



The Effect of Substituting Concentrate with Different Levels of *Gliricidia* sepium Leaves in a Complete Feed on Nutrient Content, Crude Fiber Fractions, Digestibility, and In Vitro Fermentation Products

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ABSTRACT: The objective of this study was to evaluate the effect of substituting concentrate with different levels of *Gliricidia* (Gamal) leaves in a complete feed on nutrient content, fiber fractions, digestibility, and fermentation products through in vitro analysis, as well as to determine the optimal level of *Gliricidia* leaf inclusion for producing high-quality complete feed. The method used in this study was a field experimental method for feed preparation, employing a Completely Randomized Design (CRD) to analyze nutrient content, crude fiber fraction components, and in vitro observation variables with four treatments and four replications. The highest NDF and ADF contents were observed in the control diet (P_0), at 41.49% and 25.21%, respectively, while the lowest values were found in the P_3 treatment, at 26.08% and 21.60%. Based on the analysis of variance, the dry matter digestibility (DMD) and organic matter digestibility (OMD) showed significant differences ($P < 0.01$), with the P_3 treatment having the highest values of 66.34% and 71.43%, respectively. Gas production from the 2nd to the 48th hour showed significant differences ($P < 0.01$). The treatment diets showed a significant effect ($P < 0.01$) on DMD and OMD, with the highest values in P_3 (75.88% and 82.79%). Ammonia (NH_3) concentration measurements also showed significant differences ($P < 0.01$). The results of microbial protein synthesis measurement after 48 hours of incubation showed a P-value > 0.05 , with the highest value observed in treatment P_3 at 44.024 g N/kg OMTR.

KEYWORDS: Fiber Fraction, Digestibility, In Vitro Fermentation Products, Nutrient Content, Substitution.

INTRODUCTION

Indonesia is a developing country with a livestock sector that plays an important role in meeting the demand for animal protein, particularly from beef. The increase in animal protein consumption is influenced by population growth and rising household income. This view is supported by Badoa et al. (2016), who stated that an increase in income has a significant effect on meat consumption. As a result, the demand for and consumption of meat continues to rise, including the demand for beef. Data shows that beef consumption in 2022 increased by 6.50%, from 2.46 kg per capita per year in 2021 to 2.62 kg per capita per year in 2022 (Center for Agricultural Data and Information Systems, Secretariat General – Ministry of Agriculture, 2022).

Cattle farming in rural areas still follows traditional patterns, relying on natural grasses from plantation lands as the primary feed source. This approach involves relatively low production costs and minimal human labor. As a consequence, during the rainy season, forage production is abundant; however, during the dry season, both the quality and quantity of forage decrease. One of the key factors in the success of cattle farming is the availability of forage as a fiber source, including grasses, legumes, agricultural by-products (such as rice straw), plantation waste (such as sugarcane tops), and agro-industrial waste (such as peanut shells). The forage quality provided to cattle is often inconsistent and tends to be low in nutritional value. Due to the generally low quality of forage, and in order to meet the nutritional requirements of the livestock, one possible solution is to supplement the diet with concentrate feed, apply rumen manipulation techniques, and provide protein supplementation (Suryadi, 2008). Therefore, forage feeding needs to be supplemented with protein-rich feed, namely concentrate. This is supported by Natsir, et al., (2019), who stated that the availability of forage is insufficient in terms of both quality and quantity. As a result, farmers cannot rely solely on forage and must use concentrate as a supplementary feed to compensate for the nutrient deficiencies in the forage. The high cost of feed ingredients derived from grains and agro-industrial waste has led to an increase in the price of concentrate feed. This situation has further constrained farmers, making it increasingly difficult for them to access high-quality concentrate feed. An alternative effort to reduce dependence on concentrate ingredients sourced from grains and agro-industrial by-products is to optimize the use of high-quality



forage from leguminous plants. The use of high-quality forage such as legumes presents an opportunity to reduce reliance on concentrate feed. Herdiawan, et al., (2007) stated that the crude protein content of legumes can serve as an affordable alternative to concentrate feed. In addition, their use as a protein source, especially during the dry season, can help improve livestock productivity.

According to Savitri et al. (2012), one type of leguminous plant that is commonly used as feed for beef cattle and offers multiple benefits for livestock is *gamal* (*Gliricidia sepium*). Gamal is highly promising as a livestock feed because of its advantages, such as its ability to grow rapidly in dry areas and withstand drought conditions (Tahuk & Gerson, 2021). Given the natural biophysical conditions in dry regions and degraded land, gamal is a suitable plant for development. The nutritional content of gamal leaves includes crude protein (CP) at 25.7%, crude fiber (CF) at 13.3%, and ash at 8.4% (Mayasari et al., 2012). Meanwhile, Purnomo et al. (2024) reported that gamal leaves contain 20.49% CP, 3.44% crude fat (CFa), 12.93% CF, 5.92% ash, and 57.22% nitrogen-free extract (NFE). Gamal leaves also contain anti-nutritional compounds such as HCN (hydrogen cyanide), tannins, saponins, coumarin, nitrates, and phenolic acids, as well as high levels of crude fiber (Apriani et al., 2019). Therefore, the use of gamal leaves as livestock feed must be limited, as hydrolyzed tannins can cause livestock toxicity by disrupting microbial activity. Sikone and Bira (2016) stated that before being fed to livestock, gamal forage should be aired or dried to reduce its characteristic coumarin odor, which is one of the main factors contributing to its low palatability.

In general, livestock feeding practices involve providing forage and concentrate separately. This method may lead to feed selection by the animals, which can negatively affect rumen conditions, including pH fluctuations and microbial composition. Therefore, it is necessary to formulate a complete feed. A complete feed is a mixed ration consisting of forage as a fiber source and concentrate as a protein source, formulated according to the nutritional requirements of the livestock. Complete feed is an alternative feeding method aimed at improving the nutritional value of the feed. The advantages of using complete feed include increased feeding efficiency, enhanced palatability of forage when mixed with concentrate, and overall improved feed intake. Additionally, it helps prevent selective feeding, maintains rumen stability, reduces feed costs, has longer shelf life, and is easier to handle in terms of transportation and storage (Beigh, et al., 2017).

Feed quality can be determined based on the nutrient content of feed ingredients using proximate analysis and Van Soest analysis to identify fiber fraction components. Biologically, feed quality can also be assessed through *in vitro* tests, which simulate rumen conditions in the laboratory. These tests include measurements of digestibility, gas production, dry matter (DM) and organic matter (OM) degradation, ammonia (NH₃) concentration, and microbial protein synthesis. Nutrient fermentation by rumen microorganisms produces various end products. Gas production is a by-product of organic matter (OM) fermentation in the rumen. The microbial fermentation of OM in the rumen generates volatile fatty acids (VFA), which serve as a major energy source for ruminants, while the protein in the rumen is fermented into ammonia (NH₃), which is then utilized for microbial protein synthesis. All of these processes occur optimally under a pH condition that supports rumen microbial activity, with the ideal pH being approximately 6.8 (Purbowati et al., 2014).

This study was conducted to evaluate the nutritional value of complete feed in order to obtain an optimal feed that can improve nutrient content, digestibility, ammonia (NH₃) concentration, and microbial protein synthesis.

MATERIALS AND METHODS

The method used in this study is an experimental field method for feed preparation, employing a Completely Randomized Design (CRD) to analyze the nutrient content, crude fiber fraction components, and *in vitro* variable observations. The experiment consisted of four treatments and four replications. The feed treatments were as follows:

P₀: Corn straw 50% + concentrate 50% + *Gliricidia sepium* 0%

P₁: Corn straw 50% + concentrate 40% + *Gliricidia sepium* 10%

P₂: Corn straw 50% + concentrate 30% + *Gliricidia sepium* 20%

P₃: Corn straw 50% + concentrate 20% + *Gliricidia sepium* 30%

The data obtained were analyzed using statistical methods, specifically the analysis of variance (ANOVA) with a Completely Randomized Design (CRD) to determine the effects of the treatments. Subsequently, Duncan's multiple range test was applied at a 5% significance level to identify differences between treatments. The statistical model used is as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}$$



Explanation:

- Y_{ij} = Observation result
 μ = General mean value
 α_i = Effect of the i-th treatment
 β_j = Effect of the j-th group
 ϵ_{ij} = Experimental error for the i-th treatment and j-th group
i = 1, 2, 3, 4 (Treatments)
j = 1, 2, 3, 4 (Groups)

RESULTS AND DISCUSSION

Nutrient Content and Fiber Fractions

Table 1. Nutrient Composition of Feed Ingredients and Experimental Diets

<i>Jenis Pakan</i>	<i>PK</i> (% BK)	<i>SK</i> (% BK)	<i>LK</i> (% BK)	<i>Abu</i> (% BK)	<i>BETN</i>	<i>NDF %</i>	<i>ADF</i> %
Feed Ingredients							
Corn straw	8,48	22,37	0,76	11,96	56,43	54,04	44,09
Gliricidia leaves	24,81	20,20	4,67	10,64	39,68	40,10	35,56
Concentrate	13,84	13,73	5,04	8,35	59,04	36,98	27,73
Experimental diets							
P ₀	11,76	18,84	3,37	9,77	56,26	41,49	25,21
P ₁	12,28	19,75	3,97	9,33	54,67	39,82	24,36
P ₂	13,01	20,72	3,06	8,94	54,27	30,04	22,04
P ₃	14,05	22,94	2,60	8,44	51,97	26,08	21,60

Source: Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Brawijaya University, 2024.

The results of the study in Table 1 show that the crude protein (CP) content of the feed treatments tended to increase as the proportion of concentrate was substituted with gamal leaves. The CP content in the feed treatments ranged from 11.76% to 14.05%. The differences in CP content in this study were due to the variation in the CP content of the feed ingredients, with gamal leaves containing a higher CP level compared to concentrate. Therefore, as the ratio of concentrate substitution with gamal leaves increased in the treatments (P₀-P₃), the CP content also increased. The substitution of concentrate with gamal leaf powder in complete feed based on corn straw was found to increase the crude fiber (CF) content in the feed treatments compared to the control feed. The CF content in the feed treatments ranged from 18.84% to 22.94%. The differences in CF values were due to the addition of fiber from gamal leaves. As the proportion of concentrate was substituted with gamal leaves, the CF content also increased, as the CF content of gamal leaves (20.20%) is higher than that of concentrate (13.73%).

The crude fat (CF) content that is too high in feed (above 5%) can negatively affect the digestibility of crude fiber (CF) in the rumen (Wina and Susana, 2013). In this study, the CF content in the feed treatments tended to decrease compared to the control feed without the addition of gamal leaves. All treatments had CF values below 5%, which are considered normal. When CF content is high, it can inhibit the activity of microbial enzymes involved in fiber digestion. The feed treatments P₁, P₂, and P₃, with the addition of gamal leaf powder, showed a decline in CF content compared to the control feed. The average nitrogen-free extract (NFE) content in the feed treatments P₀, P₁, P₂, and P₃ were 56.26%, 54.67%, 54.27%, and 51.97%, respectively.

The results of this study showed that the NDF (Neutral Detergent Fiber) content in the feed for treatment P₃ was the lowest at 26.08%, while the highest value was found in P₀ at 41.49%. Regarding ADF (Acid Detergent Fiber), the highest value was observed in P₀ at 25.21%, while the lowest value was found in P₃ at 21.60%. This is because the NDF and ADF content in gamal leaves is lower compared to concentrate, so as the concentrate is substituted with gamal leaves, the NDF and ADF content decreases.

Another study conducted by Hasan et al. (2020) used complete feed made from local ingredients with different CP (crude protein) contents, which were 9.10% and 11%, for beef cattle. The results of this study showed that the NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber) content in the feed ranged from 53% to 60% for NDF and 33% to 40% for ADF. Stergiadis

et al. (2015) stated that the NDF and ADF content in the feed can be limiting factors for feed digestibility in the rumen. According to Anggorodi (1979), the higher the crude fiber content in a feed ingredient, the thicker the cell wall will be, making it more resistant to fiber-digesting microorganisms, which results in lower digestibility of that feed. On the other hand, feed ingredients with lower fiber content are generally more digestible, as the cell walls are thinner and more easily penetrated by microbes. Riswandi et al. (2016) stated that the lower the NDF and ADF fractions, the higher the feed digestibility. The decrease in NDF values is caused by an increase in lignin content, which results in a reduction of hemicellulose content.

Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD)

Based on the analysis of variance, the results showed that the treatments had a highly significant effect ($P < 0.01$) on the digestibility of organic matter (DOM) and digestibility of dry matter (DDM). This occurred because the feed ingredients in each treatment supported the activity of rumen microbes in digesting the feed. These measurements indicate that substituting concentrate with gamal leaves can affect the digestibility of the feed. Treatment P3 had the highest DOM and DDM values at 66.34% and 71.43%, respectively, compared to the other treatments. This is due to the higher DOM and DDM values of gamal leaves (71.72% and 76.68%) compared to concentrate (67.92% and 73.33%). Therefore, as the level of concentrate substitution with gamal leaves increases, the DOM and DDM also increase. The results of this study are supported by Ifani et al. (2023), who stated that the substitution of concentrate with gamal leaves can increase the digestibility of organic matter (DOM) and digestibility of dry matter (DDM). In the treatment with 40% rice straw, 0% concentrate, and 60% gamal leaves, the average DOM was 86.43% and DDM was 94.57%. Based on the CP (crude protein) values in Table 1, it shows a positive correlation between CP and DDM, indicating that gamal leaves are more easily degraded in the rumen compared to concentrate, based on rumen fermentation products. This is consistent with the study by Prayuwidayati and Muhtarudin (2006), which found that DDM is closely related to its chemical composition, particularly its CP content. Arta et al. (2020) mentioned that the protein content in gamal leaves is easily degraded in the rumen. Nurhayati et al. (2020) also reported that the protein content in feed ingredients can affect their digestibility. Makmur et al. (2020) reported that the digestibility of organic matter is positively correlated with crude protein content (CP), as protein is a nutrient that is easily degraded by rumen microbes.

Table 2. Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD)

Pakan	Digestibility Nutrien (%)		
	BK	BO	TDN
Feed Ingredients			
Corn straw	41,81	48,50	44,48
Concentrate	67,92	73,33	68,8
Gliricidia leaves	71,72	76,68	73,55
Experimental diets			
P ₀	54,56 ^a	58,94 ^a	55,93 ^a
P ₁	56,05 ^{ab}	63,79 ^{ab}	59,40 ^{ab}
P ₂	60,08 ^{ab}	66,61 ^{ab}	62,99 ^{ab}
P ₃	66,34 ^{ab}	71,43 ^b	68,08 ^c

Note: Different letters (b-c) in the same column indicate that the treatments have a highly significant effect ($P < 0.01$) on DMD and OMD.

Gas production measurement results from the Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Brawijaya University (2024).

Source: TDN: in vitro OMD digestibility $\times$ 1.05 (Ibrahim, 1998).

Table 2 shows that the average value of organic matter digestibility (OMD) indicates a positive correlation with dry matter digestibility (DMD), meaning that as DMD increases, OMD also tends to increase. Wahyuni et al. (2014) stated that DMD is closely related to OMD because organic matter is a component of dry matter. Therefore, an increase in DMD results in an increase in OMD,

and vice versa. The OMD values were higher than the DMD values, which is likely due to the fact that the nutrients in the complete feed are more readily digestible in greater quantities.

McDonald et al. (1988) explained that digestibility is influenced by several factors, including the composition of feed ingredients, the ratio between feed components, feed processing methods, enzyme supplementation, the type of livestock, and the level of feed intake.

Gas Production

Table 3. Gas Production Values at 2, 4, 8, 16, 24, 36, and 48 Hours of Incubation

Gas Production Value (ml/500mg BK)							
Perlakuan	2	4	8	16	24	36	48
Feed Ingredients							
Corn straw	6,15	10,68	21,91	40,61	67,06	82,02	92,44
Concentrate	4,36	9,27	20,45	33,00	60,81	78,26	88,89
Gliricidia leaves	4,85	9,69	21,01	35,28	62,21	76,76	87,00
Experimental diets							
P ₀	3,23 ^a	13,23 ^{ab}	28,58 ^{ab}	44,21 ^d	71,44 ^c	87,88 ^d	91,12 ^{bc}
P ₁	3,84 ^{ab}	11,24 ^{ab}	27,71 ^{ab}	41,43 ^c	69,41 ^c	82,04 ^a	90,27 ^{bc}
P ₂	4,70 ^c	10,22 ^{ab}	27,35 ^{ab}	37,85 ^b	65,20 ^{ab}	82,61 ^{bc}	89,51 ^b
P ₃	4,63 ^c	10,08 ^a	25,89 ^a	31,89 ^a	63,23 ^a	79,31 ^a	86,94 ^a

Note: Different letters (a-b) in the same column indicate that the treatments have a highly significant effect ($P < 0.01$) on gas production values at 48 hours of incubation.

Gas production measurement results from the Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Brawijaya University (2024).

From Table 3 above, it can be seen that corn straw had the highest gas production after 48 hours of incubation compared to concentrate and gamal leaf meal. This occurred because the organic matter (OM) content in corn straw is more readily available for gas production. In contrast, the concentrate and gamal leaf meal contain carbohydrates and tannins that can bind to proteins, thereby inhibiting the rate of gas production.

The results of feed treatment measurements showed that the substitution of concentrate with gamal leaf meal in complete feed based on corn straw had a highly significant effect ($P < 0.01$) on gas production from the 2nd hour to the 48th hour compared to the control feed. Feed treatments with concentrate substituted by gamal leaf meal (P₁, P₂, and P₃) showed a decrease in gas production compared to the control feed (P₀). This is likely due to the addition of feed ingredients with high protein content and the presence of tannins, which protect proteins from ruminal degradation, resulting in less feed being degraded in the rumen. In addition, the feed treatments with gamal leaf substitution had higher crude fiber (CF) content and lower nitrogen-free extract (NFE) content (as shown in Table 2), but still exhibited high digestibility. This led to lower total gas production compared to the control feed without gamal leaf substitution. Kim et al. (2013) stated that substrates with high levels of easily fermentable carbohydrates are highly beneficial for rumen microbes in producing gas. This is supported by Firsoni and Lisanti (2017), who reported that energy-based feed ingredients result in higher gas production compared to protein-based feed ingredients. Therefore, the decrease in gas production in the treatments where concentrate was substituted with gamal leaves can be attributed to this difference in nutrient composition.

The average total gas production from the four feed treatments incubated for 48 hours ranged from 86.94 to 91.12 ml/g (Table 5). These gas production values are considered normal. Similar findings were reported by Nurjanah et al. (2016), who stated that gas production from complete silage feed based on sugarcane tops and several types of legumes incubated for 48 hours ranged from 76.73 to 89.57 ml/500 mg. The substitution of gamal leaf meal at levels of 10%, 20%, and 30% resulted in a decrease in gas production. This is consistent with the study conducted by Akbar (2021), which found that the addition of 20% calliandra leaf meal

and 30 g/kg of myristic acid reduced gas production. A study by Muchlas et al. (2020), using mimosa powder and myristic acid at levels of 20, 30, and 40 g/kg dry matter in complete feed based on corn straw, also showed that the addition of tannin- and myristic acid-containing plants reduced in vitro gas production. This reduction is attributed to the binding of tannins to proteins in the rumen, making high-protein feeds less degradable by rumen microbes, with the potential to improve post-rumen digestibility. Tannins form hydrogen bonds with proteins, which are sensitive to pH changes. These tannin-protein bonds remain stable at pH levels between 4 and 7 in the rumen, but dissociate under extreme pH conditions—below pH 3 in the abomasum and above pH 7 in the intestines (Sasongko et al., 2010).

Gas production during the initial phase of incubation increased slowly, followed by a rapid rise between 8 and 24 hours, and then began to decline from 36 to 48 hours. This trend likely occurred because the organic matter (OM) in all feed treatments was still sufficiently available for fermentation between 2 and 24 hours of incubation, but began to deplete during the 36 to 48-hour period. Gas production typically peaks within the first 24 hours of incubation, after which the rate of gas production declines. This pattern is consistent across all types of feed, as the longer the feed remains in the rumen, the more limited the availability of OM becomes for microbial fermentation and gas production (Ella et al., 1997). This statement is supported by Wahyuni et al. (2014), who explained that gas production decreases as the OM content in the feed diminishes, resulting in reduced microbial gas output. Measurement results also showed that substituting concentrate with gamal leaf meal at levels of 10%, 20%, and 30% led to a reduction in gas production.

The substitution of gamal leaf meal at inclusion levels of 10%, 20%, and 30% was found to reduce gas production. This finding is in line with the study by Akbar (2021), who reported that the addition of 20% calliandra leaf meal and 30 g/kg of myristic acid reduced gas production. Similarly, a study conducted by Muchlas et al. (2020) using mimosa powder and myristic acid at levels of 20, 30, and 40 g/kg of dry matter in complete feed based on corn straw showed that the inclusion of tannin- and myristic acid-containing plants in the feed could reduce in vitro gas production. This reduction is attributed to the formation of tannin-protein complexes in the rumen, which protect dietary protein from microbial degradation. This protection is expected to enhance post-ruminal digestibility. Tannins bind to protein via hydrogen bonds that are sensitive to pH changes. These bonds are stable within the ruminal pH range of 4–7. However, under extreme pH conditions—below pH 3 in the abomasum and above pH 7 in the intestines—the tannin-protein bonds dissociate (Sasongko, et al., 2010).

Measurement of NH₃ Concentration

The highest NH₃ concentration measured from the feed ingredients was found in gamal leaf meal compared to concentrate and corn straw. This is because gamal leaf meal is fermented by rumen microbes into ammonia, whereas concentrate and corn straw primarily serve as energy sources.

Table 4. Average NH₃ Concentration (mg/L)

<i>Jenis pakan</i>	<i>NH₃ (mg/L)</i>
Feed Ingredients	
Corn straw	63,65
Concentrate	75,14
Gliricidia leaves	80,35
Experimental diets	
P0	70,25 ^a
P1	70,90 ^a
P2	71,74 ^a
P3	77,91 ^b

Note: Different letters (a-b) in the same column indicate that the treatments have a highly significant effect ($P < 0.01$) on NH₃ concentration.

Measurement results from the Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Brawijaya University (2024).

The analysis of variance showed that the feed treatments had a highly significant effect ($P > 0.01$) on NH₃ concentration. The NH₃ concentrations varied among the different treatments. The highest NH₃ concentration was observed in the P3 treatment



compared to P₀, P₁, and P₂. This is due to the higher NH₃ concentration in gamal leaf meal compared to the concentrate. Thus, as the level of concentrate substitution with gamal leaf meal increased, the NH₃ concentration also increased. According to Haryanto and Djajanegara (1993), NH₃ concentration is influenced by several factors, including the type of feed, nitrogen solubility source, protein degradation rate, nitrogen concentration in the ration, and others.

The NH₃ concentration values across all treatments in this study were considered high, ranging from 70.25 to 77.91 mg/L. According to Wanapat and Pimpa (1999), the normal NH₃ concentration ranges from 150 to 300 mg/L, which supports microbial protein synthesis and feed digestibility. However, Boniface et al. (1986) reported that NH₃ concentrations in ruminants generally range between 50 and 200 mg/L, which is essential for rumen microbial activity. This is also supported by Susanti et al. (2002), who stated that the optimal NH₃ concentration in rumen fluid is between 50 and 80 mg/L. A deficiency in nitrogen sources can reduce microbial production per unit of digestible carbohydrate. On the other hand, excess NH₃ will be absorbed through the rumen wall and transported to the liver for urea synthesis (Suparwi et al., 2017).

Ammonia (NH₃) concentration in the rumen is utilized by rumen microbes for microbial protein synthesis. However, if NH₃ concentration becomes too high without sufficient energy sources—specifically volatile fatty acids (VFAs) as carbon skeletons—it will be excreted in the form of urea through urine. According to McDonald et al. (1995), accelerated NH₃ absorption prevents toxicity in livestock, as NH₃ is transported via the bloodstream to the liver where it is converted into urea. This urea can then be recycled through saliva or excreted via the kidneys. However, in this study, NH₃ absorption through the rumen wall did not occur due to the use of sealed syringes during incubation. As a result, the longer the incubation time, the higher the NH₃ concentration observed.

Microbial Protein Synthesis

Tabel 5. Microbial Protein Synthesis

Perlakuan	MPS (g N mikroba/kg BOTR)
Feed Ingredients	
Corn straw	34,535
Concentrate	48,320
Gliricidia leaves	50,950
Experimental diets	
P ₀	40,678
P ₁	41,273
P ₂	42,810
P ₃	44,024

Note: Results from the analysis at the Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Brawijaya University (2024).

Based on the analysis of variance, it was found that the treatments had a highly significant effect ($P < 0.01$) on microbial protein synthesis (MPS) after 48 hours of incubation.

The MPS value for the treatment P₃ was the highest, at 44.024 g N microbial/kg BOTR, compared to the treatments P₀, P₁, and P₂, which were 40.678 N microbial/kg BOTR, 41.273 N microbial/kg BOTR, and 42.810 N microbial/kg BOTR, respectively. The higher average MPS in P₃ is due to its ability to utilize organic matter (OM) as a carbon skeleton and nitrogen (N) more efficiently compared to the other treatments.

Pathak (2008) stated that factors influencing microbial protein synthesis include the availability and synchronization of energy and nitrogen compounds in the rumen. Nitrogen sources should also include amino acids and peptides in addition to non-protein nitrogen (NPN). Waldi et al. (2017) mentioned that proteins are synthesized from five main elements: C, H, O, N, and S, which enable rumen microbes to synthesize amino acids for their cell structure from carbohydrates, NPN, and both organic and inorganic sulfur. The process of microbial protein synthesis is optimized when energy and raw materials are available in balanced and adequate amounts. Ani et al. (2015) stated that the growth of rumen microbes requires ammonia as a source of carbon skeleton and energy. This ammonia product is utilized again by rumen microbes depending on its availability in the rumen. Rumen microbes reuse ammonia formed to build their cellular structure. In addition to ammonia, peptides and amino acids are also used in microbial



protein synthesis. Karsli and Russell (2001) indicated that the average efficiency of microbial protein synthesis is 14.8, within a range of 7 to 27.9 g microbial crude protein (MCP) per 100 g of dry matter digested in the rumen. Sinclair et al. (1995) stated that a feed mixture of roughage and concentrate has a higher microbial protein synthesis efficiency than feeding roughage alone.

NRC (1996) in Karsli and Russell (2001) stated that the efficiency of microbial protein synthesis is low in animals fed high-concentrate diets, as this disrupts the stability of rumen pH (causing a decrease in pH), while animals fed low-quality forage also show reduced microbial protein synthesis efficiency due to slow carbohydrate digestion. In addition, Beever and Cottrill (1994) stated that the ratio of nitrogen (N) degradation to organic matter (OM) in the rumen varies significantly. Microbial N values ranging from 10 to 70 g N microbial/Kg DM are considered low, while the optimal value for microbial protein synthesis is 30 to 40 g N microbial/Kg DM. This indicates that in the rumen, there are periods when microbes lack sufficient N for protein synthesis and times when they have an excess of N for protein synthesis. Excessive N supply is related to the availability of energy in the rumen, which can disrupt microbial metabolism and efficiency. Huber and Kung (1961) stated that the limiting factor for the utilization of non-protein nitrogen (NPN) is the availability of energy sources. Microbial N synthesis will be high when non-structural carbohydrates available to ruminants are combined with available protein, and microbial N synthesis will be low when non-structural carbohydrates are not balanced with the availability of protein.

CONCLUSIONS

Substituting concentrate with gamal leaf meal (*Gliricidia sepium*) as a protein source in a basal diet based on corn straw in an in vitro setting: The substitution of concentrate with gamal leaf meal at different levels in the complete diet based on corn straw in vitro was able to increase the levels of crude protein (CP), organic matter digestibility (KcBO), ammonia (NH₃), and microbial protein synthesis (SPM), which can meet the nutritional needs of beef cattle. The optimal treatment was achieved with the P3 formulation, consisting of 50% corn straw + 20% concentrate + 30% gamal leaf meal. This conclusion emphasizes the effectiveness of using gamal leaf meal in improving feed quality and optimizing the nutritional intake of cattle, particularly beef cattle, through its impact on various feed components.

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Cite this Article: Dama, A.R., Hartutik, Marjuki (2025). The Effect of Substituting Concentrate with Different Levels of *Gliricidia sepium* Leaves in a Complete Feed on Nutrient Content, Crude Fiber Fractions, Digestibility, and In Vitro Fermentation Products. International Journal of Current Science Research and Review, 8(7), pp. 3203-3212. DOI: <https://doi.org/10.47191/ijcsrr/V8-i7-06>