EUROPEAN FRONTIERS IN CURRENT SCIENCE AND RESEARCH

EVALUATING GLIRICIDIA SEPIUM LEAF INCLUSION AS A CONCENTRATE REPLACEMENT IN COMPLETE RATIONS: EFFECTS ON NUTRIENT PROFILE, FIBER COMPONENTS, DIGESTIBILITY, AND IN VITRO RUMEN FERMENTATION

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V0LUME01 ISSUE01 (2024)
Published Date: 09 December 2024 // Page no.: - 07-19

ABSTRACT

Imagine a world where livestock farming is more affordable and kinder to our planet. This study dives into how we can make that happen by using Gliricidia sepium (also known as Gamal) leaves as a substitute for expensive traditional concentrates in animal feed. We set up an experiment with four different feed mixes, gradually increasing the amount of Gliricidia sepium from 0% (our control) to 10%, 20%, and finally 30%. Our findings showed that adding more Gliricidia sepium significantly boosted the protein content of the feed and surprisingly, even lowered the tough-to-digest fiber components (NDF and ADF), while slightly reducing easily digestible carbohydrates (NFE).

When we looked at how well the feed was digested in a lab setting (in vitro), we found that moderate amounts of Gliricidia sepium (10% and 20%) kept digestibility levels strong, even slightly improving them. However, pushing it to 30% actually caused a noticeable drop in digestibility. Similarly, the rumen microbes, which are essential for digestion, were most active and efficient at the 10% Gliricidia sepium level, producing more gas, ammonia, and beneficial fatty acids, and synthesizing more microbial protein. But at 30%, their activity dipped.

What does this all mean? It suggests that Gliricidia sepium leaves can be a fantastic, cost-effective, and sustainable alternative to traditional concentrates for ruminants. The sweet spot seems to be around 10-20% inclusion, where we get the best nutritional benefits and efficient digestion without running into problems from natural compounds in the leaves. This research offers exciting possibilities for farmers looking to cut costs and improve their animals' diets in a sustainable way.

Keywords: Gliricidia sepium, complete feed, nutrient content, crude fiber, digestibility, in vitro fermentation, ruminants.

INTRODUCTION

Have you ever thought about where your meat and milk come from? It's a big question, and feeding the animals that provide these products is a huge part of the answer. Around the world, the demand for animal protein is constantly growing, driven by a rising global population and improving living standards [8, 33]. In places like Indonesia, livestock farming is absolutely vital for putting food on tables [33].

But here's a challenge: many farmers, especially in rural areas, still rely on traditional methods. They often feed their cattle natural grasses, which are great when plentiful, but during dry seasons, both the quality and quantity of this forage can drop significantly [26]. This leaves animals with nutritional gaps, and farmers often have to buy expensive concentrate feeds to make up the difference [26, 42].

These traditional concentrates, often made from grains and industrial by-products, come with a hefty price tag [2]. This puts a real financial strain on farmers, making it tough to give their animals the best nutrition and limiting how much they can produce [2]. So, there's a clear and urgent need to find new, more affordable, and sustainable ways to feed our livestock. We need alternatives that can either replace or at least reduce our reliance on these costly concentrates.

That's where leguminous forages come into play – they're like nature's protein powerhouses! These plants are packed with protein and have the amazing ability to pull nitrogen from the air, which actually enriches the soil where they grow [34]. One such superstar is *Gliricidia sepium*, commonly known as Gamal. This tree is a true survivor; it grows quickly, handles droughts like a champ, and thrives in tropical and subtropical regions [6, 21, 44]. Its toughness

makes it a fantastic, sustainable feed option, especially in dry or degraded areas [36, 44]. What's more, *Gliricidia sepium* leaves are incredibly nutritious, boasting high levels of crude protein (CP) and essential minerals, with CP content often ranging from 20.49% to 25.7% [21, 32].

Now, let's talk about "complete feeds." This is a smart way to feed animals, where we mix all the necessary ingredients – both roughage (for fiber) and concentrate (for protein and energy) – into one perfectly balanced meal [10]. The benefits are huge: animals eat more efficiently, even less palatable forages become tastier when mixed in, and they stop picking out only their favorite bits, ensuring a balanced diet [10]. Plus, complete feeds help keep the rumen (a cow's main stomach) stable, can lower overall feed costs, last longer on the shelf, and are easier to handle and store [10].

However, even a promising ingredient like *Gliricidia sepium* has its quirks. Its leaves contain natural compounds like hydrogen cyanide (HCN), tannins, saponins, and others [6]. Tannins, in particular, can be a bit tricky. They can bind with proteins and carbohydrates, making them harder for the rumen microbes to digest [4, 20, 35]. Too many tannins can even upset the microbes and reduce how much feed an animal eats [47]. But here's the fascinating part: tannins are a bit like a double-edged sword. At low to moderate levels, they can actually be beneficial! They can "protect" some dietary protein from being broken down too quickly in the rumen, allowing it to bypass the rumen and be absorbed later in the small intestine, which is a more efficient way for the animal to get its protein [9, 50]. So, the trick is to find that perfect balance – enough *Gliricidia sepium* to get the benefits, but not so much that it causes problems.

While we know a good deal about *Gliricidia sepium* as a feed [6, 21, 36], we still need more detailed studies on exactly how much to include in complete feeds and what that does to nutrient content, fiber, digestibility, and the fermentation process in the rumen. This study aims to shed light on these very questions. Specifically, we wanted to:

1. See how replacing concentrate with different amounts of *Gliricidia sepium* leaves changes the nutrient makeup (like protein, fiber, and fat) of the complete feed.
2. Figure out how well these new feed mixes are digested in a laboratory simulation of the rumen.
3. Understand the impact of *Gliricidia sepium* on the actual fermentation process in the rumen, looking at things like gas production, ammonia levels, and the production of beneficial fatty acids and microbial protein.
4. Ultimately, pinpoint the ideal amount of *Gliricidia sepium* leaves to include in complete feeds to get the best nutritional bang for our buck and the most efficient digestion.

We believe the insights from this research will be incredibly valuable, offering practical advice to farmers and contributing to more affordable, sustainable, and nutritious feeding strategies for ruminant livestock. This, in turn, can help boost the productivity and economic well-being of the entire livestock sector.

MATERIALS AND METHODS

Where and How Long We Worked

Our study took place at the Laboratory of Animal Nutrition and Feed at Brawijaya University in Indonesia. We dedicated about three months to this project, meticulously preparing the feed, running chemical analyses, and conducting the in vitro (lab-based) fermentation experiments. This timeframe allowed us to gather all our data carefully and thoroughly.

What Went Into the Feed and How We Prepared It

We used three main ingredients for our feed mixes: corn straw, a standard commercial concentrate, and, of course, *Gliricidia sepium* leaves.

- **Corn Straw**: We collected fresh corn straw from local farms, chopped it into small pieces (about 2-3 cm long), and then air-dried it until it had a consistent, low moisture content. After drying, we ground it into a meal. Corn straw is a common and readily available agricultural leftover that provides essential fiber for ruminants [25].
- **Commercial Concentrate**: We bought a typical commercial concentrate feed, the kind beef cattle farmers in the area often use. Its ingredients were pretty standard, providing the necessary protein and energy [14].
- **Gliricidia sepium Leaves**: We carefully harvested *Gliricidia sepium* leaves from mature trees, making sure to separate them from the stems. Then, we air-dried them in the shade. This drying process helps keep their nutrients intact and reduces natural compounds like coumarin, which can sometimes make the feed less appealing to animals [36, 37]. Once dried, we ground the leaves into a fine meal.

Our Experimental Setup: The Different Feed Mixes

To really understand the effects of *Gliricidia sepium*, we used a scientific approach called a Completely Randomized Design (CRD). This means we had four different feed mixes (treatments), and we repeated each mix four times to ensure our results were reliable. We kept the amount of corn straw constant at 50% in all mixes, focusing on how different levels of *Gliricidia sepium* replaced the concentrate. Here's how we formulated our experimental diets:

- **P0 (Our Control Group)**: This mix had 50% Corn Straw + 50% Commercial Concentrate + 0% *Gliricidia sepium* leaves. This was our baseline to compare everything against.
- **P1**: This mix had 50% Corn Straw + 40% Commercial

Concentrate + 10% *Gliricidia sepium* leaves. We started with a small amount of *Gliricidia sepium* here.

- **P2**: This mix had 50% Corn Straw + 30% Commercial Concentrate + 20% *Gliricidia sepium* leaves. Doubled the *Gliricidia sepium* here.
- **P3**: This mix had 50% Corn Straw + 20% Commercial Concentrate + 30% *Gliricidia sepium* leaves. This was our highest *Gliricidia sepium* inclusion level.

After measuring out all the ingredients, we mixed them thoroughly for each treatment to make sure every bite of feed was consistent [10]. Then, we stored these mixes in airtight containers to keep them fresh and prevent any nutrient loss until we were ready for analysis.

What We Looked For in the Feed (Chemical Analysis)

Before we even started the in vitro digestion part, we meticulously analyzed the nutritional content of all our ingredients (corn straw, concentrate, *Gliricidia sepium* leaves) and each of our four complete feed mixes. We followed standard laboratory procedures for this:

- **Proximate Analysis**: This is like a general nutritional breakdown. We measured:
  - **Dry Matter (DM)**: How much solid material is left after all the water is removed. We did this by drying samples in an oven [23, 24].
  - **Crude Protein (CP)**: The total protein content. We used the Kjeldahl method, which measures nitrogen and converts it to protein [23, 24].
  - **Crude Fiber (CF)**: The indigestible, fibrous parts of the feed. We used a special acid and alkali digestion process [23, 24].
  - **Ether Extract (EE)**: The fat content. We extracted the lipids using diethyl ether [23, 24].
  - **Ash**: The mineral content, determined by burning the sample in a furnace [23, 24].
  - **Nitrogen-Free Extract (NFE)**: This is the readily digestible carbohydrate portion, calculated by subtracting all the other components from 100% [23, 24].
- **Fiber Fraction Analysis (Van Soest Method)**: This gives us a more detailed look at the fiber, which is super important for ruminants:
  - **Neutral Detergent Fiber (NDF)**: This tells us the total cell wall components, including hemicellulose, cellulose, and lignin. High NDF usually means animals can't eat as much [7, 22].
  - **Acid Detergent Fiber (ADF)**: This specifically measures cellulose and lignin. High ADF generally means lower digestibility [7, 22].
  - **Acid Detergent Lignin (ADL)**: This is the truly indigestible part of the fiber, which significantly limits how much of the fiber can be digested [7, 22].

We ran all these tests three times for each sample to make sure our results were super accurate and could be reproduced.

Simulating Digestion in the Lab (In Vitro Fermentation)

To understand how our feed mixes would behave inside a cow's rumen, we used a widely accepted lab technique called in vitro gas production. It's basically a mini-rumen simulation [12, 13, 19].

- **Collecting Rumen Fluid**: We carefully collected rumen fluid from three healthy beef cattle that had a special opening (fistula) to their rumen. These animals were fed a standard diet, so their rumen microbes were typical and healthy. We collected the fluid before their morning meal, strained it to remove any feed particles, and quickly transported it to the lab in warm, oxygen-free containers [5, 19]. Keeping it oxygen-free is crucial because rumen microbes are anaerobic, meaning they can't survive with oxygen.
- **Setting Up the Incubation**:
  - We precisely weighed out about 0.5 grams of each feed mix (P0, P1, P2, P3) into 100 mL glass bottles. We did this four times for each mix.
  - Then, we prepared a special liquid mixture: rumen fluid combined with a buffer solution (two parts buffer to one part rumen fluid). This buffer mimics the rumen environment, providing essential minerals and keeping the pH stable [5].
  - While continuously flushing the bottles with carbon dioxide (to keep oxygen out), we added 30 mL of our rumen fluid-buffer mixture to each bottle containing the feed sample [5, 12].
  - We also prepared "blank" bottles with just rumen fluid and buffer (no feed) to account for any gas produced by the microbes themselves, not from the feed.
  - Finally, we sealed the bottles tightly and placed them in a water bath shaker at 39°C (the normal temperature inside a cow's rumen) for 24 hours [19].
- **Measuring Gas Production**:
  - At specific times (2, 4, 8, 16, 24, 36, and 48 hours after starting the incubation), we measured the gas produced in each bottle.
  - We used a special pressure sensor hooked up to a digital display to read the gas pressure in the bottles [5, 12]. We then converted these pressure readings into actual gas volumes using a pre-established curve.
  - After each measurement, we released the gas from the bottles to prevent too much pressure from building up.
- **Analyzing What Was Left (Post-Incubation)**: After 48 hours, we stopped the fermentation by putting the

bottles in an ice bath. Then, we analyzed the contents for several important factors:

- **In vitro Dry Matter Digestibility (IVDMD) and In vitro Organic Matter Digestibility (IVOMD)**: We filtered the fermented liquid, dried the leftover solids, and weighed them to see how much dry matter wasn't digested. We calculated IVDMD as the percentage of dry matter that disappeared. For IVOMD, we also burned the dried leftovers to measure the organic matter that was digested [17, 28].
- **Ammonia-Nitrogen (NH3-N) Concentration**: We spun down some of the fermented liquid in a centrifuge and then measured the ammonia-nitrogen. This tells us how much protein was broken down and how much nitrogen was available for the microbes to use [11, 16, 18, 30, 49].
- **Total Volatile Fatty Acid (VFA) Concentration**: We acidified another portion of the liquid and used a gas chromatograph to measure the total volatile fatty acids. VFAs are the main energy source for cows, produced when microbes break down carbohydrates [16, 41].
- **Microbial Protein Synthesis (MPS)**: We estimated how much new microbial protein was made by measuring specific compounds (purine derivatives) that are unique to microbial cells [16, 18, 38]. This gives us an idea of how efficiently the microbes are using nitrogen to grow [18, 30, 48].

Making Sense of the Numbers (Statistical Analysis)

Once we had all our data, we put it through rigorous statistical analysis using specialized software. We used a technique called one-way Analysis of Variance (ANOVA) to see if the different levels of *Gliricidia sepium* had a significant impact on any of our measurements. If we found a significant difference (meaning the results weren't just due to chance, typically $P < 0.05$), we then used Duncan's Multiple Range Test (DMRT) to pinpoint exactly which feed mixes were different from each other. Our statistical model looked like this:

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

In plain language:

- Y_{ij} represents the specific measurement we got for a particular feed mix and repetition.
- mu is the overall average value across all measurements.
- $alpha_i$ shows the effect of each different feed mix (P0, P1, P2, P3).
- $beta_j$ accounts for any variation between our repetitions.
- $epsilon_{ij}$ is the random experimental error.

This careful statistical approach helped us be confident in the conclusions we drew from our experiment.

RESULTS

What We Found in the Feed: Nutrients and Fiber

First, let's look at the basic building blocks of our feed mixes. Table 1 shows you the nutritional content of each individual ingredient (corn straw, concentrate, and *Gliricidia sepium* leaves) and then how those nutrients came together in our four experimental diets (P0, P1, P2, P3). This table is key to understanding how our choices impacted the final feed quality.

Table 1: Nutrient Composition of Feed Ingredients and Experimental Diets (DM Basis, %)

Feed Component	Crude Protein (CP)	Crude Fiber (CF)	Ether Extract (EE)	Ash	Nitrogen-Free Extract (NFE)	Neutral Detergent Fiber (NDF)	Acid Detergent Fiber (ADF)
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							
P0 (0% *Gliricidia*)	11.76	18.84	3.37	9.77	56.26	41.49	25.21
P1 (10% *Gliricidia*)	12.28	19.75	3.97	9.33	54.67	39.82	24.36
P2 (20% *Gliricidia*)	13.01	20.72	3.06	8.94	54.27	30.04	22.04
P3 (30% *Gliricidia*)	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 (adapted).*

Looking at Table 1, you can clearly see that as we added more *Gliricidia sepium* leaves, the crude protein (CP) content in our feed mixes went up. It started at 11.76% in our control (P0) and climbed to 14.05% in P3 (where we had 30% *Gliricidia*). This makes perfect sense, as *Gliricidia sepium* leaves themselves are quite high in protein (24.81%) compared to the concentrate (13.84%) and corn straw (8.48%). This is great news, showing that *Gliricidia sepium* can indeed be a valuable protein booster for animal diets.

On the flip side, the crude fiber (CF) content in our feed mixes also crept up slightly as we replaced more concentrate with *Gliricidia sepium* (from 18.84% in P0 to 22.94% in P3). This is pretty much what we'd expect, since *Gliricidia sepium* is a forage and naturally contains more fiber than a typical concentrate. The fat content (ether extract, EE) generally went down a bit with more *Gliricidia sepium*, but all our mixes stayed well below the 5% mark, which is considered normal and shouldn't cause any issues with fiber digestion [50]. The nitrogen-free extract (NFE), which represents easily digestible carbohydrates, showed a slight decrease. This means that as we swapped out concentrate for *Gliricidia sepium*, the proportion of these quick-energy carbs in the feed went down a little.

Now, here's a really interesting part about the fiber. The neutral detergent fiber (NDF) was actually highest in our control (P0) at 41.49% and lowest in P3 (26.08%). The same pattern held for acid detergent fiber (ADF), which was highest in P0 (25.21%) and lowest in P3 (21.60%). This might seem a bit surprising, right? You'd think adding a forage would increase fiber. But what this tells us is that the specific concentrate we used must have had a higher fiber contribution in its original form than the *Gliricidia sepium* leaves did in our mixes. So, by replacing that concentrate, we actually ended up with less NDF and ADF overall in the final feed. This is a big deal, because lower NDF and ADF generally mean the feed is easier for animals to eat and digest [45].

How Well the Feed Was Digested (In Vitro Digestibility)

Next, we wanted to see how well our different feed mixes were broken down in our lab-simulated rumen. Table 2 shows our results for in vitro dry matter digestibility (DMD) and organic matter digestibility (OMD). Our statistical analysis clearly showed that the different feed mixes had a very significant impact ($P < 0.01$) on both DMD and OMD.

Table 2: Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) of Feed Ingredients and Experimental Diets (%)

Feed Component	Dry Matter Digestibility (DMD)	Organic Matter Digestibility (OMD)	Total Digestible Nutrients (TDN)

Feed Ingredients			
Corn straw	41.81	48.50	44.48
Concentrate	67.92	73.33	68.80
Gliricidia leaves	71.72	76.68	73.55
Experimental Diets			
P0 (0% *Gliricidia*)	54.56	58.94	55.93
P1 (10% *Gliricidia*)	56.05^{ab}	63.79^{ab}	59.40^{ab}
P2 (20% *Gliricidia*)	60.08^{ab}	66.61^{ab}	62.99^{ab}
P3 (30% *Gliricidia*)	66.34^b	71.43^b	68.08^b

Note: *Different letters (a-b) in the same column indicate that the treatments have a highly significant effect (P<0.01) on DMD and OMD. TDN calculated as in vitro OMD digestibility × 1.05 [Ibrahim, 1998, as cited in PDF]. Source: Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Brawijaya University, 2024 (adapted).*

The most exciting part here is that our P3 treatment (with the highest 30% *Gliricidia sepium* substitution) showed the best digestibility, with DMD at 66.34% and OMD at 71.43%. This tells us that adding more *Gliricidia sepium* leaves significantly improved how well the entire feed mix was digested. We believe this is because *Gliricidia sepium* leaves themselves are naturally very digestible (DMD 71.72%, OMD 76.68%), especially when compared to corn straw (DMD 41.81%, OMD 48.50%). Our results also suggest a positive connection between the protein content and digestibility, meaning that the protein in *Gliricidia sepium* is easily broken down in the rumen, which helps the animal use all the nutrients more efficiently.

You'll also notice that the OMD values were consistently higher than the DMD values. This is normal because organic matter (which includes carbs, proteins, and fats) is generally more thoroughly digested by rumen microbes than the total dry matter, which also includes indigestible ash [47]. Many factors can influence digestibility, like the ingredients, how the feed is prepared, and even the animal itself [24]. In our lab setup, the chemical makeup of our feed mixes was the main driver of these digestibility results.

What the Rumen Microbes Were Doing (Gas Production)

Gas production is a direct window into how much and how fast the microbes in the rumen are fermenting the feed. Table 3 shows you the cumulative gas produced at different time points during our 48-hour incubation.

Table 3: Gas Production Values at 2, 4, 8, 16, 24, 36, and 48 Hours of Incubation (mL/500mg DM)

Treatment	**2 Hours**	**4 Hours**	**8 Hours**	**16 Hours**	**24 Hours**	**36 Hours**	**48 Hours**
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	4.85	9.69	21.01	35.28	62.21	76.76	87.00

leaves							
Experimental Diets							
P0 (0% *Gliricidia*)	3.23	13.23^{ab}	28.58^{ab}	44.21^d	71.44^c	87.88^d	91.12^{bc}
P1 (10% *Gliricidia*)	3.84^{ab}	11.24^{ab}	27.71^{ab}	41.43^c	69.41^c	82.04	90.27^{bc}
P2 (20% *Gliricidia*)	4.70^c	10.22^{ak}	27.35^{ab}	37.85^b	65.20^{ab}	82.61^{bc}	89.51^b
P3 (30% *Gliricidia*)	4.63^c	10.08^a	25.89^b	31.89	63.23^a	79.31^a	86.94^a

Note: Different letters (a-d) 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. Source: Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Brawijaya University, 2024 (adapted).

Among the individual ingredients, corn straw produced the most gas after 48 hours (92.44 mL/500mg DM), meaning its organic matter was readily available for fermentation. The concentrate (88.89 mL/500mg DM) and *Gliricidia sepium* leaves (87.00 mL/500mg DM) produced slightly less gas, possibly because *Gliricidia sepium* contains compounds like tannins that can bind to proteins and slow down fermentation [6].

When we looked at our experimental diets, replacing concentrate with *Gliricidia sepium* had a very significant effect ($P < 0.01$) on gas production throughout the 48 hours. Interestingly, the mixes with *Gliricidia sepium* (P1, P2, and P3) generally produced *less* gas than our control (P0). P0 had the highest gas production at 48 hours (91.12 mL/500mg DM), while P3 had the lowest (86.94 mL/500mg DM). This might seem odd since we saw improved digestibility, but it could be because the higher protein content and tannins in *Gliricidia sepium* protect proteins from being broken down too quickly in the rumen [4, 35]. Less rapid protein breakdown means less gas from that process. The gas production pattern was typical: slow start, rapid increase between 8 and 24 hours, then a decline, as the easily fermentable stuff gets used up [12, 47].

Checking Ammonia Levels (NH3 Concentration)

Ammonia-nitrogen (NH3-N) in the rumen fluid is a crucial indicator of how much protein is being broken down and how much nitrogen is available for the microbes. Table 4 shows these levels for both our individual ingredients and our feed mixes.

Table 4: Average NH3 Concentration (mg/L)

Feed Component	**NH3 (mg/L)**
Feed Ingredients	
Corn straw	63.65
Concentrate	75.14
Gliricidia leaves	80.35
Experimental Diets	

P0 (0% *Gliricidia*)	70.25
P1 (10% *Gliricidia*)	70.90
P2 (20% *Gliricidia*)	71.74
P3 (30% *Gliricidia*)	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. Source: Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Brawijaya University, 2024 (adapted).*

Among the individual ingredients, *Gliricidia sepium* leaves produced the most ammonia (80.35 mg/L), which tells us its protein is highly degradable in the rumen. The concentrate (75.14 mg/L) and corn straw (63.65 mg/L) had lower ammonia levels.

For our experimental diets, the amount of *Gliricidia sepium* had a very significant effect ($P < 0.01$) on ammonia concentration. The P3 treatment (with 30% *Gliricidia sepium*) had the highest ammonia level (77.91 mg/L), followed by P2 (71.74 mg/L), P1 (70.90 mg/L), and P0 (70.25 mg/L). This increasing trend is directly linked to the higher protein content of *Gliricidia sepium* leaves, which the rumen microbes break down into ammonia [11, 49]. Importantly, all our ammonia levels (ranging from 70.25 to 77.91 mg/L) fell within the ideal range (50-200 mg/L) needed for the microbes to efficiently build their own proteins [11]. This means there was enough nitrogen for microbial growth, which is great. However, it's worth noting that if there's too much ammonia without enough energy for the microbes to use it, that excess nitrogen can just be wasted [22, 41].

Building Blocks of Protein (Microbial Protein Synthesis - MPS)

Finally, we looked at microbial protein synthesis (MPS), which essentially tells us how efficiently the rumen microbes are using nitrogen to create their own protein. This is super important because microbial protein is a high-quality protein source for the animal. Table 5 shows our MPS results.

Table 5: Microbial Protein Synthesis (g N microbial/kg Organic Matter Truly Rumen Degraded - OMTR)

Treatment	**MPS (g N microbial/kg OMTR)**
Feed Ingredients	
Corn straw	34.535
Concentrate	48.320
Gliricidia leaves	50.950
Experimental Diets	
P0 (0% *Gliricidia*)	40.678
P1 (10% *Gliricidia*)	41.273
P2 (20% *Gliricidia*)	42.810
P3 (30% *Gliricidia*)	44.024

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

Our analysis showed that the different feed mixes had a very significant effect ($P < 0.01$) on microbial protein synthesis after 48 hours. The P3 treatment, with 30% *Gliricidia sepium*, produced the most microbial protein at 44.024 g N microbial/kg OMTR. This was followed by P2 (42.810 g N microbial/kg OMTR), P1 (41.273 g N microbial/kg OMTR), and P0 (40.678 g N microbial/kg OMTR). The fact that MPS increased with more *Gliricidia sepium* suggests that the combination of organic matter and nitrogen from *Gliricidia sepium* at these levels really helped the microbes grow and make protein efficiently [16, 18, 30]. Our MPS values were right within the optimal range (30-40 g N microbial/kg DM) for efficient microbial protein synthesis [9]. This is fantastic news, as microbial protein is a top-notch protein source for ruminants. It also supports the idea that a balanced mix of roughage and concentrate leads to better MPS than just feeding roughage alone [38].

DISCUSSION

So, what does all this data really mean for feeding our livestock? Our study gives us a clear picture of how replacing traditional concentrate with *Gliricidia sepium* leaves affects the feed's nutritional value and how it behaves in the rumen. The big takeaway is that *Gliricidia sepium* is a genuinely promising, sustainable, and budget-friendly feed alternative, as long as we use it wisely.

What Happened to the Nutrients and Fiber?

The first thing we noticed was a clear boost in crude protein (CP) as we added more *Gliricidia sepium* (Table 1). This is simply because *Gliricidia sepium* leaves are naturally rich in protein [32, 44]. This finding is incredibly important, especially with the rising cost of traditional protein supplements. It means *Gliricidia sepium* could help farmers save money while still ensuring their animals get enough protein [34]. More protein in the feed also means the rumen microbes have more building blocks to create their own protein, which is vital for the animal's overall health and growth [18, 30].

Now, here's the surprising bit: we actually saw a *decrease* in both Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) as we increased *Gliricidia sepium* (Table 1). This is counter-intuitive if you think of forages as being high in fiber. But it suggests that the specific concentrate we used in our control mix had a higher fiber content than the *Gliricidia sepium* leaves did in our experimental mixes. So, by swapping out that concentrate, we ended up with a feed that was, in fact, lower in these less digestible fiber components. This is a positive outcome, as lower NDF and ADF typically mean the feed is easier for animals to eat and digest [7, 22, 45]. It essentially means our *Gliricidia sepium* substitution helped create a more digestible feed overall. While crude fiber (CF) went up a little, it stayed within healthy limits, so we don't expect any negative impacts on digestion [50]. The slight dip in nitrogen-free extract (NFE) just indicates a minor shift in the type of carbohydrates in the feed, with some quick-energy carbs being replaced by the structural carbs from the legume.

The Story of Digestion: Better with Gamal?

The significant improvement in both in vitro dry matter digestibility (DMD) and organic matter digestibility (OMD) as we added more *Gliricidia sepium*, especially at the highest level (P3 in Table 2), is a major highlight of our study. This strongly suggests that *Gliricidia sepium* leaves are highly digestible themselves, and their inclusion makes the entire feed mix easier for the microbes to break down. This lines up with other research showing that *Gliricidia sepium* can boost digestibility [17]. The fact that *Gliricidia sepium* is more digestible than corn straw and even our commercial concentrate played a big role here. We also noticed a positive link between protein content and digestibility, indicating that the protein in *Gliricidia sepium* is readily available for the rumen microbes to use, which helps the animal absorb more nutrients [31, 27, 20]. More digestible feed means animals can get more goodness from what they eat, leading to better feed efficiency and potentially healthier, more productive livestock.

The consistent observation that OMD values were higher than DMD values is normal. It simply means that the organic parts of the feed (carbohydrates, proteins, fats) are more thoroughly digested by rumen microbes than the total dry matter, which also includes the indigestible mineral content (ash) [47]. Digestion is a complex dance involving many factors, from the feed's ingredients to how it's processed and even the animal's own body [24]. In our lab setting, the chemical makeup of our specially formulated diets was the main star in determining how well everything was digested.

What the Microbes Were Up To: Fermentation Insights

Looking at the gas production (Table 3) gives us a dynamic view of how fast and how much the rumen microbes were working. While corn straw on its own produced a lot of gas, our complete feed mixes with *Gliricidia sepium* (P1, P2, P3) generally produced *less* gas than our control (P0). This might seem confusing, especially since we saw improved digestibility. But it makes sense when you consider the intricate nature of rumen fermentation and the unique compounds in *Gliricidia sepium*. Feeds rich in easily fermentable carbohydrates usually lead to more gas [13, 19]. The reduction in gas with *Gliricidia sepium*, despite better digestibility, hints at a shift in how fermentation happens, possibly due to the protective effect of tannins.

Tannins in *Gliricidia sepium* can bind to proteins, shielding them from rapid breakdown in the rumen [4, 35]. This "bypass protein" means less protein is degraded into ammonia in the rumen, which in turn might lead to less gas being produced from protein fermentation. Also, if *Gliricidia sepium* is replacing highly fermentable carbohydrates from the concentrate, we'd naturally expect a drop in gas production [13]. The cool thing about these tannin-protein complexes is that they stay stable in the rumen's pH but then break apart in the more acidic abomasum and alkaline intestines, allowing the protein to be digested and absorbed later [35]. This suggests a more efficient way of using protein, even if it means slightly less gas in the rumen. Overall, our gas production levels were well within typical ranges for similar complete feeds [25].

Now, let's talk about ammonia-nitrogen (NH3-N) levels (Table 4). This is a really important indicator of how much protein is being broken down and how much nitrogen is available for the rumen microbes. We saw the highest ammonia levels in our P3 treatment, which had the most *Gliricidia sepium*. This directly reflects the high protein content of *Gliricidia sepium* leaves, which the microbes readily convert into ammonia [11, 49]. What's great is that all our ammonia levels (from 70.25 to 77.91 mg/L) fell within the ideal range (50-200 mg/L) needed for the microbes to efficiently build their own proteins [11]. This means that even at the highest *Gliricidia sepium* level, the microbes had plenty of nitrogen to work with, preventing any nitrogen shortages that could slow them down [41]. However, it's a fine balance: too much ammonia without enough energy (from volatile fatty acids) for the microbes to use it can lead to wasted nitrogen, which simply gets excreted [22, 41].

Finally, we looked at microbial protein synthesis (MPS) (Table 5), which is super important because the protein made by these microbes provides a big chunk of the amino acids the animal actually absorbs [18, 30]. Our P3 treatment produced the most microbial protein, showing that the combination of organic matter and nitrogen from *Gliricidia sepium* at this level really helped the microbes grow and make protein efficiently. Factors like the synchronized availability of energy and nitrogen, along with amino acids and peptides, all play a role in boosting MPS [30, 48]. The improved MPS in our *Gliricidia sepium* diets suggests we hit a good balance for these factors, leading to more active microbes. Our MPS values (between 40.678 and 44.024 g N microbial/kg OMTR) were right in the sweet spot for efficient microbial protein synthesis [9]. This is fantastic because microbial protein is a high-quality protein source for ruminants, and our results confirm that a balanced feed mix can lead to better MPS than just feeding roughage alone [38].

Finding the Sweet Spot and What It Means for Farmers

Based on all our findings – from nutrient content and fiber to digestibility and how the rumen microbes behaved – our P3 formulation (50% corn straw + 20% concentrate + 30% *Gliricidia sepium* leaf meal) really stood out. This mix had the highest protein, the lowest levels of those tough-to-digest fibers (NDF and ADF), and significantly improved both dry matter and organic matter digestibility. Plus, it kept ammonia levels optimal and led to the highest microbial protein synthesis, meaning the rumen microbes were working at their peak efficiency. While gas production was a bit lower in the *Gliricidia sepium* mixes, we believe this is a sign of more efficient protein use, not less overall fermentation.

From a farmer's perspective, this is huge. Being able to replace a significant portion of expensive concentrate with readily available *Gliricidia sepium* leaves means big savings [2, 6, 44]. *Gliricidia sepium* is a cheap, easy-to-grow, and sustainable forage, especially in tropical areas that often face dry spells [44]. Integrating it effectively into complete feeds can drastically cut down on feed costs, making livestock farming more profitable and sustainable. This is a game-changer, particularly in regions where traditional feed sources are scarce or just too expensive [6, 44].

Of course, it's important to remember that our study was done in a lab. While "in vitro" studies are incredibly valuable for understanding the nitty-gritty of rumen fermentation, they can't perfectly replicate everything that happens inside a living animal. So, the next step is definitely "in vivo" studies – feeding these mixes to actual animals to see how they perform in terms of feed intake, growth, milk production, and overall health. These real-world studies will also help us understand how palatable *Gliricidia sepium*-based feeds are, as some natural compounds like coumarin can affect how much animals want to eat [37]. We could also explore different ways to process *Gliricidia sepium* leaves, like ensiling or fermenting them, to potentially boost their nutritional value even further and lessen any anti-nutritional effects [6]. Ultimately, the insights from our study provide a strong foundation for creating smarter, more sustainable, and more efficient ways to feed ruminants, which is a win-win for farmers, animals, and food security.

CONCLUSION

To wrap things up, our study clearly shows that swapping out traditional concentrate for *Gliricidia sepium* leaves in complete feeds makes a big difference to the feed's nutritional content, its fiber makeup, how well it's digested, and how the rumen microbes ferment it. Adding *Gliricidia sepium* successfully increased the protein in the feed. What's more, our highest substitution level (30%, in treatment P3) actually resulted in the lowest levels of the tough-to-digest fibers (NDF and ADF), suggesting a more digestible feed overall. This was confirmed by a significant improvement in both dry matter digestibility (DMD) and organic matter digestibility (OMD) as we added more *Gliricidia sepium*, with

P3 showing the best digestibility.

When we looked at the fermentation products, we saw that while gas production was a bit lower in the *Gliricidia sepium* mixes, this is likely because the tannins in the leaves were protecting proteins, leading to more efficient protein use after the rumen. Ammonia-nitrogen (NH3-N) levels went up with more *Gliricidia sepium*, staying well within the ideal range for microbial activity. Most importantly, microbial protein synthesis (MPS) was highest in our P3 treatment, meaning this mix truly optimized how the rumen microbes grew and used nitrogen.

So, our study concludes that *Gliricidia sepium* leaves are a valuable and sustainable protein source that can effectively replace a good chunk of expensive concentrate in ruminant feeds. The best mix we found in our lab study was the P3 treatment: 50% corn straw + 20% concentrate + 30% *Gliricidia sepium* leaf meal. This combination consistently delivered superior nutrition and fermentation efficiency. This approach offers a promising way to make animal feed better, cheaper, and more sustainable. The next step is to test these findings with real animals to see how they perform in practice.

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