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Nyamplung seed cake as a dietary supplement for dairy goats: *In vitro* digestibility and fermentability analysis

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ABSTRACT

The crude oil industry derived from Nyamplung seeds (*Calophyllum inophyllum*) in Indonesia is rapidly developing. A byproduct of crude oil industry from Nyamplung seed is called Nyamplung Seed Cake (NSC). This research was undertaken to examine the biochemical constituents and *In vitro* digestibility and fermentability analysis of NSC, alongside the effects of NSC supplementation on dairy goat feed. In terms of biochemical composition, NSC exhibited 90.59% dry matter, 84.87% organic matter, 21.93% crude protein, 6.70% ether extract, 10.07% hemicellulose, 42.32% neutral detergent fiber, and 32.25% acid detergent fiber. Regarding secondary plant metabolites, NSC contained a total flavonoid content of 1.65%, 6.58% total phenols, and 0.87% saponins. The total tannin content in NSC was 0.95%, comprising 0.44% condensed tannins and 0.44% hydrolyzable tannins. NSC supplementation at 15% and 20% resulted in dry matter digestibility, organic matter digestibility, and fermentation metrics similar to those of standard feed. Meanwhile, supplementation at the level of 25% significantly increased the digestibility of dry matter to 77.38% and the digestibility of organic matter to 74.99% and produced the lowest methane emission of 48,756.06 ppm compared with other feed treatments. This study concluded that the addition of NSC to dairy goat feed improved feed digestibility and lowered methane emissions without negatively affecting rumen digestion. This study supports the use of NSC as a substitute for protein source diet ingredients in dairy goat feed, recommending its inclusion at levels of up to 25% of the total ration.

Introduction

The Nyamplung, also known as Tamanu (*Calophyllum inophyllum*), is identified by various names across different regions: Alexandrian laurel in English, Nyamplung in Java and Sunda, Nyamplong or Camplong in Madura, Punaga in Minangkabau and Bali, and Kanaga or Panaga in Dayak. This plant is indigenous to Indonesia and flourishes abundantly from Sumatra to the Papua Islands (Ansori *et al*., 2019). The seeds of the Nyamplung plant produce kernels that can be processed to extract Tamanu oil, which serves as an alternative biofuel and cosmetic ingredient (Krishnappa *et al*., 2024). Tamanu oil is extracted from Nyamplung seeds using pressing methods and is used in cosmetics and herbal medicine in Indonesia. Given its significant benefits and applications, the Tamanu oil industry in Indonesia has expanded to meet the wide market demand (Hadiyanto *et al*., 2020)

The TCO industry generates waste known as Nyamplung Seed Cake (NSC), a byproduct remaining after the extraction of TCO. The extraction process of nyamplung seeds involves drying and shelling, followed by pressing, which produces TCO and NSC in a 50:50 ratio (Rahman *et al*., 2019). Tamanu oil processing industries can produce up to 4–6 tons of NSC monthly, depending on their production capacities. The nyamplung plant is notably productive, capable of yielding 3,000–10,000 seeds per tree per season, or approximately 100–200 kg per tree per season, which can be utilized in the CTO processing industry (Hasnah *et al*., 2021).

The increasing demand for TCO suggests that NSC waste production will continue to rise. In practice, local farmers have already applied NSC to ruminants. With a crude protein content of approximately 21.0-23.6%, NSC holds promise as an alternative protein feed source (Restiani, 2017), offering significant potential as a nonconventional feed option for livestock. However, no studies have explored the use of NSC as alternative feed for dairy goats. As a byproduct of TCO, NKC may contain secondary metabolites that could either act as anti-nutritional factors or help reduce methane ($CH4$) emissions when used as ruminant feed. The Nyamplung kernel is rich in saponins, alkaloids, flavonoids, and tannins, all of which are present in NKC. Furthermore, NKC can serve as a protein source while also providing plant secondary metabolites that function as rumen modifiers to lower methane emissions (Yanti and Yayota, 2017)

The procurement of protein source feed has always been a concern for local farmers because of its high price. The sustainable production of NSC and its relatively low price make it a promising alternative protein for animal feed. This study aimed to explore the biochemical content, secondary metabolites of plants, and digestibility of NSC *In vitro*. NSC was used in dairy goat feed rations at various levels (0%, 15%, 20%, and 25%) to evaluate its fermentability and digestibility index *In vitro*. This research is important to conduct considering that there is very little information and references on the use of NSC, especially for dairy goat feed.

Materials and methods

Biochemical Constituents and Secondary Metabolites of Plants

The material used in this study was NSC from nyamplung seed extraction in the CTO industry in Cilacap Regency, Central Java Province, Indonesia. Composites from three production batches of NSC were collected as much as 30 kg, and then a 1 kg sample was taken for chemical composition analysis. The process of making nyamplung oil / Crude Tamanu Oil (CTO) and nyamplung seed cake (NSC) is shown in Fig. 1. Four different types of feed for dairy goats were prepared, consisting of standard feed (0%NSC) and feed using NSC at levels (15%, 20%, and 25%). To analyze the NSC and ration samples, they were placed in a drying oven set at 60°C for 48 h. Once dried, the samples were ground to a size of 1 mm. To assess the dry matter (DM), a 5 g specimen was desiccated at 105°C for 24 hours, in accordance with AOAC Method 934.01. The organic matter (OM) was quantified by incinerating the sample in a furnace

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at 550°C for 5 hours. The crude protein (CP) content was determined using the Kjeldahl method, as per AOAC Method 984.13. The ether extract (EE) was evaluated employing the Soxhlet procedure, in conformity with AOAC Method 920.39, 2005. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were scrutinized utilizing an Fiber Analyzer Ankom 200, complying with AOAC protocols (AOAC, 2005; Method 2002.04 and Method 973.18), with heat-stable amylase utilized for the NDF evaluation. Hemicellulose was calculated by subtracting ADF from NDF. Secondary metabolites, including phenols, flavonoids, saponins, and tannins, were examined using spectrophotometry (UV-1800; Shimadzu). Saponin analysis was conducted according to Noviyanty (2021), while total phenol analysis and flavonoid content was determined using a previously described procedure (González and Herrador, 2007). The analysis of hydrolyzable and condensed tannins followed the procedure described by Makkar *et al*. (1993). All materials consisted of NSC, and the four research feeds were analyzed for biochemical contstituents and plant secondary metabolites, with the results presented in Tables 1 and 2.

Table 1. Biochemical constituents and ruminal *In vitro* incubation of NSC[1] (%, DM[2]).

Chemical composition	
Item	Precentage
Dry matter	90.59
Ash	5.72
Crude protein	21.93
Neutral detergent fiber	42.32
Acid detergent fiber	32.25
Hemicellulose	10.07
Ether extract	6.7
Non-fibrous carbohydrate	56.24
Ca	0.33
P	0.13
Ruminal *In vitro* incubation	
Digestibility of dry matter (%)	78.22
Digestibility of organik matter (%)	81.56
Total VFA (mM)	80.11
Ammonia-N NH_3 (mM)	4.57
Methane emission in the rumen (ppm)	47,50.,72

[1]NSC: Nyamplung seed cake; [2]DM: Dry matter

Ruminal In vitro incubation

NSC and experimental feed were incubated under rumen conditions using an *In vitro* method. To assess digestion *In vitro*, rumen liquid was acquired from the butchering of two-year-old Sapera goats reared on a standard diet. The rumen liquid gathered was strained through filtering fabrics. Subsequently, a rumen buffer was formulated by combining the rumen liquid with a buffering solution at a 1:4 proportion, as detailed by Tilley and Terry (1963). The buffering solution was concocted in accordance with the method outlined by Mcdougall (1948). The buffer was aerated with carbon dioxide (CO2) to preserve anaerobic environments. Ruminal incubation was performed using glass serum bottles containing dry samples and rumen buffer (Tilley and Terry, 1963). The glass containers were securely closed with rubber plugs and aluminum closures. Incubation was conducted at 39°C for 48 hours in an aerobic incubator. The glass containers were securely closed with rubber closures and aluminium seals. Incubation was conducted at 39°C for 48 h in an incubator. The methane levels were expressed in ppm.

Fig. 1. The process of making nyamplung oil / Crude Tamanu Oil (CTO) and Nyamplung seed cake (NSC).

Table 2. Chemical compositions of diet in the present study (%, DM[1] basis).

Parameter	Diet Standart (0% NSC[2])	T1	T2	T3
		(15% NSC[2])	(20% NSC[2])	(25% NSC[2])
Dry matter (%)	88.81	88.85	88.86	88.87
Ash (%)	11.37	10.82	10.54	10.26
Crude protein (%)	14.02	15.05	16.07	17.01
Neutral Detergent Fiber (%)	40.75	41.45	46.19	49.32
Acid Detergent Fiber (%)	29.25	29.72	29.7	28.18
Hemicellulose (%)	11.5	11.73	16.49	21.14
Ether extract (%)	5.98	6.39	6.58	6.76
Non-fibrous carbohydrate (%)	51.56	58.57	59.3	57.74
Ca (%)	1.55	1.78	1.66	1.69
P (%)	0.74	0.89	0.83	0.87

[1]DM: dry matter, [2]NSC : nyamplung seed cake

Fiberglass wool was employed to maintain the separation of the specimen from the rumen buffer. Once gas was collected, all the bottles were opened, and the contents were filtered through a crucible. The pH value of the rumen buffer was ascertained utilizing a pH meter (OHAUS, China), whereas volatile fatty acids (VFA) were analyzed through gas chromatography (GC, Shimadzu, Japan) as delineated by Hidayah *et al.* (2022). The ammonia-N concentration within the rumen buffer was quantified employing the colorimetric technique articulated by Chaney and Marbach (1962), as follows.

Experimental design and data analysis

Biochemical constituents and secondary metabolite data of the NSC plants were investigated descriptively. The effect of NSC supplementation levels on dairy goat feed was was assessed *In vitro* employing a completely randomized design consisting of four types of feed treatments and six replications. Four different dairy goat feeds were formulated using NSC, with varying supplementation levels (0%, 15%, 20%, and 25% NSC). Ruminal incubation data, as well as dry matter digestibility and organic matter digestibility, were examined using analysis of variance (ANOVA) at a 5% significance threshold using SPSS (IBM Corp., Version 26). Duncan's Multiple Range Test (DMRT) was used when the treatment had a significant effect ($p < 0.05$) among the treatments.

Results

Biochemical constituents and secondary metabolites of plants

The compositions of dry matter, crude protein, ether extract, neutral detergent fiber, acid detergent fiber, and hemicellulose in NSC were 90.59%, 21.93%, 6.70%, 42.32%, 32.25% and 10.07% respectively (Table 1). The oil extracted from nyamplung seeds is rich in protein. The oil extraction process decreases the fat content in the kernel, consequently augmenting the protein concentration in the residual cake. NSC are abundant in various secondary metabolites, encompassing phenols, flavonoids, saponins, and tannins. The concentrations of total flavonoids, phenols, and saponins in NSC were 1.65%, 6.58%, and 0.87%, respectively. The total tannin content in the NSC was 0.95%, with condensed tannins accounting for 0.44% and hydrolyzable tannins accounting for 0.51%.

NSC was used as a supplement in dairy goat feed at up to 25%, and the resulting chemical composition is shown in Table 2. NSC supplementation increased the levels of crude protein, NDF, ADF, hemicellulose, and ether extract compared to standard feed.

Ruminal In vitro incubation

In this study, NSC in rumen incubation showed digestibility of dry matter (DMDigs) and digestibility of organic matter (OMDigs), total VFA, ammonia, and methane emission at 78.22%, 81.56%, 80.11 mM, 4.57 mM, and 47,500.72 ppm, respectively (Table 1). The NSC was compared with other experimental feeds to evaluate rumen digestibility (Fig. 2). In general, supplementation of NSC up to 20% (0%NSC vs. 15%NSC, 20%NSC) did not affect DMDigs and OMDigs values compared to the standard feed ($p > 0.05$). The DMDigs of the experimental feeds with 0%NSC, 15%NSC, and 20%NSC were 66.22%, 70.00%, and 71.02%, respectively. The OMDig values were 65.56%, 67.87%, and 69.29%. The highest DMDigs and OMDigs were produced by the feed with 25% NSC, at 77.38% and 74.99%, respectively, whereas the lowest values were produced by the standard feed (0% NSC) ($p < 0.05$).

Rumen incubation produced Total VFA and Ammonia levels that were relatively similar across all feeds studied ($p > 0.05$). Supplementation with 25% NSC culminated in the minimal methane emissions in comparison to all other feeds ($p < 0.05$) (Fig. 3). In contrast, feeds supplemented with 15% and 20% NSC produced methane emissions similar to those of the standard feed ($p > 0.05$). During the 48-hour rumen incubation, NSC produced 47,500.72 ppm of methane, while methane emissions from feeds with 0% NSC, 15% NSC, 20% NSC, and 25% NSC were 57,140.76; 56,751.30; 58,731.25; and 48,756.06 ppm, respectively.

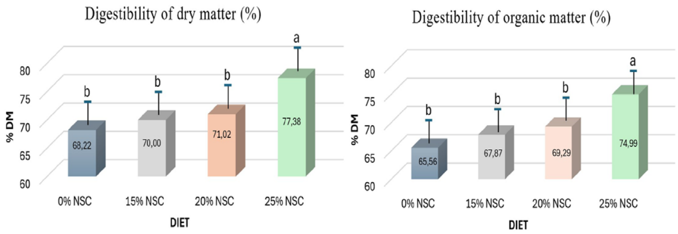

Fig. 2. Investigation of the *In vitro* digestibility of dry matter (A) and organic matter (B) in feed with various levels of non-structural carbohydrate (NSC) supplementation. a,b Mean values with different superscripts differ significantly ($p < 0.05$).

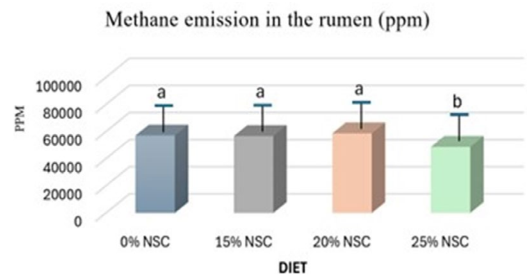

Fig. 3. Methane emissions in the rumen from different levels of NSC supplementation. a,b Mean values with different superscripts differ significantly ($p < 0.05$).

Discussion

NSC's biochemical constituents of NSC is similar to that of oilseed by-products, such as palm kernel meal (PKM) and Copra Cake (CC). Visually, NSC resembles PKM and CC, sharing a brown colour and dry texture. The ether extract level in NSC is largely determined by the processing method used. Fig. 1 depicts the process of transforming the nyamplung fruit into crude tamanu oil (CTO). Importantly, NSC contains a higher ether extract content than other feed ingredients, which could elevate the risk of rancidity (Tinello *et al.*, 2017; Siddique *et al.*, 2010).

Nyamplung seeds, like secondary plant metabolites (Rojas-Sandoval, 2022), may remain in their byproducts after oil extraction. Therefore, further study is needed to confirm this, as secondary plant metabolites can act as antinutritional factors in the ruminant feed. Previous studies have examined the concentrations of secondary plant metabolites in several oil-extraction byproducts. By-products of palm oil, such as palm kernel cake and copra cake contain a total phenol content of less than 0.50% (Rakita *et al.*, 2023). The total concentrations of phenols, tannins, and saponins in soybean meal, rapeseed meal, and sunseed meals are very low, at less than 0.20% (Nehmeh *et al.*, 2022). NSC contains a higher concentration of secondary plant metabolites, namely 6.58% total phenol, 0.87% total saponins, total tannins 0.95%. This shows that the secondary metabolite content of NSC is higher than that of conventional feed ingredients produced by oil extraction.

Based on *In vitro* investigations, NSC supplementation as dairy goat alimentation exhibited a substantial influence on DMDigs values ($P < 0.05$). Duncan's test outcomes revealed significant variances in DMDigs among treatments, implying that 25% NSC yielded elevated DMDigs in comparison to 0% NSC, 15% NSC, and 20% NSC ($P < 0.05$), whereas DMDigs of 0% NSC, 15% NSC, and 20% NSC did not exhibit significant discrepancies.

These studies indicate that NSC supplementation may affect ruminal digestion. The chemical composition of the substituted feed ingredient plays a crucial role. Higher use of NSC in the diet results in different ingredients. Ingredients with higher crude protein, ether extract, and digestible organic matter can enhance the overall digestibility of the diet. Supplementation at a proportion of 25% can enhance DMDigs of diet.

The Nyamplung seed cake used in this study had a relatively high protein concentration of 21.93%. Previous investigations have reported that nyamplung seed cake containing protein ranging from 21.7% to 23.6% and ether extract levels of 14.9%–15.3% (Leksono *et al*., 2017). Substitutes that provide essential nutrients for maximal microbial activity in the rumen can improve diet digestibility. For instance, urea-fermented rumen digesta has a higher crude protein content and digestible organic matter, which enhances feed digestibility. The proteins and extract ether in nyamplung seed cakes are highly digestible, allowing rumen microbes to readily break them down. Sangadji (2022) reported that rumen microbes degrade feed proteins, turning them into peptides and subsequently into simpler compounds. Luminata *et al*. (2024) suggests that the substitution of feed ingredients can alter the microbial population in the rumen, which in turn affects digestion. For example, diets high in concentrates increase the population of amylolytic and proteolytic bacteria, enhancing the breakdown of carbohydrates and proteins.

Neutral Detergent Fiber (NDF) substantially influences the digestibility of aliment. The digestibility metric for dry matter is influenced by the feed's biochemical profile, especially the concentration of NDF and ADF. Feed formulations containing 25% Non-Structural Carbohydrates (NSC) culminated in an augmented NDF concentration. Nyamplung seed cake is procured from the extraction of desiccated nyamplung seeds, which are rich in essential nutrients such as carbohydrates and NDF. The concentrations of neutral detergent fiber and non-fibrous carbohydrates (NFC) impact the fermentation byproducts and *In vitro* NDF digestibility (Ahmed *et al*., 2021; Silva *et al*., 2025). This is congruent with the findings of this investigation, wherein the reduction in Dry Matter Digestibility (DMDigs) value was influenced by the incorporation of NSC at a 25% level. Ma *et al*. (2020) highlighted that when ADF levels rise in diet components, it usually means that digestibility is compromised, as ADF consists of lignin that is quite resistant to microbial breakdown in the rumen. Conversely, feed substances with reduced ADF content possess thinner cellular walls that are more readily decomposed by rumen microorganisms, thereby facilitating digestion and enhancing digestibility and feed utilization efficiency.

This study showed that the use of NSC at the 25% level resulted in the lowest methane emissions compared to other treatments. This is likely due to the NSC content contributing to improved feed quality, as reflected by the higher protein and carbohydrate contents and lower fiber content. The higher the level of NSC used, the higher was the NDF content and the lower was the ADF content. An increase in the concentration of fibrous carbohydrates can lead to increased propionate production, which utilizes H2 and reduces emissions of methane (Bosher *et al*. 2024; Sun *et al*., 2022). In addition, the 25% NSC feed presented a higher ether extract concentration compared to the other tested diets, which may help explain the lower methane emissions observed. Fat supplementation has been shown to reduce protozoal counts significantly. Lauric acid, for example, decreased protozoal counts by 96%. This reduction in protozoa can indirectly affect methanogenesis, as protozoa are associated with methanogenic archaea. Li *et al*. (2022) showed that essential fatty acids in feed can reduce methanogenic bacteria without negatively affecting the growth of fibrolitic bacteria in the rumen. Other studies have also supported this finding, stating that fat supplementation adversely affects rumen microbes such as methanogenic bacteria and ciliate protozoa (Hegarty, 1999).

The reduction of methane emissions is not only related to nutrient composition, such as protein, carbohydrates, and crude fiber, which indicate feed quality; one of the aspects also includes an increase in the concentration of secondary metabolites from plants. On the other hand, the NSC studied in this research was found to have a relatively high content of secondary metabolites, namely total flavonoids at 1.65%, saponins at 0.87%, total phenols at 6.58%, and total tannins at 0.95%. The secondary metabolite content in plants can influence the digestion that occurs in the rumen (Króliczewska *et al*., 2023). Methanogenic bacteria are affected by the presence of secondary metabolites, which limit their proliferation process of these microbes (Ku-Vera *et al*., 2020) (Kadigi *et al*., 2024). As documented by Palangi and Lackner (2022), saponins can act as antiprotozoal agents and contribute to the reduction of methane emissions in various *In vitro* and in vivo studies.

The concentration of VFA in this study increased in line with the rising levels of NSC supplementation, ranging between 75.51-81.67 mM. Meanwhile, ammonia production ranged from 3.16-3.76 mM. The increase in VFA production also has the potential to lower pH, which can suppress the growth of methanogenic bacteria (Dai and Faciola, 2019). Statistically, the production of VFA and ammonia across all treatments did not differ and remained within the normal range. In this study, the fermentation index showed that there were no adverse effects from using NSC in the diet. Another interesting finding was that NSC supplementation at the 25% level resulted in the lowest emission, 48,756.06 ppm. The study of Jayanegara *et al*. (2020) support the results of this study, stating that tannins have been shown to reduce methane emissions by modulating the ruminal microbiota, specifically by decreasing the populations. This reduction is achieved through both direct inhibition of methanogens and indirect effects such as reduced nutrient digestibility. Therefore, NSC supplementation in this study contributed to improving feed quality by demonstrating superior feed digestibility and reducing methane emissions.

Conclusion

Nyamplung seed cake (NSC) can be categorized as an alternative protein source for dairy goat feed. Using NSC in the diet results in ingredients with higher crude protein, ether extract, and digestible organic matter, which can enhance the overall digestibility of the diet. Moreover, NSC contains secondary plant metabolites that have a positive effect on reducing methane production during rumen fermentation. without negatively impacting rumen digestion. This study supports the use of NSC as a substitute for protein source feed ingredients in dairy goat feed, recommending its inclusion at levels of up to 25% of the total ration.

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Conflict of interest

The authors have no conflict of interest to declare.

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