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# Research Article
# Optimization of Rumen Microbial Protein Synthesis by Addition of Gambier Leaf Residue to Cattle Feed Supplement

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## Abstract

**Background and Objective:** This study aimed to achieve optimal microbial protein synthesis by adding various levels of gambier leaf residue (GLR) to cattle feed supplement (CFS). **Materials and Methods:** Gambier (*Uncaria gambir* Roxb) leaf residue containing 9.96% condensed tannins was added at levels of 0, 2.5, 5.0 and 7.5% (DM) to CFS formula containing 29% crude protein and then tested *in vitro* rumen digestion. **Results:** Addition of 5% GLR significantly decreased the rumen protozoan population by 24.43% ($p<0.05$) and decreased the $NH_3$-N content ($p<0.01$) but did not affect the pH or total volatile fatty acid (VFA) content ($p>0.05$). The mean of the microbial biomass was 111-285 mg $100^{-1}$ mL of rumen fluid and the microbial protein concentration was 59-157 mg $100^{-1}$ mL of rumen fluid, which was highest at 5.0 GLR ($p<0.01$). Overall, the rate of microbial biomass production, rate of microbial protein synthesis and efficiency of microbial protein synthesis were 7.30-10.18 mg $100^{-1}$ mL $h^{-1}$, 3.86-5.62 mg $100^{-1}$ mL $h^{-1}$ and 16-47 mg microbial protein $100^{-1}$ mg digested organic matter, respectively, with the highest rate at 5.0% GLR and the lowest rate in controls ($p<0.01$). **Conclusion:** Addition of 5.0% GLR to CFS can optimize microbial protein synthesis in the rumen.

Key words: Condensed tannin, protozoa, microbial biomass, cattle feed supplement, gambier

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The presence of microbes in the rumen of cattle confers a mutualistic symbiotic effect to the host. Normal concentrations of ruminal microbes are bacteria at $10^{10}$-$10^{11}$ cells $g^{-1}$ and protozoa at $10^5$-$10^6$ cells $g^{-1}$ of rumen fluid; anaerobic fungi, mycoplasmas and bacteriophages may also be present[1]. Ruminal microbes assist in the digestive process and the microbial biomass continues along the flow of feed into the small intestine, becoming a source of protein for livestock that is known as microbial protein. Indeed, microbial proteins are a major source of protein for cattle that receive poor-quality forage because almost all protein is degraded in the rumen[2].

The growth of rumen microbes is largely determined by the availability of essential nutrients, e.g., soluble carbohydrates, protein, nitrogen sources and certain minerals[3]. Agricultural Polytechnic of Payakumbuh has developed a cattle feed supplement (CFS) known as "Permen Sapi"[4] that provide nutrients essential for microbial growth. This CFS, which is provided to the community and produced commercially[5], has satisfactory results and increase the daily gain of beef cattle from 0.68-1.02 kg $day^{-1}$ [4]. However, the benefits of CFS are reduced in cattle receiving low-quality rations. There is an interaction effect of CFS addition and the concentrate in ration to rumen degradation of fiber fraction in low-quality forage[6] because some bacterial species are preyed upon by protozoa; thus, it is necessary to add defaunation compounds[7-8].

Ruminal protozoa tend to prey on bacteria in the absence of soluble carbohydrates (starch, sugar, pectin) and reduced available nitrogen from proteins; for example, a protozoan cell can prey on 250 bacterial cells $day^{-1}$ [9]. Accordingly, controlling the population and growth of protozoa in the rumen (partial defaunation) can improve the rumen digestibility of feed and increase the synthesis of microbial proteins in the rumen[10]. Many natural compounds, such as condensed tannin have been developed as defaunation compounds.

Tannin is a polyphenolic compound with a high molecular weight that can function as a defaunation agent and protect protein at a specific concentration. Protozoan populations declined significantly as a result of tannin addition[11,12]. For instance, the use of tannins from pistachio nuts increased the daily gain of Holstein bulls[13]. A potential tannin resource in West Sumatera, Indonesia is the gambier plant (*Uncaria gambir* Roxb), which is rich in catechin (catechin acid or catechu acid) and tannic acid (catechin anhydride)[14]. Gambier leaf residue (GLR) is the waste from gambier leaves that have been extracted. GLR contains 9.96% condensed tannins and has the potential to be used as a material for rumen protozoan defaunation[15]. Tannins from GLR can increase digestibility and propionate production in rumen[16], reduce methane production and increase daily gain of beef cattle[16] but there have been no reports whether this positive effect is related to microbial protein synthesis in the rumen. In this study the effects of adding GLR to CFS on protozoan populations, rumen fermentation and microbial protein synthesis via *in vitro* rumen digestion was examined.

## MATERIALS AND METHODS

**Materials:** The materials used in this study consisted of CFS ingredients, GLR, rumen fluid inoculum and McDougalls buffer. The equipment used consisted of a 100 mL serum bottle, a shaking water-bath, (Precision, USA) a pH meter (Hi9807-phep, Singapore), a centrifuge (Hitachi CR21, Japan), Eppendorf tubes, analytical scales and a spectrophotometer (Jasco V-530, Japan). The CFS was prepared according to a formula[4], with adding 4 levels of GLR (0, 2.5, 5.0 and 7.5%, DM basis) in iso-protein and energy. The composition of the CFS and the addition of GLR are presented in Table 1. The CFS was prepared by cooking brown sugar with 50% water until melted, pouring into a stirred mixture of batter, then forming with a pellet machine and drying at 60°C.

**Implementation of research:** The *in vitro* studies followed the procedure of Tilley and Terry's[17]. In a 100 mL serum bottle, a CFS sample of 0.5 g plus 50 mL of McDougalls buffer and rumen fluid mixture at a ratio of 4:1, was treated with $CO_2$ gas for 30 sec. The serum bottle was then placed in a shaking water-bath at a temperature of 39°C and incubated for 3, 6, 12, 24, 36 and 48 h of incubation.

**Parameters evaluated:** After completion incubation, ruminal pH measurements were obtained using a Hanna pH meter (Hi9807-phep). Rumen fluid (0.5 mL) was poured into a dark bottle and 0.5 mL of MFS (methyl-green formalin saline) solution was added and allowed to stand for 1 h according to the procedure described by Ogimoto and Imai[9]. Protozoan populations were calculated under a microscope using Neubauer chambers.

The rumen fluid was centrifuged at 3,000 rpm for 5 min at 4°C and the supernatant was used for N-$NH_3$ analysis with the Conway micro diffusion method and volatile fatty acid (VFA) analysis via steam distillation method[20]. The residue was filtered through a Whatman 41 paper for organic matter analysis[20].

Table 1: The composition of cattle feed supplement with the addition of gambier leaf residue

| | Level of gambier leaf residue (DM%) | | | |
|---|---|---|---|---|
| Ingredients | 0 | 2.5 | 5.0 | 7.5 |
| Brown sugar | 15 | 15 | 15 | 15 |
| Bran | 29 | 28 | 27 | 26 |
| Coconut meal | 15 | 14 | 12 | 11 |
| Soybean meal | 15 | 15 | 15 | 15 |
| Tapioca | 15 | 15 | 15 | 15 |
| Urea | 5 | 5 | 5 | 5 |
| Salt | 3 | 2.5 | 3 | 2.5 |
| Mineral | 3 | 3 | 3 | 3 |
| Gambier leaf residue | 0 | 2.5 | 5 | 7.5 |
| **Chemical composition DM (%)** | | | | |
| Organic matter | 84.32 | 84.51 | 85.95 | 85.96 |
| Crude protein | 29.83 | 29.64 | 29.36 | 29.30 |
| Fat | 4.91 | 4.69 | 4.59 | 4.09 |
| Crude fiber | 27.01 | 28.16 | 29.30 | 29.45 |
| Tannin | 0 | 0.68 | 1.17 | 1.60 |

The protein (TCA-soluble protein) content was analyzed following the Shultz and Shultz procedure[18] using 10 mL of rumen fluid plus 10 mL of trichloroacetic acid (Merch, Darmstadt, Germany) 20% and 10 mL of sulfoacetic acid (Merch, Darmstadt, Germany) 2%, which was allowed to stand for 1 h. The mixture was then centrifuged at 3,000 rpm for 20 min at 4°C and filtered through Whatman 41 paper (Whatman, China). The protein residue was analyzed using the Kjeldahl method[20].

The rumen microbial biomass was determined following the Griswold procedure[19]. A 1.5 mL supernatant from the above step was added to an Eppendorf tube, precipitated by centrifugation at 15,000 rpm for 30 min at 4°C, washed with 0.85% NaCl solution and centrifuged again in the same manner. The supernatant was removed and the precipitate was dried in a 60°C oven for 48 h and weighed. The dry microbial biomass in the Eppendorf tube was suspended in 1 mL of 1N NaOH in a water-bath at 60°C for 10 min. After cooling, the tube centrifuged at 15,000 rpm at 4°C for 30 min. The supernatant obtained was considered the soluble microbial protein and was analyzed with the biuret method using a UV spectrophotometer at 540 nm and bovine serum albumin (BSA) as a standard.

**Statistical analysis:** The response parameters measured were analyzed using a randomized block design[22] with 4 treatment levels of GLR addition to the CFS and the 4 cattle rumen fluid donors as blocks. Data were analyzed using Duncan's new multiple range tests (DNMRT) at the 5% level of significance. Data processing was performed using Statistica software version 10 (Statsoft Inc.)[21].

## RESULTS AND DISCUSSION

**Protozoan population:** The result of 48 h incubation *in vitro* assay (Table 2) showed that the addition of 2.5% GLR to the CFS decreased the rumen protozoan population by 24.43% compared to controls ($p<0.05$). However, no significant differences were found with higher levels of GLR. Such a decline in the protozoan population occurs due to the content of catechin, a class of condensed tannins that negatively affects ruminal protozoa[11,23,12], of GLR. Condensed tannins have a stronger negative effect on protozoa than do hydrolyzable tannins, as demonstrated in a previous study in which a protozoan population decreased by 11.6% due to an addition of hydrolyzable tannins compared to 28.6% due to an addition of condensed tannins[24].

In the present study, the protozoan population decreased linearly with an increase in GLR in the CFS, following the equation $Y = 1.1034-0.0638x$, with $R^2 = 0.8276$. This finding indicates that higher levels of GLR, from 2.5-7.5% in the CFS results in linearly smaller protozoan populations. Negative effects of tannins on protozoan populations *in vitro* are reported to occur from the first hour of incubation and to continue until the 6th h[25].

The antiprotozoal effect of condensed tannins is caused by the reaction of tannins with the protozoan cell wall, altering cell wall permeability and, leading to cell death[26]. Condensed tannins have a higher antiprotozoal effect than do hydrolyzable tannin[24] and holotrichs protozoa are more sensitive to condensed tannins than are entodiniomorphs[27].

**Characteristics of rumen fermentation:** The pH and total VFA content of rumen fluid did not differ significantly after

Table 2: Protozoan populations, fermented products and rumen microbial protein synthesis

| | Level of gambier leaf residue (DM%) | | | | | |
|---|---|---|---|---|---|---|
| Parameters | 0 | 2.5 | 5.0 | 7.5 | SEM | p-value |
| Protozoan population (cells×$10^5$) | 1.175[a] | 0.888[b] | 0.681[b] | 0.712[b] | 0.08 | 0.04 |
| Ruminal pH | 6.85 | 6.95 | 6.93 | 6.95 | 0.03 | 0.09 |
| Total VFAs (mM) | 108 | 102 | 108 | 121 | 7.02 | 0.15 |
| $NH_3$-N (mg $100^{-1}$ mL) | 14.75[a] | 15.17[a] | 13.95[b] | 11.92[c] | 0.33 | <0.01 |
| N-utilization (mg MP $mg^{-1}$ $NH_3$-N[1]) | 6.79[c] | 6.51[c] | 7.63[b] | 8.71[a] | 0.21 | <0.01 |
| TCA-soluble protein (mg $100^{-1}$ mL[2]) | 319[b] | 345[b] | 465[a] | 433[a] | 26.0 | <0.01 |
| Microbial biomass production rate (mg $100^{-1}$ mL $h^{-1}$) | 7.30[c] | 8.73[b] | 10.18[a] | 10.18[a] | 0.15 | <0.01 |
| Microbial protein synthesis rate (mg $100^{-1}$ mL $h^{-1}$) | 3.86[d] | 4.72[b] | 5.62[a] | 4.30[c] | 0.09 | <0.01 |

Means with different letters in the same row are significantly different, SEM: Standard error of the mean, [1]N-utilization: Ratio of microbial protein (MP)/ $NH_3$-N in rumen fluid, [2]Total soluble protein in 10% (vol/vol) trichloroacetic acid

treatment of CFS with GLR (p>0.05). The average pH of the rumen fluid was 6.92±0.07, an optimal pH (5.5-7.5) for survival and rumen microbial activity[28]. The same finding has also been reported by other researchers; for example, the effect of a tannin monomer (catechin) had no significant effect on rumen pH *in vitro*, with a mean of 6.82[29].

As mentioned above, ruminal protozoa play a role in maintaining the stability of the rumen pH by rapidly degrading soluble carbohydrates, thereby reducing the conversion of carbohydrates to lactic acid by bacteria[3]. In this study, the protozoan population decline up to 24.43% of the control population did not affect the pH of rumen fluid. It should be noted that the protozoan populations in this study were not eliminated, as GLR only suppresses the protozoan population and growth in rumen fluid (partial defaunation).

The mean total VFA content was 110.10±14.59 mM, which was within the optimal range of 80-160 mM to support the growth of ruminal microbes[30]. Other researchers have also reported no effect of tannin on VFA production in the rumen[31]. However, several other researchers observed a decrease in total VFA production in the rumen due to the influence of condensed tannin in the ration[23, 31].

The ammonia ($NH_3$-N) concentration of the rumen fluid decreased by the addition of GLR to the CFS at 5.0 and 7.5% (p<0.05). $NH_3$ is the final product of crude protein degradation in the rumen. The condensed tannin content in GLR serves to protect proteins from degradation by rumen microbes by binding to and precipitating the proteins. One gram of condensed tannin is able to bind 28.89 g of protein[33].

The crude protein contained in the CFS consists of non-protein nitrogen (urea) and rumen-degraded proteins of soybean meal and coconut meal. It is expected that urea will be hydrolyzed to ammonia to meet the nitrogen requirement for ruminal microbes and that the rumen-degraded proteins will be protected, that they will be available during post-rumen digestion. At 5.0 and 7.5% GLR, the $NH_3$-N concentration decreased to 13.95 and 11.92 mg $100^{-1}$ mL of rumen fluid; this concentration is higher than needed to support the optimal growth of ruminal microbes (11.2 mg $100^{-1}$ mL)[35].

Ammonia is the main source of nitrogen utilized by ruminal microbes for survival and growth. The efficiency of nitrogen use increased at GLR levels of 5.0 and 7.5% (p<0.05), at 8.71 and 7.63 mg microbial protein $mg^{-1}$ $NH_3$-N, respectively. The observed lower $NH_3$-N concentrations at 5.0 and 7.5% GLR levels suggest that both can result in higher nitrogen use efficiency and that a nitrogen supply of 11.92 or 13.95 mg $100^{-1}$ mL of rumen fluid is sufficient for microbes to grow optimally.

**Production of biomass and microbial proteins:** The production of microbial biomass (Table 3) as a result of the addition of GLR to the CFS showed a significant effect (p<0.01) after incubation for 3, 6, 12, 24 and 36 h but was not significant (p>0.05) after 48 h. The CFS without GLR addition (control) showed the lowest microbial biomass production (p<0.01) was found with 5.0% GLR addition, except at 36 and 48 h. The addition of GLR at the highest level (7.5%), had a negative effect of lowering (p = 0.18) microbial biomass production.

The average microbial biomass ranged from 111-285 mg $100^{-1}$ mL of rumen fluid, with the lowest production found after 36 h and the highest after 24 h. The highest biomass production in this study was greater than that reported by Bretschneidera *et al.*[32], whereby maize silage supplementation in cows resulted in microbial biomass production ranging from 170-191 mg $100^{-1}$ mL of rumen fluid. However, microbial biomass production in goats supplemented with urea-molasses ranged from 41.2-56.8 mg $100^{-1}$ mL of rumen fluid[36].

The microbial biomass of rumen fluid contains protein in the range of 52.30-55.20% (dry base) for microbial protein synthesis, as shown in Table 4. Microbial protein synthesis

Table 3: Microbial biomass (mg $100^{-1}$ mL) in relation to the level of gambier leaf residue addition in cattle feed supplement

| | Level of gambier leaf residue (DM%) | | | | | |
|---|---|---|---|---|---|---|
| Incubation time (h) | 0 | 2.5 | 5.0 | 7.5 | SEM | p-value |
| 3 | 191.05[b] | 214.15[a] | 219.60[a] | 210.99[a] | 6.04 | 0.04 |
| 6 | 165.32[b] | 245.31[a] | 281.00[a] | 164.18[b] | 11.72 | <0.01 |
| 12 | 111.07[d] | 164.46[b] | 208.83[a] | 144.43[c] | 5.42 | <0.01 |
| 24 | 218.60[c] | 252.24[b] | 285.32[a] | 225.34[c] | 3.65 | <0.01 |
| 36 | 114.38[d] | 171.12[a] | 137.61[c] | 157.60[b] | 3.56 | <0.01 |
| 48 | 167.52 | 157.61 | 170.93 | 164.35 | 5.42 | 0.18 |

Means with different letters in the same row are significantly different, SEM: Standard error of the mean

Table 4: Microbial protein (mg $100^{-1}$ mL) in relation to the level of gambier leaf residue addition in cattle feed supplement

| | Level of gambier leaf residue (DM%) | | | | | |
|---|---|---|---|---|---|---|
| Incubation time (h) | 0 | 2.5 | 5.0 | 7.5 | SEM | p-value |
| 3 | 100.53[b] | 114.89[a] | 119.09[a] | 117.25[a] | 3.66 | 0.04 |
| 6 | 87.24[c] | 131.86[b] | 153.95[a] | 91.44[c] | 6.07 | <0.01 |
| 12 | 58.51[c] | 88.44[b] | 114.44[a] | 80.39[b] | 2.96 | <0.01 |
| 24 | 115.30[d] | 135.70[b] | 156.53[a] | 125.57[c] | 2.21 | <0.01 |
| 36 | 60.21[b] | 91.93[a] | 75.31[b] | 87.69[a] | 1.97 | <0.01 |
| 48 | 88.28 | 84.69 | 93.59 | 91.48 | 2.93 | 0.11 |

Means with different letters in the same row are significantly different, SEM: Standard error of the mean

Table 5: Microbial protein synthesis efficiency (mg microbial protein $100^{-1}$ mg digested organic matter) in relation to the level of gambier leaf residue addition in cattle feed supplement

| | Level of gambier leaf residue (DM%) | | | | | |
|---|---|---|---|---|---|---|
| Incubation time (h) | 0 | 2.5 | 5.0 | 7.5 | SEM | p-value |
| 3 | 41.87 | 43.01 | 43.88 | 47.30 | 2.74 | 0.27 |
| 6 | 29.08[c] | 20.65[b] | 24.26[a] | 35.06[c] | 0.91 | <0.01 |
| 12 | 16.72[c] | 22.88[b] | 29.63[a] | 25.05[b] | 2.16 | 0.03 |
| 24 | 28.86[b] | 27.86[b] | 37.70[a] | 31.28[b] | 0.78 | <0.01 |
| 36 | 22.33[b] | 22.47[a] | 19.63[b] | 22.68[a] | 3.02 | 0.85 |
| 48 | 15.88[b] | 16.10[b] | 19.72[a] | 18.43[ab] | 0.94 | 0.05 |

Means with different letters in the same row are significantly different, SEM: Standard error of the mean

ranged from 59-157 mg $100^{-1}$ $mL^{-1}$ of rumen fluid, with the lowest obtained in controls after 12 h and highest with 5.0% GLR addition after 24 h. Similarly, Mao *et al.*[34] reported the highest microbial protein synthesis of 118-148 mg $100^{-1}$ mL after 24 h of incubation and Kardaya *et al.*[35] obtained 108-157 mg $100^{-1}$ mL of microbial proteins after the same duration.

In our study, an addition of 5.0% GLR resulted in the highest microbial protein synthesis ($p<0.01$) at 3, 6, 12 and 24 h, whereas the lowest was found in the controls. The addition of GLR to the CFS showed the following polynomial relationship: $Y = -1,7101x^2+14,936x+ 84,077$ with $R^2 = 0.9714$. Based on this equation, the highest microbial protein synthesis is 168.69 mg $100^{-1}$ mL of rumen fluid will be achieved with 5.76% GLR addition to the CFS.

Increased biomass and microbial proteins in rumen fluid due to the addition of GLR are due to the condensed tannins in GLR, leading to a decrease in protozoan populations (partial defaunation) and resulting in reduced bacterial predators in the rumen fluid. Decreased protozoan populations do not result in a decrease in rumen fermentation characteristics, with optimal rumen bacterial growth that is reflected in increased rumen fluid microbial biomass. In addition, condensed tannins are reportedly able to reduce the loss of fermentation gas, thus increasing the VFA profile and the efficiency of microbial protein synthesis[37].

The efficiency of microbial protein synthesis (Table 5) ranged from 16-47 mg of microbial protein MP $100^{-1}$ mg of digested organic matter (DOM). Addition of 5.0% GLR yielded the highest efficiency ($p<0.01$), (37.70 mg MP $100^{-1}$ mg DOM), after 24 h of incubation. This value is higher than that of ration mixed forage and concentrates, at 27.9 mg MP $100^{-1}$ mg DOM[38], as well as that of an ammoniated straw supplemented legume extract (28.75 mg MP $100^{-1}$ mg DOM)[39] and a ration with highly degraded proteins *in vivo* (20.88 mg MP $100^{-1}$ mg DOM)[40].

Table 6: Rate of microbial protein synthesis (mg $100^{-1}$ mL $h^{-1}$) in relation to the level of gambier leaf residue addition in cattle feed supplement

| | Level of gambier leaf residue (DM%) | | | | | |
|---|---|---|---|---|---|---|
| Incobation time (h) | 0 | 2.5 | 5.0 | 7.5 | SEM | p-value |
| 3 | 23.78[b] | 28.04[a] | 29.62[a] | 28.54[a] | 1.17 | 0.04 |
| 6 | 10.85[c] | 17.77[b] | 21.25[a] | 11.21[c] | 0.96 | <0.01 |
| 12 | 2.74[c] | 5.36[b] | 7.43[a] | 4.55[b] | 0.24 | <0.01 |
| 24 | 3.86[d] | 4.72[b] | 5.62[a] | 4.30[c] | 0.09 | <0.01 |
| 36 | 0.93[c] | 1.82[a] | 1.33[b] | 1.69[a] | 0.05 | <0.01 |
| 48 | 1.31 | 1.23 | 1.39 | 1.37 | 0.06 | 0.13 |

Means with different letters in the same row are significantly different, SEM: Standard error of the mean

Table 2 shows the highest biomass production after 24 h, the addition of 5.0% GLR yielded the highest ($p<0.01$) rate of microbial biomass production (10.18 mg $100^{-1}$ mL $h^{-1}$) and the highest rate of microbial protein synthesis (5.62 mg $100^{-1}$ mL $h^{-1}$) ($p<0.01$). These values are higher than those reported by Kardaya *et al.*[35], reporting that the rate of rumen microbial biomass production in cattle with rice straw rations receiving urea-molasses supplementation ranged from 2.9-4.7 mg $100^{-1}$ mL $h^{-1}$ and that, the rate of rumen microbial protein synthesis ranged from 1.58-2.50 mg $100^{-1}$ mL $h^{-1}$.

As shown in Table 6, the rate of microbial protein synthesis exhibited a decreasing pattern with the duration of incubation. The highest rate of microbial protein synthesis, 23.78-29.62 mg $100^{-1}$ mL $h^{-1}$, was after 3 h, with the highest rate with 5.0% GLR treatment and the lowest in the control ($p<0.05$). At the end of incubation, 48 h, a very low growth rate of 1.23-1.39 mg $100^{-1}$ mL $h^{-1}$ was observed, with no significant effect found ($p>0.05$) due to the addition of GLR. The decrease in the rate of microbial protein production along with the increase of the time of incubation is due to the decrease of fermentation substrate available so that the impact on the decrease of microbial biomass production[40].

## CONCLUSION

The addition of gambier leaf residue (GLR) to cattle feed supplements (CFS) may suppress rumen protozoan populations to levels 24.43% below control levels. GLR addition results in rumen fermentation characteristics that support microbial growth, such that microbial biomass production and microbial protein synthesis are optimally obtained. The highest-rates of microbial biomass production and microbial protein synthesis were found in CFS with 5.0% GLR added.

## SIGNIFICANCE STATEMENT

This study demonstrates that the addition of gambier leaf residue to cattle feed supplements can suppress rumen protozoan populations and support the rumen environment to obtain optimal levels of microbial protein synthesis. This study will help researchers identify the critical level of rumen microbial protein synthesis with the addition of gambier leaf residue to cattle feed supplements, which was previously unexplored. Thus, a new hypothesis about cattle feed supplementation with gambier leaf residues addition can be developed.

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