Effects of spinach tree leaves and high concentrates diets supplemented with micro minerals on *in vitro* rumen fermentation profiles

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Abstract. The effects of different level Spinach tree leaves *Cnidoscolus aconitifolius*, and high concentrate diets supplemented with micro minerals on in vitro rumen fermentation profiles were investigated. The basal diets consisted of 30% king grass (*Pennisetum* hybrid), and 70% concentrate. The mineral mix consists of FeCl₃.4H₂O; MnCl₂.4H₂O; CuSO₄.5H₂O; ZnSO₄.7H₂O; and CoCl₂.6H₂O. The treatments were Basal Ration (B), B+5% *C. aconitifolius* leaf (BC), B+2% mineral mix (BM), and B + 5% *C. aconitifolius* leaf + 2% mineral mix (BCM). The treatment diets were incubated for 48 h. Completely Randomized Design with one-factor consists of four treatments, and three replications were used in this experiment followed by One Way Analysis of Variance for statistical analysis. The gas production, N-ammonia, propionate, dry matter, and organic matter digestibility decreased significantly (p<0.05) by the treatment of mineral mix BM, BCM with the lowest value was found on BCM. Rumen microbial protein synthesis and pH was not influenced by the treatments of spinach tree alone (BC) (p>0.05) but increased significantly (p<0.05) when mineral mix treatments BM and BCM with the highest value at BCM. No effect (p>0.05) were observed for Protozoa population, methane production, acetate, and butyrate proportion. It was concluded that supplementation of *C. aconitifolius* leaves to the high concentrate diet has no effect on the fermentation variables whereas it combination with micro minerals reduced the fermentability and increased microbial protein synthesis in the rumen.

1. Introduction

Spinach tree leaf (*Cnidoscolus aconitifolius*) has a high protein content (around 18.74%) [1], and hence it is potential to be used as a feed for nitrogen source for ruminants. On the other hand, it also has a tannin content which has the potential as an anti-nutrient that could limit the usage as animal feed [2]. Besides the common anti-nutritive, i.e., the capability to bind with other feed compounds mostly protein so could alter the digestibility, the presence of tannin could lead to others function in the ruminant such us anti-microbial, anti-parasitic and antioxidant [3]. With almost the same function, tannin is also used in monogastric animals [4].

Feeding too much concentrates diet that has less fiber content to ruminant may lead to rumen upset and may disrupt the rumen functions like rumen acidosis, the condition which distinguished by the rapid declining acidic value of rumen liquid, bothering the optimal state for most rumen microbes. The conditions that occur due to the fast production of lactic acid as the impact of
degrading high concentration of fermentable carbohydrate by rumen microbes. Some of the ruminant microbes, which could influence rumen acidosis such as fibrolytic, amylolytic, were altered by tannin [5]. Addition of buffering mineral mix in concentrated feed for early lactating cows could prevent the subacute rumen acidosis [6]. At this study, we reported the result of in vitro ruminal fermentation characterization of high concentrate ration consists of spinach tree (*Cnidoscolus aconitifolius*) leaves and micro minerals supplementation.

2. Materials and methods
An *in vitro* gas production technique using glass syringes for 48 hours incubation time as described by [7] which was arranged on the completely randomized design. Rumen liquid was prepared from two fistulated Ongole Crossbreed cattle daily feeding with king grass (*P. hybrid*), wheat meal, molasses, urea, and minerals. The samples incubated were the samples consist of 4 treatments i.e. Basal ration (B), B + 5% Spinach tree leaf (*C. aconitifolius*) (BC), B + 2% mineral mix (BM), B + (5% Spinach tree leaf (*C. aconitifolius*) + 2% micro minerals mix (BCM) as described in Table 1, while the composition of the mineral mix was formulated according to Sofyan *et al.* [8] and presented in Table 2. All the treatments sample was repeated triplicate. By Using Neway Excel Program ver.6 [9], the ten times point for total 48 hours observation was fitted by an exponential model [10] to measure the complete gas production from the incubation. The response variables measured were gas production, dry matter digestibility, organic matter, pH, partial volatile fatty acid (by gas chromatography), i.e., acetic acid, propionic acid, butyric acid, N-ammonia [11], microbial protein synthesis [12], protozoa population [13] and methane production [14]. The data results were statistically analyzed by one way analysis of Variance using CoStat statistical software [15].

Table 1. Basal ration formula

| Feedstuff                          | Unit | B   | BC  | BM  | BCM |
|-----------------------------------|------|-----|-----|-----|-----|
| King grass (*P. purpureoides*)     | % DM | 30.00| 30.00| 30.00| 30.00|
| Spinach tree leaf (*C. aconitifolius*) | % DM | 0.00| 5.00| 0.00| 5.00|
| Wheat meal                        | % DM | 10.50| 8.50| 10.50| 8.50|
| Soybean meal                      | % DM | 14.30| 12.10| 14.30| 12.10|
| Corn                              | % DM | 44.20| 43.40| 44.20| 43.40|
| CaCO₃                             | % DM | 1.00| 1.00| 1.00| 1.00|
| Mineral mix                       | % DM | 2.00| 2.00|     |     |

B = basal ration; BC = B + 5% spinach tree (*C. aconitifolius*); BM = B + 2% mineral mix; BCM = B (5% spinach tree (*C. aconitifolius*) + 2% mineral mix

Table 2. Composition of mineral mix composition

| Micro-mineral   | (%)  |
|----------------|------|
| FeCl₂.4H₂O     | 0.59 |
| MnCl₂.4H₂O     | 23.80|
| CuSO₄.5H₂O     | 32.75|
| ZnSO₄.7H₂O     | 42.22|
| CoCl₂.6H₂O     | 0.64 |
| Amount         | 100.00|
3. Results and discussions

All the variables respond result was described in table 3. Gas production values showed a different group at B, and BC treatment with higher gas production versus BM, and BCM treatment with lower gas production, it indicated that the mineral mix treatment provided a significant result (p<0.05) decreasing the gas production. N-Ammonia, Dry Matter, and Organic Matter digestibility were also decreased (p<0.05) by the treatment of mineral mix (BM, BCM). Acetic acid, microbial protein synthesis was decreased (p<0.05) by the treatment of mineral mix (BM, BCM). C3 (propionate proportion) was decreased by BCM treatments. Protozoa population, and estimated methane production were not affected by the treatment (p>0.05). Supplementation of C. aconitifolius alone on high concentrate ration does not provide significant effect (p>0.05) in all variables compared to the control ration (B).

Table 3, Effect of high concentrate diet consisting of spinach tree (Cnidoscolus aconitifolius) leaves supplemented with micro minerals on rumen fermentation

| Variables    | Unit | B          | BC         | BM          | BCM         |
|--------------|------|------------|------------|-------------|-------------|
| Gas Production | ml/200 mg DM | 88.87 ± 0.977<sup>a</sup> | 85.98 ± 2.538<sup>a</sup> | 49.56 ± 7.919<sup>b</sup> | 48.01 ± 2.338<sup>b</sup> |
| pH           |      | 6.70 ± 0.031<sup>c</sup> | 6.68 ± 0.015<sup>a</sup> | 6.81 ± 0.061<sup>b</sup> | 6.88 ± 0.052<sup>c</sup> |
| NH<sub>3</sub> | mM   | 29.73 ± 3.371<sup>a</sup> | 29.45 ± 1.233<sup>a</sup> | 8.44 ± 0.935<sup>b</sup> | 12.36 ± 2.269<sup>b</sup> |
| MPS          | mg/ml | 0.57 ± 0.267<sup>b</sup> | 0.46 ± 0.289<sup>b</sup> | 2.69 ± 0.478<sup>b</sup> | 2.24 ± 0.191<sup>c</sup> |
| Protozoa     | Log cell/ml | 4.951 ± 0.0605 | 5.133 ± 0.1985 | 4.81 ± 0.3880 | 4.773 ± 0.3708 |
| Methane      | mM   | 9.31 ± 1.914 | 8.76 ± 1.403 | 6.57 ± 1.807 | 6.32 ± 0.923 |
| DM digestibility | %       | 62.01 ± 6.080 | 60.56 ± 9.647<sup>a,b</sup> | 47.56 ± 3.601<sup>c</sup> | 38.13 ± 8.330<sup>c</sup> |
| OM digestibility | %       | 61.07 ± 6.420 | 60.10 ± 10.758<sup>a,b</sup> | 44.97 ± 4.805<sup>c</sup> | 34.15 ± 9.055<sup>c</sup> |
| VFA partial  |      | 49.16 ± 1.105 | 49.77 ± 1.110 | 49.01 ± 2.642 | 53.42 ± 2.159 |
| C2           | %    | 35.43 ± 1.091<sup>a</sup> | 34.07 ± 1.501<sup>a,b</sup> | 35.03 ± 0.932<sup>a</sup> | 32.16 ± 1.081<sup>b</sup> |
| C4           | %    | 15.41 ± 0.302 | 16.16 ± 1.114 | 15.96 ± 1.721 | 14.42 ± 1.597 |

Mean with different superscript in the same row differ significantly (P<0.05). B = basal ration; BC= B + 5% spinach tree (C. aconitifolius); BM= B + 2% mineral mix; BCM= B (5% spinach tree (C. aconitifolius)) + 2% mineral mix, NH<sub>3</sub> = N-ammonium, MPS= Microbial Protein Synthesis, DM = Dry Matter, OM = Organic Matter VFA=Volatile Fatty Acid; C2=Acetic Acid, C3=Propionic Acid; C4=Butyric Acid. * the estimated methane was calculated by the formula [14].

Supplementation of C. aconitifolius at 5% DM in singular form was not affecting the ruminal fermentation parameter, although it contributed more tannin compound than control ration in this experiment, we measured the tannin content of C. aconitifolius, and found the level at 0.14% and 0.32% for tannin, and condensed tannin respectively. Similar results in previous study [16] reported that supplemented tannin on high grain diet was not affected ruminal fermentation parameter, nor the supplementation of condensed tannin extract of quebracho on low and high forage diet on the dairy cow was not influenced the dry matter digestibility [5]. Effectivity anti-microbial activity of tannin compound depends on the chemical structure, and species-specific microbes [3] this could be the reason why C. aconitifolius has not affected the variables. Metal sulfate bound has been known to have lower solubility in rumen liquid than hydroxyl form, causing lower dry matter digestibility of the diet [17] thus adding CuSO<sub>4</sub>·5H<sub>2</sub>O; ZnSO<sub>4</sub>·7H<sub>2</sub>O as a part of mineral mix in BM, and BCM ration can restore the decrease in dry matter, which is also directly related with decreasing organic matter, gas production, and also propionate proportion. The same result was explaining by the diminished of dry matter disappearance when in-sacco observation of the supplementation of trace mineral Cu, Zn, Mn on corn silage based diet for steer [18]. On the other hand, the addition of catalytic supplement consisting of gelatin, sago, Co, Zn, and ammonium sulfate could support the growth of rumen microbes [19] this explained the increased of microbial protein synthesized of the BM, and BCM
treatments in the present study. Meanwhile, lower degrading dry matter process can produce a stable condition of rumen pH value that could support the growth of rumen microbes. Rumen microbes will use N sourced from available N-ammonia to increase their populations, which in turn could reduce the N-ammonia concentration [20], and this explains why the same condition appears at BM, and BCM treatments.

4. Conclusion
Supplementation of C. aconitifolius leaf on the high concentrate diet does not affect the in vitro ruminal fermentation parameters while its combination with micro-minerals could reduced the fermentability and improve rumen microbial protein synthesis.

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