Effect of Pollard and Soybean Meal Protected with Condensed Tannin in Concentrate on In Vitro Gas Production

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Abstract. The method used in this study was in vitro gas production. Data were analyzed using a nested randomized block design with 4 inclusion levels of condensed tannin (CT) in pollard (P) and soybean meal (SBM). The treatments consisted of P0: pollard + 0% CT, P2: pollard + 2% CT, P4: pollard + 4% CT, P6: pollard + 6% CT, SBM0 = SBM + 0% CT, SBM2: SBM + 2% CT, SBM4: SBM + 4% CT, SBM6: SBM + 6% CT. The diet consisted of maize stover and concentrate. Variables measured were in vitro cumulative gas production, b value (gas production from undissolved material but potentially degraded), and c value (gas production rate from potentially degraded material). The results indicated the highest cumulative gas production was achieved in P0 and SBM0 with 140.89 and 125.83 ml/500 mg dry matter, respectively. While the lowest one was in P6 and SBM6 with 106.10 and 100.26 ml/500 mg dry matter, respectively. The highest b value was observed in P0 and SBM0 with 154.14 and 139.01 ml/500 mg dry matter, respectively. The c value was not significantly different among treatments. It could be concluded that the CT inclusion is beneficial to decrease in vitro cumulative gas production and b value of pollard and soybean meal.

1. Introduction
Forage is the principal feed for ruminants. However, most of the available forages in Indonesia have low nutrient content so that it cannot fulfill the nutrient requirement of livestock for maintenance and production. For this reason, concentrate feed is supplied as a forage complement to meet nutrient requirements of livestock. Generally, concentrate feed is derived from energy and protein sources such as wheat pollard (Triticum aestivum) and soybean meal (Glycine max), respectively.

Pollard is a by-product of wheat flour processing. According to [13], pollard is a combination of wheat husk and endosperm. Pollard has a high potency to be used as an animal feed because of its abundant availability and good nutrient content. The dry matter (DM), ash, crude protein (CP), ether extract (EE), and crude fiber (CF) contents of pollard were 89.66%, 3.49%, 15.15%, 5.18%, and 7.08%, respectively [3]. On the other hand, soybean meal is a by-product of soybean oil processing. Soybean meal has a high nutrient content so that it can be used as a protein source in the feed. According to [14], the DM, organic matter (OM), CP, EE, CF, nitrogen-free extract (NFE), and total digestible nutrient (TDN) contents of soybean meal were 87.16%, 80.35%, 49.09%, 2.66%, 5.94%, 35.30%, and 83.20%,
respectively. The high nutrient content in soybean meal, particularly CP content, will not be optimally utilized for livestock productivity if it has a high rumen degradable protein (RDP) value. Previous studies showed that the RDP value of soybean meal was 92% [4], while the RDP value of pollard was 89.49% [2]. A high RDP value indicates that a lot of CP content in feed will be degraded in the rumen to ammonia, which then will be converted to urea in the liver and eventually excreted through urine [11]. One strategy that can be used to protect the CP content in the feed is by using tannin treatment, especially condensed tannin (CT). The CT or proanthocyanidins are polymeric bonds that are not branches of the flavonoid group (Flavan-3-ol, Flavan-3,4-diol), with a molecular weight of 1000-20000 Dalton [6]. The CT could bind peptide protein, ionic groups, and hydroxyl groups from the feed. The dominant bond of the CT-Protein complex is peptide bonds that are very unstable at pH < 3.0 and pH > 8.0 [7]. This condition causes the CT-Protein bond is stable in the rumen and becomes unstable when it reaches abomasum. Therefore, the use of CT in feed is very efficient to reduce RDP value, which will contribute to the increase in the conversion of amino acids in the small intestine [10].

2. Materials and methods

2.1. Materials

The materials used in this study were pollard, soybean meal, and mimosa bark extract (CT source). Mimosa bark extract was usually used as a leather tanning material (South African products). One kg of pollard or soybean meal was mixed with 250 ml of water. After that, CT in the form of mimosa bark extract was added according to the treatment. The mixture was then oven-dried at 60°C for 12 hours. The treatments used were:

- P0 = pollard + 0% CT (control)
- P2 = pollard + 2% CT
- P4 = pollard + 4% CT
- P6 = pollard + 6% CT
- SBM0 = soybean meal + 0% CT (control)
- SBM2 = soybean meal + 2% CT
- SBM4 = soybean meal + 4% CT
- SBM6 = soybean meal + 6% CT

2.2. Methods

The method used in this study was in vitro gas production with a nested randomized block design. The variables measured in this study were cumulative gas production, b value (gas production from undissolved material but potentially degraded), and c value (gas production rate from potentially degraded material). Incubation intervals used during gas production measurements were 0, 2, 4, 8, 16, 24, 36, 48, and 72 hours. The formula for calculating gas production was as follow:

\[ G_t = (V_t - V_0 - V_{blank}) - C_f \]

Notes:
- \( G_t \) = gas production at t hour (ml)
- \( V_t \) = volume at t hour
- \( V_0 \) = volume at 0 hour
- \( V_{blank} \) = volume of blank
- \( C_f \) = correction factor
2.3. Data analysis
Data were analyzed using analysis of variance, with the use of error value between 1 to 5%. If the analysis of variance showed significant differences, the data were further analyzed proceeded with Duncan’s multiple range test.

3. Results and discussion

3.1. Cumulative gas production
The results of in vitro cumulative gas production in pollard and soybean meal treated with 0, 2, 4, and 6% CT with the incubation period of 2, 4, 8, 16, 24, 36, 48 and 72 hours are presented in Table 1 and 2, respectively. Based on the data in Tables 1 and 2, CT treatment showed a lower cumulative gas production compared to the control. This result indicates that CT could protect protein content in pollard and soybean meal so that it can reduce the fermentation process in the rumen.

**Table 1.** Cumulative gas production (ml/500 mg DM) of pollard treated with different levels of CT (0, 2, 4, and 6%)

| Incubation period | P0     | P2     | P4     | P6     |
|-------------------|--------|--------|--------|--------|
| 2 h               | 3.19±0.42 | 2.64±0.14 | 4.32±1.25 | 2.36±1.25 |
| 4 h               | 16.38±1.94 | 15.44±2.34 | 14.63±3.75 | 12.79±4.45 |
| 8 h               | 59.97±9.99 | 59.40±2.34 | 49.74±14.60 | 47.02±13.64 |
| 16 h              | 108.96±4.03<sup>b</sup> | 105.18±0.96<sup>b</sup> | 90.88±9.70<sup>b</sup> | 85.94±10.03<sup>a</sup> |
| 24 h              | 118.82±1.11<sup>b</sup> | 114.23±1.09<sup>b</sup> | 98.41±9.13<sup>a</sup> | 92.89±9.75<sup>a</sup> |
| 36 h              | 130.48±0.56<sup>c</sup> | 199.65±1.50<sup>b</sup> | 103.73±9.40<sup>a</sup> | 99.01±9.75<sup>a</sup> |
| 48 h              | 136.30±0.04<sup>c</sup> | 124.68±1.24<sup>b</sup> | 108.73±9.40<sup>a</sup> | 103.60±10.73<sup>a</sup> |
| 72 h              | 140.89±0.97<sup>c</sup> | 128.00±0.93<sup>b</sup> | 112.36±9.12<sup>a</sup> | 106.10±10.45<sup>a</sup> |

<sup>a,b,c</sup>different superscript within the same row indicates a significant difference (P<0.05)

The addition of CT in pollard had a significant effect (P<0.05) on cumulative gas production at the incubation period of 16, 24, 36, 48 and 72 hours, with the lowest value observed in 6% CT treatment (Table 1). The CT treatment on soybean meal also showed a significant effect (P<0.05) on cumulative gas production in the incubation period of 2, 4, 24, 36, 48, and 72 hours, where 6% CT could reduce the fermentation value compared to the control (Table 2). The decrease in cumulative gas production in the treated sample showed that CT could protect protein content so that it can by-pass to the small intestine.

**Table 2.** Cumulative gas production (ml/500 mg DM) of soybean meal treated with different levels of CT (0, 2, 4, and 6%)

| Incubation period | SBM0     | SBM2     | SBM4     | SBM6     |
|-------------------|----------|----------|----------|----------|
| 2 h               | 4.04±0.70<sup>b</sup> | 2.93±0.42<sup>a</sup> | 4.32±0.97<sup>b</sup> | 2.23±0.83<sup>a</sup> |
| 4 h               | 13.66±0.27<sup>b</sup> | 10.88±1.11<sup>a</sup> | 15.33±1.12<sup>b</sup> | 8.87±0.97<sup>b</sup> |
| 8 h               | 39.29±4.47 | 35.57±6.83 | 36.80±4.20 | 30.13±2.26 |
| 16 h              | 73.85±10.89 | 69.32±14.07 | 66.34±12.85 | 57.87±8.55 |
| 24 h              | 94.63±7.69<sup>c</sup> | 87.45±5.42<sup>b</sup> | 82.65±5.47<sup>b</sup> | 70.14±4.10<sup>a</sup> |
| 36 h              | 112.73±3.52<sup>c</sup> | 103.79±7.78<sup>b</sup> | 101.06±5.78<sup>b</sup> | 83.41±2.30<sup>a</sup> |
| 48 h              | 121.37±3.51<sup>c</sup> | 113.11±5.97<sup>b</sup> | 110.38±4.51<sup>b</sup> | 92.03±0.63<sup>a</sup> |
| 72 h              | 125.83±2.40<sup>b</sup> | 118.83±4.99<sup>b</sup> | 117.49±2.98<sup>b</sup> | 100.26±0.34<sup>a</sup> |

<sup>a,b,c</sup>different superscript within the same row indicates a significant difference (P<0.05)

According to [1], CT is an anti-nutritional compound that could bind protein into the complex compound which is resistant to protease. Therefore, CT can be used as an additional feed ingredient to protect the protein from microbial degradation in the rumen. Additionally, CT can also act as a chelating agent with a spasmylytic effect, which shrinks the intestine resulting in the reduction of intestinal peristalsis. The existence of this spasmylytic effect might also be able to shrink the cell wall or cell membrane so that it could alter the bacterial cell permeability. The impairment of cell permeability could...
inhibit bacterial activities, thus resulting in bacterial growth is hampered or even dead. CT also has antibacterial activity through protein precipitation because CT has the same effect as phenolic compounds. Antibacterial effects of CT include reacting with cell membranes, enzyme inactivation, and destruction or inactivation of genetic material functions [5]. CT can bind to proteins with hydrogen bonds that are sensitive to pH changes. CT-protein bond will stable at pH 4 to 7 in the rumen, whereas at extreme pH ie. pH less than 3 in the abomasum and pH more than 7 in the intestine, CT-protein bond will be released [12].

3.2 Gas production kinetics

The results for gas production from insoluble and potentially degraded fraction and gas production rate of pollard and soybean meal are shown in Tables 3 and 4, respectively. These parameters were used to observe how much feed material that degraded in the rumen as well as to present the degradation rate of CT-protected pollard and soybean meal.

**Table 3.** The b value (gas production from undissolved material but potentially degraded) and c value (gas production rate from potentially degraded material) of pollard treated with different levels of CT (0, 2, 4, and 6%)

| Parameters | P0                | P2                | P4                | P6                |
|------------|-------------------|-------------------|-------------------|-------------------|
| b (ml/500 mg DM) | 154.14±0.19c   | 141.63±0.13b    | 122.44±8.55a   | 117.25±9.63a   |
| c (ml/h)   | 0.078±0.009       | 0.086±0.004     | 0.084±0.010     | 0.083±0.009     |

A-c different superscript within the same row indicates a highly significant difference (P<0.01)

Gas production potency is the value used to see the potency of OM that can be digested in the rumen. Tables 3 and 4 show that CT had a highly significant effect (P<0.01) on the b value of pollard and soybean meal, respectively. The highest b value was found in P0 and SBM0 with 154.14 and 139.01 ml/500 mg DM, respectively. This result because of those treatments had no tannin addition so that they were easily degraded in the rumen. In the previous studies, it was found that pollard and soybean meal were easily degraded in the rumen with the RDP value of 89.49% [2] and 92% [4], respectively. On the other hand, the lowest b value in this study was observed in P6 and SBM6 with 117.25 and 108.81 ml/500 mg DM, respectively. The lowest b value indicates that the feed is not easily degraded in the rumen so that their nutrient content will by-pass to the small intestine.

**Table 4.** The b value (gas production from undissolved material but potentially degraded) and c value (gas production rate from potentially degraded material) of soybean meal treated with different levels of CT (0, 2, 4, and 6%)

| Parameters | SBM0                 | SBM2                 | SBM4                 | SBM6                 |
|------------|----------------------|----------------------|----------------------|----------------------|
| b (ml/500 mg DM) | 139.01±1.95c | 135.73±5.39bc | 127.07±1.49b | 108.81±1.59a |
| c (ml/h) | 0.053±0.008         | 0.064±0.016         | 0.049±0.009         | 0.049±0.009         |

A-c different superscript within the same row indicates a highly significant difference (P<0.01)

According to [16], the presence of CT in the feed will form a complex bond with protein, resulting in the reduction of b value. In a study by [8], it was showed that the average b value of saponin-protected feedstuffs ranged from 130.58 to 136.63 ml/500 mg DM. This result indicates a decrease as well as an increase in b value that occurs from CT addition to the feed, which may be due to differences in tannin sources and supplementation level [15].

As can be seen in Tables 3 and 4, CT treatment had no significant effect (P>0.05) on c value of pollard and soybean meal, respectively. The highest c value in pollard and soybean meal was observed in P2 and SBM2 treatments with 0.086 and 0.064 ml/h, respectively. Previously, [9] stated that the high gas production rate indicating the high feed degradation by microbes in the rumen. Therefore, the treatment with a low c value is preferable. In pollard, P0 gave the best results with c value of 0.78 ml/h, while in soybean meal, SBM4 and SBM6 gave the best result with c value of 0.049 ml/h.
4. Conclusion
The inclusion of condensed tannin in pollard and soybean meal could decrease their degradability in the rumen. The use of 6% condensed tannin provides the best result on the reduction of cumulative gas production. In pollard, the use of condensed tannin at such level beneficially reduces cumulative gas production from 140.89 ml/500 mg dry matter, while in soybean meal reduce from 125.83 become 100.26 ml/500 mg dry matter.

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