Effect of tannin supplementation from *Uncaria gambir* extract on rumen fermentation, microbial protein and *in vitro* gas production

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**Abstract.** Gambir (*Uncaria gambir*) is a tropical plant which contains tannin as a main phenolic compounds. This research was aimed to quantify the ability of tannin to bind protein and evaluate the addition of tannin from gambir extract on rumen fermentation, microbial protein and *in vitro* gas production. The treatments consisted of RC0 (0%), RC2 (2%), RC4 (4%) and RC6 (6%) of gambir extract level in mix rations with 70:30 ratio (concentrate:forage). Parameters measured were protein precipitation, VFA production, NH₃ concentration, microbial protein and kinetics of gas production. The result showed that tannin from gambir extract had a significant ability (P<0.05) to precipitate protein *Bovine Serum Albumin*. Supplementation of tannin from gambir extract significantly increased (P<0.05) NH₃ concentration and microbial protein. There was no significant effect on VFA total production (P>0.05). Gas production in RC0, RC2, RC4 and RC6 was decreased (P<0.05) after 48 hours incubation by 49.28 ml, 48.41 ml, 47.40 ml and 47.07 ml, respectively. It was concluded that the addition of tannin from gambir extract with level 6% in rations can be used to increased *in vitro* rumen fermentation.

1. **Introduction**

Cattle fed with too high protein feed can cause an increase degradation of proteins in the rumen due to the activity of rumen microbial fermentation that overrides the protein, so that the protein cannot reach the small intestine to be absorbed as amino acids. The degradation of proteins in the rumen should be able to provide the amount of N needed to increase microbial growth and influence nutrient digestibility. One technology that can be used to protect feed proteins (by pass protein) from rumen degradation is by adding tannins.

Tannin is a phenolic compound found in plants and is known as a secondary plant metabolite. According to [1] in general, tannins are divided into two types, namely condensed tannins (CT) and hydrolysable tannins (HT). Condensed Tannins (CT) have a high level of stability, are difficult to digest by enzymes, heat, or acid and are commonly used as a bypass nutrient, whereas HT stability tends to be low so that it is easily broken down into simple phenol and sugar groups. Condensed...
Tannins (CT) has the ability to bind proteins in the rumen and can reduce protein degradation [2]. The level of using tannin as an additive in feed is very influential on its activity. The use of tannins at low levels could maximize microbial protein synthesis so that the efficiency of microbial protein synthesis increases and protein degradation in the rumen decreases [1].

One of the raw materials, which has high tannin content and has the potential as a bypass protein agent is gambir (Uncaria gambir). Gambir is a plant that is widely grown in tropical countries such as Indonesia and Malaysia [3]. The main compounds in gambir plants according to [4] are pseudotannin catechin and phlobaltannin catechutannat acid (commonly called tannins) with the percentage of each compound is 7-30% and 22-55%. According to [5] stated that tannins contained in gambir are condensed tannins. This research was aimed to quantify ability of tannin to bind protein and evaluate the addition of tannin from gambir extract on rumen fermentation, microbial protein and in vitro gas production.

2. Materials and methods

2.1. Materials

The materials used in this study were gambir extract, soybean meal, wheat bran, king grass (Pennisetum hybrid), CaCO₃ mineral, Bovine Serum Albumin (BSA), McDougall solution, rumen fluid, tannin acid. The tools used were water bath, gas syringe, and conway petri dish, spectrophotometer, and centrifuge. This research was conducted at the BPTBA LIPI, Gunungkidul, Special Region of Yogyakarta.

2.2. Methods

2.2.1. Optimization of BSA protein binding with tannin from gambir extract. Protein binding capacity testing used tannin protein BSA solution precipitation method according to [1]. Samples were incubated for 15 min, read the absorbance at a wavelength of 510 nm. The reading results was entered into the regression equation Y=2.0987X-0.0088 which was obtained from the standard tannin acid to determine protein precipitation with tannins.

2.2.2. In vitro incubation. The ration used is a mixture of concentrates and forage of Pennisetum hybrid with a ratio of 70:30 using an iso protein system which considers the protein content of 14% and 65% of TDN according to [6]. In vitro incubation of feed samples refers to [7] method. Samples were incubated for 4 hours at a water bath temperature of 39°C. Supernatant results of incubation in the second and fourth hours were used for analysis of VFA production, NH₃ concentration and microbial protein. Gas production measurement refers to the [8] method. The estimation of this calculation used Orskov exponential equations [9]. Where P value was cumulative gas production (ml). Maximum gas production in ml (a+b), production gas rate in ml/h (c), and incubation time (t).

\[ P = a + b \left( 1 - \exp(-ct) \right) \]

2.2.3. Statistical analysis. This experiment was used factorial Randomized Block Design (RBD) with first factor was level supplementation of gambir extract, treatments consisted of: RC0 (0%), RC2 (2%), RC4 (4%) and RC6 (6%) of gambir extract level in mix rations. The second factor is the duration of fermentation of 2 and 4 hours and rumen fluid from different cattle as a group. The variables observed were: 1) production of volatile fatty acids (VFA) using gas chromatography (GC 2010 Plus, Shimadzu Crop., Kyoto, Japan) [10], 2) NH₃ concentrations referring to the Conway micro diffusion method [10], and 3) microbial proteins [11] followed by the Lowry method [12]. While for the in vitro gas production kinetics parameters using a Completely Randomized Design (CRD) with the treatment factor the level of gambir extract supplementation. Each treatment was repeated 5 times. The data obtained were analyzed using ANOVA and continue to post tests using of Least Significant Differences (LSD) [13].
3. Result and discussion
Gambir extract in this study had a tannin content of 52.23% of wb. Previous research stated that the type of tannin contained in gambir extract is condensed tannin (CT). [5] reported that the CT content of gambir extract was 43.15 g/kg. Gambier extracted using hot water has a condensed tannin content of 66.96% of wt [14]. The results of the binding optimization of BSA protein with tannins from gambir extract showed very significant results (P<0.05) shown in Table 1. The concentration of BSA protein that was successfully bound increased with increasing levels of gambir extract which was 10.09 mg/ml of BSA protein at the level of gambir extract 180 mg.

| Table 1. Determination of protein concentration was binding with gambir extract |
|-------------------------------------------------|
| Gambir extract (mg) | Concentration of BSA protein was bound (mg/ml) |
|---------------------|-----------------------------------------------|
| 0                   | 0.01\(^a\)                                    |
| 60                  | 3.36\(^b\)                                    |
| 120                 | 6.88\(^c\)                                    |
| 180                 | 10.09\(^d\)                                   |

Description: Different superscripts in the same column showed significantly different (P<0.05).

Tannin is a secondary plant metabolites compound that plays a role in reducing the quality of materials by forming complex bonds with proteins. Tannin-protein complexes occur because of the presence of hydrogen bonds, hydrophobic interactions, and covalent bonds between these compounds. The existence of a number of functional groups on tannins causes the deposition of proteins. Complex compounds between tannins and proteins formed do not dissolve in the rumen, but dissolve in the acidic atmosphere in the abomasum, the complex undergoes enzymatic digestion so that proteins can be utilized by animal [15].

Different feed formulations have a significant effect on total gas production (P), maximum gas production (a+b), and cumulative gas production rate (c) (P<0.05). There was a tendency to decrease gas production along with increasing levels of gambir extract. The highest total gas production during 48 hours incubation was found in RC0 feed (without the addition of gambir extract) as much as 49.28 ml, while the lowest gas production was produced by RC6 feed which was 47.07 ml shown in Table 2. Gambir extract supplement at level 2% (RC2) has a better influence on total gas production and maximum gas production compared to other gambir extract level treatments. This can be seen from the higher gas production speed (c) which is equal to 0.082 ml/hour. Similar results have also been reported by [16] that in vitro gas production decreased with increasing tannin levels from Acacia nilotica on feed during 24-hour incubation (P<0.05). [17] reported that condensed tannin found in plants can reduce gas production compared to the hydrolized tannin.

| Table 2. Gas production (48 h), maximum gas production (a+b), and gas production rate (c) |
|-------------------------------|---------------------------------|-----------------|
| RC0  | 49.28±0.39\(^a\) | 50.23±0.42\(^a\) | 0.083±0.003\(^a\) |
| RC2  | 48.41±1.33\(^ab\) | 49.40±1.46\(^ab\) | 0.082±0.004\(^a\) |
| RC4  | 47.40±1.01\(^bc\) | 48.46±1.00\(^b\) | 0.079±0.001\(^ab\) |
| RC6  | 47.07±0.66\(^c\) | 48.28±0.71\(^b\) | 0.077±0.003\(^b\) |

Description: RC0 (0%); RC2 (2%); RC4 (4%) and RC6 (6%) of gambir extract level in mix rations; P= cumulative gas production after incubation 48 h (ml); a+b=maximum gas production (ml); c= production gas rate (ml/hour). Different superscripts in the same column showed significantly different (P<0.05).

Supplementation of gambir extract tends to reduce gas production kinetics during incubation, it was due to tannins interact with feed components such as protein and fiber [18]. The slowing down degradation of proteins and fibers results in the inhibition of gas production which is a by-product of nutrient fermentation [19]. Feed treatment without addition of gambir extract has the highest gas production. Gas production will reach a faster peak if more soluble and easily degraded fractions [20].
The effect of supplementation of gambir extract in rations on ammonia (NH$_3$) concentrations, microbial proteins, and total VFA is shown in Table 3. Ammonia (NH$_3$) and microbial protein concentration increased with increasing levels of gambir extract at fermentation time of 2 h and 4 h (P<0.05). The increased concentration of NH$_3$ is thought to be a result of the high protein content in the ration mixture and the contribution of protein values from gambir extract, so that rumen microbes can degrade protein. Ammonia concentration reflects a large amount of protein ration in the rumen and its value is strongly influenced by the ability of rumen microbes to degrade protein rations [21].

Table 3. Effect of gambir extract supplementation on NH$_3$ concentration, microbial protein, and VFA

| Fermentation time | NH$_3$ concentration (mM) | Mean | Microbial Protein (mg/100 ml) | VFA Total (mM) | Mean |
|-------------------|---------------------------|------|-------------------------------|----------------|------|
|                   | RC0 | RC2 | RC4 | RC6 | Mean | 2 h | RC0 | RC2 | RC4 | RC6 | Mean | 2 h | RC0 | RC2 | RC4 | RC6 | Mean | 4 h | RC0 | RC2 | RC4 | RC6 | Mean | 4 h |
| 2 h               | 3.53 | 4.41 | 4.90 | 4.39 | 4.31$^c$ | 20.50 | 22.90 | 32.30 | 36.90 | 28.15$^c$ | 15.51 | 13.21 | 13.57 | 14.68 | 14.24 |
| 4 h               | 5.12 | 4.41 | 5.24 | 5.70 | 5.12$^d$ | 22.90 | 21.20 | 28.00 | 27.40 | 24.88$^d$ | 14.75 | 14.38 | 15.08 | 16.15 | 15.09 |
| Mean              | 4.32$^a$ | 4.41$^a$ | 5.07$^b$ | 5.04$^b$ | | 21.7$^a$ | 22.05$^a$ | 30.15$^b$ | 32.15$^b$ | |

Description: RC0 (0%), RC2 (2%), RC4 (4%) and RC6 (6%) of gambir extract level in mix rations. Different superscripts in the same column and row in each parameters showed significantly different (P<0.05).

Ammonia (NH$_3$) produced in the rumen was used for the synthesis of rumen microbial proteins [22]. Microbial proteins may supply from 50 to 100% of the metabolism protein required by beef cattle and it considered of quality due to its intestinal digestibility and amino acid profiles [6]. Total Volatile Fatty Acid (VFA) is not influenced by the level of addition of gambir extract (P>0.05). According to [23] reported that rations containing CT from quebracho extract did not affect the total VFA concentration in the rumen, but affected the proportion of propionic acid.

4. Conclusion
Gambir extract has tannin which has the ability to bind protein BSA with highest concentration 10.09 mg/ml in 180 mg of gambir extract. Supplementation of gambir extract levels up to 6% can increase the concentration of ammonia and microbial proteins, however it decreases in vitro gas production.

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