

Effect of Lime (*Citrus aurantifolia*) Waste Powder Supplementation on *in Vitro* Fermentability and Digestibility in Male Goats

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ABSTRACT

Goat production in tropical areas faces significant challenges due to seasonal forage scarcity and high feed costs, necessitating alternative feed resources. This study aimed to evaluate the effect of lime (*Citrus aurantifolia*) peel meal supplementation on the digestibility and fermentability of male goat rations *in vitro*. The experiment was conducted using a Completely Randomized Design with five supplementation levels: R0 (0%), R1 (5%), R2 (10%), R3 (15%), and R4 (20%) using a basal diet of Taiwan Napier grass and concentrate. The parameters evaluated included *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD), total volatile fatty acids (total VFAs) concentration, and rumen ammonia-nitrogen (NH₃). The results showed that the treatments significantly affected ($P < 0.05$) all parameters. The R3 treatment (15%) provided the most optimal results, yielding the highest IVDMD (64.08%), maximum IVOMD (72.06%), peak total VFA (171.89 mM), and ideal NH₃ (10.08 mM). This efficiency was triggered by a substrate shift, where the high crude fiber of Taiwan Napier grass (27.12%) was substituted by lime peel meal which had lower crude fiber (11.92%), thereby reducing the total lignin proportion of the ration and optimizing fermentation. However, the 20% level (R4) induced a threshold effect that decreased the IVOMD (64.24%) and total VFA (141.92 mM) values due to the accumulation of bioactive compounds (saponins and limonene) that began to inhibit microbial activity. In conclusion, supplementing male goat rations with 15% lime peel waste meal optimizes rumen fermentation and feed digestibility, offering a sustainable alternative feed strategy to mitigate feed price instability.

Keywords: *Citrus aurantifolia*, Rumen Fermentation, Digestibility, Goat, *In Vitro*.

INTRODUCTION

Goats are a livestock commodity widely found in Indonesia and play a strategic role in developing national food security. According to the Indonesian Central Bureau of Statistics (2025), the goat population in West Java accounts for 5.46% of the national total, equivalent to 864,027 heads out of 15,824,305 heads across Indonesia. However, this large population size is not yet directly proportional to optimal productivity. This gap is primarily driven by constraints frequently encountered by farmers during the rearing process, such as limitations in forage quantity and quality during the dry season, as well as fluctuations in feed ingredient prices. This uncertainty often forces farmers to provide suboptimal feed without considering the fulfillment of the livestock's nutritional requirements.

Efforts that can be made to minimize the impact of such price fluctuations include optimizing the utilization of local alternative feed ingredients available year-round. Recently, numerous studies have highlighted agro-industrial waste as a potential alternative raw material. Agro-industrial waste is considered a viable alternative given its abundant availability at competitive prices due to its status as a by-product.

Indonesia, as a tropical country, possesses various types of agro-industrial products available year-round, one of which is lime. Lime (*Citrus aurantifolia*) is a type of horticultural crop that can be cultivated across various regions in Indonesia. Lime production in Indonesia reached 33,511 tons in 2021 and increased to 53,457 tons in 2022 (Ernawati et al., 2023). Meanwhile, from 2021 to 2022, West Java produced 1,117 tons and 3,500 tons of lime, respectively (BPS Jabar, 2024). These data indicate that West Java contributed 3.33% to the total national production in 2021 and 6.55% in 2022. During the 2023–2025 period, West Java consecutively produced 4,944 tons, 3,558 tons, and 9,732 tons of lime (BPS Jabar, 2026). Based on the data obtained from the Central Bureau of Statistics of West Java Province, lime production in this region exhibits an increasing trend. The amount of lime peel waste generated in Indonesia is estimated to reach 85,323.49 tons of dry matter, originating from household waste and the food industry (Sari, 2023). This fact strengthens the potential utilization of this agro-industrial waste as an alternative feed for livestock, given that the success of an alternative feed ingredient is determined not only by its nutritional content but also by its supply sustainability.

Lime peel is a by-product of the citrus juice industry that has potential as a feed additive due to its bioactive compound content. According to Adindaputri et al. (2013), lime peel contains flavonoids such as naringin, hesperidin, naringenin, hesperetin, rutin, nobiletin, and tangeretin. Quantitatively, Hindun (2017) stated that the flavonoid content is 0.667% w/w with an IC50 value of 42.11 mg/mL. In addition to flavonoids, there are also essential oils, tannins, saponins, phenols, and alkaloids (Pratiwi et al., 2013). On the other hand, the primary compound components making up the essential oil group in lime peel are limonene (40.44%), β -pinene (23.59%), and citral (6.93%) (Putri et al., 2023). Based on previous studies, these compounds contained in lime peel are known to manipulate the rumen environment, thereby proving beneficial in optimizing fermentability and digestibility in livestock. Although the potential of lime peel is recognized as beneficial for ruminants, information regarding the optimal supplementation level in male goats remains very limited. Therefore, this study is essential to evaluate the fermentability and digestibility responses at various levels of lime peel waste meal supplementation *in vitro*.

MATERIALS AND METHODS

This study was conducted at the Laboratory of Ruminant Nutrition and Feed Chemistry, Faculty of Animal Husbandry, Padjadjaran University, Indonesia, over a period of four months.

Ethical approval: The experimental procedures were reviewed by the Padjadjaran University research committee and granted an ethical exemption. This exemption applies because no live animals were subjected to experimental procedures. The rumen fluid utilized in the *in vitro* trials was strictly collected as a by-product from goats slaughtered for commercial purposes at a local abattoir.

Feed Sample Preparation

The experimental samples used consisted of chopped 40-day-old Taiwan Napier grass, a basal ration, and dried, ground lime peel.

Table 1. Fiber Fraction of Lime Peel

Parameter	Content (% DM)
Acid Detergent Fiber (ADF)	18.67
Neutral Detergent Fiber (NDF)	24.16
Lignin	4.16
Cellulose	14.51
Hemicellulose	5.49

Source: Van Soest Analysis at Laboratory of Ruminant Animal Nutrition and Feed Chemistry Padjadjaran University (2024)

Table 2. Feed Ingredient Composition of The Basal Ration

Feed Ingredient	Feed Substances (%)				
	Moisture	Ash	Ether Extract	Crude Protein	Crude Fiber
Tofu Dregs	5.62	3.31	2.59	20.53	14.38
Soy Bean Meal	8.18	7.72	1.18	47.08	2.45
Cassava Dregs	9.70	3.40	0.45	2.26	13.55
Palm Kernel Meal	7.29	5.42	9.84	11.88	18.00
Rice Bran	8.31	11.26	9.09	15.23	10.98
Coconut Meal	8.49	8.25	1.37	24.36	6.37

Source: Proximate Analysis at Biotechnology Center, IPB University (2024)

Table 3. Feed Composition

	Feed Substances (%)				
	Moisture	Ash	Ether Extract	Crude Protein	Crude Fiber
Basal Ration	8.18	6.41	4.38	16.99	11.89
Taiwan Napier Grass	6.98	17.30	1.57	13.24	27.12
Lime Peel	9.30	5.70	0.89	10.34	11.92

Source: Proximate Analysis at Biotechnology Center, IPB University (2024)

Table 4. Feed Proportions in The Treatment Groups

Treatment	Feed Ingredient (%)		
	Grass	Basal Ration	Lime Peel
R0	60	40	0
R1	55	40	5
R2	50	40	10
R3	45	40	15
R4	40	40	20

Description of Treatment:

R0 = 60% grass + 40% basal ration + 0% lime peel

R1 = 55% grass + 40% basal ration + 5% lime peel

R2 = 50% grass + 40% basal ration + 10% lime peel

R3 = 45% grass + 40% basal ration + 15% lime peel

R4 = 40% grass + 40% basal ration + 20% lime peel

Table 5. Nutrient Profile of The Dietary Treatments

Treatment	Nutrient Content (%)				
	Moisture	Ash	Ether Extract	Crude Protein	Crude Fiber
R0	7.46	12.94	2.69	14.74	21.03
R1	7.58	12.36	2.66	14.60	20.27
R2	7.69	11.78	2.63	14.45	19.51
R3	7.81	11.20	2.59	14.31	18.75
R4	7.92	10.62	2.56	14.16	17.99

***In Vitro* Running Refers to Tilley and Terry (1963) Method**

This *in vitro* study was conducted using the Tilley and Terry (1963) method, with observations made on total VFA concentration, NH₃ production, dry matter digestibility (IVDMD), and organic matter digestibility (IVOMD). Rumen fluid was collected from three freshly slaughtered male goats, while the artificial saliva was prepared following McDougall's (1947) formulation. The chemical components used included 9.8 g NaHCO₃, 9.3 g Na₂HPO₄·7H₂O, 0.57 g KCl, 0.47 g NaCl, 0.06 g MgSO₄, and 0.04 g CaCl₂. All chemicals were added into a beaker glass, then distilled water was added to a final volume of 1000 mL and stirred using a magnetic stirrer until completely homogeneous.

A total of 0.5 grams of each sample was weighed into individual fermentation tubes, following the proportions of grass, basal ration, and lime peel as shown in Table 4. Each tube was then filled with 10 mL of a McDougall solution and rumen fluid in a 4:1 ratio, followed by the addition of carbon dioxide (CO₂) gas before sealing. The fermentation tubes containing the samples and rumen fluid were incubated in a water bath at 38–40 °C for 48 hours. During incubation, the tubes were shaken every three hours to simulate the peristaltic movements of the goat digestive tract. After 48 hours, the pH of each solution was measured, and 3–5 drops of saturated HgCl₂ solution were added to terminate rumen microbial activity. The contents were then centrifuged at 3000 rpm for 10 minutes to separate the residue from the supernatant. The supernatant was used for the analysis of NH₃ and VFA production. Meanwhile, for the digestibility analysis, the remaining residue underwent the second stage (abomasum phase) by adding a pepsin solution containing 2.47 g/L of pepsin (p.a.) and 20 mL/L of HCl in distilled water, followed by an additional 48 hours of incubation.

Observed Variables

Digestibility of Dry Matter (DM) and Digestibility of Organic Matter (OM)

After the initial 48-hour incubation period, the solution was centrifuged at 3000 rpm for 10 minutes to separate the supernatant from the residue. The supernatant was discarded, and the residue was retained. Subsequently, 50 mL of pepsin solution was added to each fermentation tube, and the samples were incubated for another 48 hours with manual shaking every six hours. Following the second incubation period, the fermentation tubes were opened, and the entire contents were filtered using pre-weighed Whatman No. 41 filter paper to separate the residue from the digested solution. Any residue remaining on the walls of the fermentation tubes was rinsed with distilled water to ensure complete recovery of the sample.

The filter paper containing the residue was placed in a pre-weighed aluminum dish and dried in an oven at 105°C for 24 hours. After drying, the dish was placed in a desiccator for 15 minutes. The aluminum dish containing the dried residue was then weighed to determine the final dry matter weight. Subsequently, the residue was transferred into a pre-weighed porcelain crucible and dried again in an oven at 105°C for another 24 hours. After drying, the crucible was placed in a desiccator for at least 15 minutes until it cooled to room temperature. The crucible and residue were then weighed to obtain the post-oven dry weight of the sample for dry matter digestibility calculation.

The dried samples were then ashed in a muffle furnace at 600°C for 4–6 hours. After cooling in a desiccator, the samples were weighed to determine the organic matter content.

Total Volatile Fatty Acids (VFA) Concentration

Total VFA concentration was measured using the Markham steam distillation technique (1942). A 5 mL aliquot of supernatant was placed into the steam distillation apparatus, followed by the addition of 1 mL of H₂SO₄, then the apparatus was sealed. Distillation continued until all VFA had evaporated and condensed into an Erlenmeyer flask, reaching a total volume of 200 mL. After condensation, 3–5 drops of phenolphthalein indicator were added and mixed by gently swirling the flask. The solution was then titrated with 0.5 N HCl until the pink color disappeared, indicating the endpoint. The volume of HCl used was recorded for further calculation.

Total VFA production was calculated using the following formula:

$$VFA_{total} (mM) = (a - b) N HCl \times \frac{1000}{5}$$

Note: a = Volume of blank titrant (5 mL NaOH)

b = Volume of sample titrant Ammonia (NH₃) production

Ammonia (NH₃) Concentration

NH₃ analysis was carried out using the Conway microdiffusion method. Conway dishes and their lids were prepared and sealed with petroleum jelly around the rims. A total of 1 mL of supernatant was added to the outer compartment (left side) of the Conway dish, while 1 mL of boric acid solution containing methyl red and bromocresol green indicators was placed in the central well. The dish was then tightly sealed and gently shaken to mix the Na₂CO₃ solution with the supernatant. The Conway dishes were left to stand at room temperature for 24 hours. After incubation, the contents of the central well were titrated with 0.005 N H₂SO₄ until the color changed from blue to pink.

NH₃ production was calculated using the following formula:

$$NH_3 (mM) = (V H_2SO_4 \times N H_2SO_4 \times 1000) mM$$

Note: V H₂SO₄ = Volume of H₂SO₄ used for titration (mL)

N H₂SO₄ = Normality of H₂SO₄

Statistical Analysis

The data were analyzed using Analysis of Variance (ANOVA) to determine the effect of treatments, followed by Duncan's Multiple Range Test (DMRT) to identify significant differences among treatment means.

RESULTS AND DISCUSSION

The results of the study regarding the evaluated parameters, including IVDMD, IVOMD, total VFAs, and NH₃ are presented in Table 6.

Table 6. Result of Lime Peel Treatment on IVDMD, IVOMD, VFAt, and NH₃

Parameter	Treatment				
	R0	R1	R2	R3	R4
IVDMD (%)	60.05 ^b ± 0.70	62.04 ^c ± 0.96	63.15 ^{cd} ± 0.72	64.08 ^d ± 1.72	57.35 ^a ± 1.04
IVOMD (%)	66.09 ^{ab} ± 0.67	67.39 ^{bc} ± 1.38	69.82 ^{cd} ± 1.46	72.06 ^d ± 2.33	64.24 ^a ± 3.29
VFAt (mM)	165.27 ^b ± 1.99	137.05 ^a ± 15.75	162.39 ^b ± 9.84	171.89 ^b ± 12.54	141.92 ^a ± 3.81
NH ₃ (mM)	10.11 ^a ± 0.51	11.22 ^b ± 0.50	10.16 ^a ± 0.46	10.08 ^a ± 0.32	10.15 ^a ± 0.41

^{abcd} Means within the same row with different superscript letters are significantly different ($P < 0.05$)

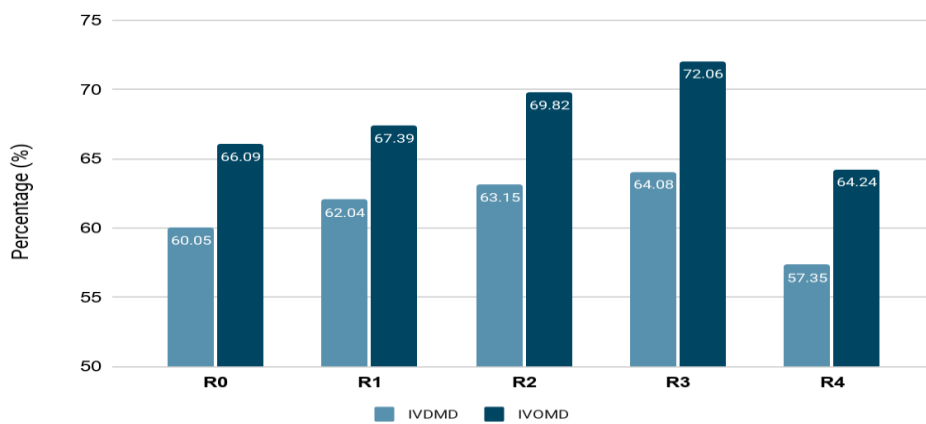


Figure 1. Effect of Lime Peel Supplementation Levels on The IVDMD and IVOMD.

Dry Matter Digestibility

Based on Table 6, the addition of lime peel waste meal significantly affected ($P < 0.05$) the *in vitro* dry matter digestibility (IVDMD) of male goats. Overall, the IVDMD values across all treatments were categorized within a good digestibility range, with the R3 treatment (15%) yielding the highest IVDMD value of $64.08 \pm 1.72\%$. This figure represents a 2.28 percentage point increase compared to the control treatment (R0: $60.05 \pm 0.70\%$). Nevertheless, the IVDMD value in the R4 treatment (20%) decreased significantly to $57.35 \pm 1.04\%$ ($P < 0.05$), representing a drop of 2.7 percentage points relative to the control.

The increase in digestibility values up to the 15% lime peel addition level (R3) is closely related to a shift in the substrate components contained within the ration. The inclusion of Taiwan Napier grass (*Pennisetum purpureum*), which contains 27.12% crude fiber, was gradually reduced and substituted with lime peel meal that possessed a lower crude fiber content of 11.92% (Table 3 and Table 5). This phenomenon aligns with the statement by Wahyuni et al. (2014), who noted that feeds with high crude fiber content tend to have low IVDMD values. This occurs because such feeds are relatively difficult for rumen microbes to digest.

Crude fiber consists of several fractional components, one of which is lignin, an indigestible element for livestock. Lignin can form lignocellulose and lignohemicellulose complexes that hinder enzymatic access to the substrate (Stypinski et al., 2024). A reduction in the crude fiber composition of the ration can decrease the total lignin component, thereby enhancing the accessibility of rumen microbial enzymes to degrade the feed. Furthermore, lignin is known to have a negative regulation with the flavonoid compounds contained in lime peel. In their study, Wang et al. (2024) revealed that lignin and flavonoids compete for the same precursor substrate, namely *p-Coumaroyl-CoA*. Therefore, flavonoid compounds are beneficial in suppressing lignin levels in the ration.

On the other hand, lime peel contains saponins, which are well-known defaunation agents. The saponin content in lime peel is suspected to function optimally at the 15% level, suppressing the protozoa population without disrupting the activity of cellulolytic bacteria. A decline in the protozoa population can reduce predation on rumen bacteria, allowing the cellulolytic bacterial population to work more optimally in degrading the feed. The IVDMD value in R3, which reached 64.08%, is categorized as high-quality feed, aligning with the statement by Tillman et al. (1991) that rations with a digestibility above 60% can efficiently meet the production needs of livestock. The decrease in the IVDMD value at the 20% level (R4) indicates an accumulation of bioactive compounds that exceeded the threshold tolerance limit, thereby disrupting the fermentation process by rumen microbes.

Organic Matter Digestibility

Based on Table 6, the addition of lime peel waste meal significantly affected ($P < 0.05$) the *in vitro* organic matter digestibility (IVOMD) of male goats. Overall, the IVOMD values across all treatments were categorized within a good digestibility range, with the R3 treatment (15%) yielding the highest IVOMD value of $72.06 \pm 2.33\%$. This figure represents a 5.97 percentage point increase compared to the control treatment (R0: $66.09 \pm 0.67\%$). Nevertheless, the IVOMD value in the R4 treatment (20%) decreased drastically to $64.24 \pm 3.29\%$ ($P < 0.05$), representing a drop of 1.85 percentage points relative to the control.

The fluctuation observed in the IVOMD values perfectly mirrored the trend in the *in vitro* dry matter digestibility (IVDMD). According to Tillman et al. (1998), an increase in dry matter digestibility will directly contribute to a proportional increase in organic matter digestibility. Therefore, it is theoretically expected that the R3 treatment successfully achieved the highest IVOMD value of 72.06%, perfectly aligning with its IVDMD peak. Although the ash supply originating from Taiwan Napier grass was gradually reduced and replaced by lime peel meal with a relatively lower ash content (Table 3 and Table 5), the total organic matter digestibility actually increased up to the 15% addition level. This phenomenon represents an excellent level of feed efficiency and reflects an optimized fermentation process.

The success in degrading organic matter up to 72.06% serves as physical evidence of excellent synchronization within the rumen. The abundance of this degraded organic matter has a direct implication on the availability of fermentation products, as demonstrated by the highest peak in total VFA concentration (171.89 mM) as an instant energy source, alongside the availability of ammonia (NH_3) within the optimum upper limit range (10.08 mM) for microbial protein synthesis.

Volatile Fatty Acid (VFA)

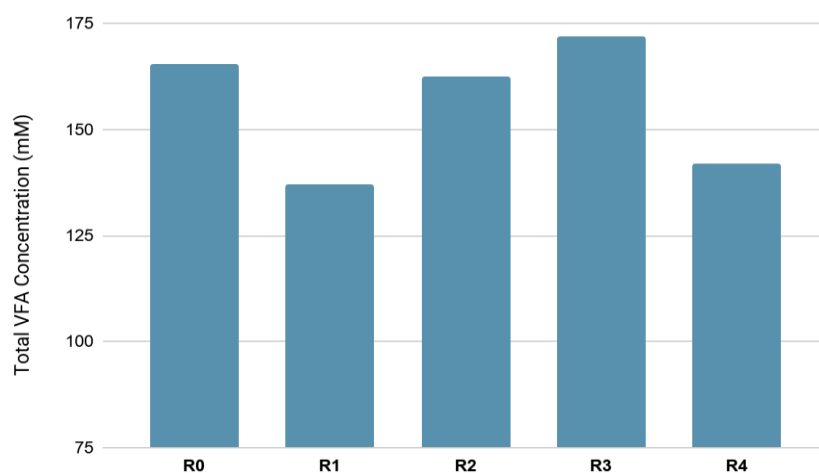


Figure 2. Effect of Lime Peel Supplementation Levels on The Total VFA Concentration.

Based on Table 6, the addition of lime peel waste meal significantly affected ($P < 0.05$) the *in vitro* total VFA concentration of male goats. Overall, the total VFA concentrations across all treatments were categorized within

a good fermentability level. Although fluctuations occurred in certain treatments, the addition of lime peel up to the 15% level (R3) successfully yielded the highest achievement at 171.89 ± 12.54 mM. This figure represents a 4.01% increase compared to the control treatment (R0: 165.27 ± 1.99 mM). Meanwhile, after the total VFA concentration peaked at the 15% level (R3), a drastic decrease occurred at the 20% supplementation level to 141.92 ± 3.81 mM. At the 20% level, there was a 17.44% ($P < 0.05$) reduction from the peak total VFA concentration, and the concentration at this level was relatively lower than that of the control treatment.

According to Sutardi (1980), the optimum VFA concentration to support rumen microbial growth ranges from 80 to 160 mM. Although the total VFA concentration in the R3 treatment exceeded this reference value, the IVDMD and IVOMD data for the R3 treatment were precisely at the peak of their increasing trend. This proves that the fermentation process proceeded optimally without any indication of rumen microbial activity disruption. The degraded organic matter was abundantly available as a raw material for microbial fermentation, thereby triggering a massive surge in total VFA concentration.

Conversely, the 20% supplementation level indicated the occurrence of a threshold effect. The bioactive compound content in lime peel, such as saponins and essential oils (particularly *limonene*), is suspected to have reached a toxicity accumulation level that began to inhibit rumen microbial activity, thereby suppressing the rate of carbohydrate fermentation.

In addition to VFA as the primary product of carbohydrate fermentation, a significant by-product formed during this process is methane (CH_4) gas. Methane is a major greenhouse gas emitted in large quantities by ruminant livestock, contributing directly to the rise in global average temperatures and serving as a key concern in environmental sustainability.

Although methane production was not directly quantified in this study, the resulting VFA profile can provide a projection regarding the dynamics of methanogenesis following lime peel waste meal supplementation. The optimal fermentation achieved at the 15% level (R3) demonstrates efficient feed energy conversion, indicating that methanogenesis remains well-controlled. However, the drastic decrease in VFA concentration at the 20% level (R4) is suspected to trigger a shift in fermentation pathways. The disruption of VFA production leads to the accumulation of hydrogen gas (H_2) within the rumen, which is subsequently redirected massively by methanogenic archaea to form methane. Therefore, restricting supplementation to the 15% level is crucial to maintain fermentation efficiency while preventing undesirable surges in methanogenesis.

Ammonia (NH_3)

Based on Table 6, the addition of lime peel waste meal significantly affected ($P < 0.05$) the *in vitro* NH_3 concentration of male goats. Overall, the NH_3 concentrations across all treatments were within the normal range, spanning from 10.08 ± 0.32 mM to 11.22 ± 0.50 mM. The results of the study indicated that the NH_3 concentration exhibited a dynamic yet stable fluctuation across treatments.

According to Sutardi (1979), the optimum NH_3 concentration produced in the rumen ranges between 4 and 12 mM. The NH_3 concentration generated by each treatment remained stable around the upper limit of the reference range, despite a gradual decline in crude protein (CP) content originating from the grass (Table 3 and Table 5). Taiwan Napier grass, which has a CP content of 13.24%, was progressively substituted with lime peel containing lower CP at 10.34%. This phenomenon demonstrates that the inclusion of lime peel up to the 20% level did not disrupt the protein degradation process, but rather triggered efficient microbial protein synthesis within the rumen.

Efficient protein synthesis can be achieved through a high level of organic matter digestibility. As stated by Kalpikorini et al. (2024), IVOMD encompasses dietary nutrients such as carbohydrates, proteins, lipids, and vitamins. A good organic matter digestibility level allows for sufficient NH_3 availability to meet the requirements of protein fermentation by rumen microbes. Furthermore, lime peel also contains tannins, which are known to form tannin-protein complexes (*bypass protein*) that can be directly absorbed post-ruminally by the livestock without undergoing rumen degradation.

Practical and Economic Implications

From a practical perspective, lime peel waste is abundantly available at virtually zero raw material cost from lime juice production centers (e.g., in Kuningan, Indonesia). The expenses are solely limited to variable transportation and minimal basic processing (drying and milling). A simple economic analysis based on the feed formulation reveals a significant cost-saving potential. By replacing 15% of the conventional basal grass, which typically costs IDR 650 per kg with this zero-cost agricultural by-product (R3 treatment), the overall reliance on purchased forages is substantially minimized. This 15% substitution effectively translates into a proportional reduction in daily basal forage expenditure for farmers. Furthermore, the significant improvement in organic matter digestibility (IVOMD) implies that goats extract greater nutritional value from this substituted diet. Economically, this means optimal energy conversion is achieved with cheaper feed inputs, potentially maximizing the profit margin for commercial ruminant production without compromising fermentation quality.

Research Limitations and Future Perspectives

Although the present *in vitro* study provides valuable baseline data regarding the nutritional potential of lime peel waste meal, it is important to acknowledge its inherent limitations. The *in vitro* method simulates rumen fermentation in a highly controlled static environment, which cannot fully replicate the complex and dynamic biological processes of a live ruminant's digestive tract. Factors such as feed palatability, voluntary feed intake, passage rate, and the actual systemic absorption of fermentation end-products (VFA and NH_3) cannot be evaluated using this approach. Therefore, future *in vivo* studies using live male goats are highly recommended to validate these findings. Subsequent research should focus on evaluating actual feed consumption, physiological responses, and animal growth performance to comprehensively determine the viability of lime peel waste meal as a sustainable feed ingredient.

CONCLUSION

The addition of lime (*Citrus aurantifolia*) peel waste meal up to the 15% level (R3 treatment) represents the best and most optimal supplementation level for enhancing the digestibility and fermentability of male goat rations *in vitro*. At this optimal level, the highest *in vitro* dry matter digestibility (IVDMD) was achieved at $64.08 \pm 1.72\%$, with a maximum *in vitro* organic matter digestibility (IVOMD) of $72.06 \pm 2.33\%$, and a peak total VFA concentration reaching 171.89 ± 12.54 mM, while maintaining an ideal ammonia (NH_3) concentration at 10.08 ± 0.32 mM. This performance enhancement was driven by a shift in feed substrate quality, where the high crude fiber portion of Taiwan Napier grass was progressively substituted by lime peel waste meal, which possessed a relatively lower crude fiber content. This substitution indirectly decreased the total lignin proportion of the ration and created an efficient energy-nitrogen synchronization for rumen microbial activity. Furthermore, supplementation beyond the 15% level (i.e., the 20% level in the R4 treatment) exhibited a threshold effect that reduced both the fermentation rate and digestibility due to the accumulation of bioactive compounds (essential oils and saponins) exceeding the tolerance limit of rumen microbes.

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