysis and revised the article draft. NQ and SS: designed the experiment, checked data analysis, and revised the article draft.

Ethical approval

The experiment was approved by the Animal Welfare Commission of the University of Jambi Animal Ethics Committee and was conducted at the Laboratory of Dairy Nutrition, Faculty of Animal Husbandry, Universitas Jambi, Indonesia. Rumen liquor was obtained from one ruminally cannulated, non-lactating Holstein dairy cow housed at the Field Experimental Station, Faculty of Animal Husbandry, Universitas Jambi, Indonesia. The animal was cared for in accordance with the University of Jambi's standards for animal welfare.

Generative AI and AI-assisted technology statement

The authors used generative AI tools (such as ChatGPT and Grammarly) solely to assist with language editing and to enhance the clarity and readability of the English text. No artificial intelligence tools were used for data analysis, interpretation, or the generation of original scientific content. The authors take full responsibility for the accuracy and integrity of all scientific aspects of this publication.

Conflict of interest

The authors have declared no conflict of interest.

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