Effects of cinnamon bark meal (Cinnamomum burmannii Ness ex Bl) as protein protection agent on in vitro rumen fermentation characteristic

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Abstract. This experiment aimed to investigate the effect of protein protection on diet contained cinnamon bark meal as cinnamaldehyde source on rumen fermentation characteristics and in vitro gas production kinetics. Five experimental diets (Pennisetum purpureum (60%): wheat pollard (30%): soybean meal (10%)) added with cinnamon bark meal equal to cinnamaldehyde level as much as 0, 200, 400, 600, 800 mg based on dry matter (DM) and each treatment was replicated for 3 times. Fermentation parameters were measured by incubating the sample in a rumen liquor buffer that was taken from a rumen fistulated Bali cattle using Menke and Steingass gas production technique. The gas produced was recorded at 2, 4, 6, 8, 12, 24, 36 and 48 h of the incubation. The kinetics of gas production was analyzed using the Fit Curve. Results of this experiment showed that there were no significant (P>0.05) different among treatments on the rumen fermentation characteristic (pH, NH₃, total volatile fatty acid (VFA) production, and molar proportions of the main VFA), total gas production and kinetics of gas production. It was concluded that adding the cinnamon bark meal as cinnamaldehyde source was no adverse effects on rumen fermentation.

1. Introduction

Dietary protein is extensively degraded in the rumen to polypeptides and amino acids and then deaminated to ammonia (NH₃). There are losing the potency of dietary protein as a source of amino acids for the ruminant animal. Protein degradation in the rumen will reduce the biological value of feed protein. Therefore, proteins need to be protected so that more amino acids are available in post-rumen digestion. Protected protein in the rumen could increase the amino acid profile of the protein reaching the small intestine for digestion and absorption [1].

Plants synthesize an array of secondary metabolite to defend themselves against exogenous abiotic or biotic constraints that to have no direct function in the plant growth [2]. Plant secondary metabolite could be exploited as natural safe feed additives without adversely affecting rumen fermentation [3]. Cinnamaldehyde is one of plant secondary metabolite of cinnamon trees to reduce the rumen biodegradability of proteins making its use possible to decrease proteases and deaminases activity [4,5]. Rumen undegradable protein can increase dietary protein to post rumen tract. However, cinnamaldehyde could form bonds with various reactive groups on protein molecules (including digestive enzymes) as it passes along the intestines, thus counteracting the benefits of rumen undegradable protein. This may probably be caused interference of cinnamaldehyde with enzymes or...
microbial activity to produce gas which is a by-product of fermentation process in the rumen. Measurement by in vitro gas production, provide valuable information about the fermentation process and kinetics of diet digestion in rumen fluid [6]. Related to this problem, cinnamon bark meal as cinnamaldehyde source is considered to be a promising natural compound to increase protein utilization of ruminant diet. This present study aimed to evaluate the effects of cinnamon bark meal as cinnamaldehyde source on rumen fermentation characteristic and in vitro gas production kinetics.

2. Materials and methods

2.1. Sample preparation
In this study, substrates of the fermentation consisted of 60% elephant grass, 30% wheat bran, and 10% soybean meal. Cinnamon bark meal contains cinnamaldehyde for 2.17% (purity >80%). Feedstuffs and cinnamon bark meal were ground to pass through a 1-mm screen. The chemical compositions of samples were analyzed by proximate analysis according to [7]. Rumen fluid was obtained from a rumen fistulated Bali cattle fed a diet consisting of Pennisetum purpureum and wheat pollard 60:40 DM basis TDN 62.01% and CP 13.16% and done in the morning prior feeding.

2.2. In vitro gas production
The mix of feed sample and cinnamon bark meal according to the treatment were incubated in syringe containing buffered rumen fluid as described by [8] in triplicate. The dietary treatments were: P0 (60% elephant grass + 30% wheat bran + 10% soybean meal), P1 (P0+1.16% cinnamon bark meal or equal to cinnamaldehyde with 200 mg/kg DM basis), P2 (P0+2.3% cinnamon bark meal or equal to cinnamaldehyde with 400 mg/kg DM basis), P3 (P0+3.5% cinnamon bark meal or equal to cinnamaldehyde with 600 mg/kg DM basis), P4 (P0+4.5% cinnamon bark meal or equal to cinnamaldehyde with 800 mg/kg DM basis). Calibrated glass syringe of 100 ml contained approximately 300 mg of sample were prewarmed at 39ºC before infused with CO2 gas and the injection of 30 ml of buffered rumen liquor a mixture of artificial rumen fluid and rumen in ratio 2:1 to follow by incubation in a water bath at 39ºC. Gas production was measured at 2, 4, 6, 8, 12, 24, 36 and 48 h post-incubation.

The fermentation kinetics were estimated using a Fit Curve program [9] to obtain the kinetics of gas production, which are: intercept value of initial gas production (a) and represent as gas produced from the fermentation of soluble fraction (%), gas production from the insoluble fraction (b), but slowly degradable fraction (%), potential extent of gas production (a+b) and fractional rate constant of gas production for the insoluble fraction (c). After 48 h incubation, pH, NH3, and volatile fatty acid (VFA) were measured from the liquid media. Determination of NH3 according to [10] and VFA was determined according to [11].

2.3. Data analysis
All of the data were analyzed as a randomized completely design using one-way ANOVA procedure of SPSS ver. 16. Duncan’s Multiple Range Test (DMRT) was used as a post-hoc analysis described by [12]. Comparison test and significance level were declared at P<0.05.

3. Results and discussion

3.1. Rumen fermentation characteristic
Rumen fermentation characteristic of cinnamon bark meal addition as cinnamaldehyde source with different level are shown in Table 1. Table 1 showed the different level of cinnamon bark meal treatments no effect on ruminal pH, NH3, total VFA, proportions (%) of acetate, propionate, butyric, acetic and butyric ratio; (Table1). The pH of the media after 48 h past of incubation was not affected by addition cinnamon bark meal as cinnamaldehyde source. pH medium ranged from 6.73 to 6.75, and it is in the physiology pH for activity rumen microbes [13]. No such imbalance was found due to
addition of cinnamon bark oil and its main component cinnamaldehyde in the diet. The results are consistent with other studies where rumen pH values did not differ on addition of cinnamaldehyde in dairy cows [14,15] and beef cattle [16].

Table 1. Rumen fermentation characteristic of cinnamon bark meal addition as cinnamaldehyde source.

| Parameter                  | T0     | T1     | T2     | T3     | T4     |
|----------------------------|--------|--------|--------|--------|--------|
| pH                        | ns     | 6.73   | 6.75   | 6.75   | 6.74   |
| VFA (mM)                  | ns     | 98.50  | 96.57  | 96.07  | 94.84  | 92.83  |
| Acetic acid (%)           | ns     | 76.40  | 76.49  | 76.18  | 76.01  | 76.24  |
| Propionic acid (%)        | ns     | 15.70  | 15.52  | 15.79  | 15.91  | 15.56  |
| Butyric acid (%)          | ns     | 7.90   | 7.99   | 8.03   | 8.07   | 8.20   |
| Acetic:propionic           | ns     | 4.87   | 4.93   | 4.83   | 4.78   | 4.90   |
| NH3 (mg/100ml)            | ns     | 54.92  | 53.54  | 53.27  | 53.11  | 52.39  |

ns Non significant.

Low rumen pH for prolonged periods below 5.5 can negatively affect feed intake, microbial metabolism, and nutrient degradation [17]. Benchar et al. [18] reported that the addition of cinnamaldehyde increased ruminal pH when dairy cows received cinnamon leaf oil at 400 mg/L. On the contrary, Vakili et al. [19] showed that the addition of cinnamon oil 5g/day reduced ruminal pH in high-concentrate diet. The observed lower concentration of rumen pH after feeding can be linked to higher VFA concentration in the rumen, which is due to the negative relationship between VFA concentration and pH in rumen fluid [20]. Their conflicting results can be partially described by the type of diets used because there is the main difference in diets among dairy cattle and beef cattle. The pH values were correlated with the high doses and could be partially described by the type of diets used of these studies. Buffer activity in the rumen was relatively high when the forage composition is greater than the concentrate in diet [19].

Total VFA concentration and molar proportions of the main VFA (acetate, propionate, and butyrate) were unaffected by cinnamon bark meal addition. The mean concentration of total VFA in experimental treatments ranged from 92.83 to 112 mM and similar in all dietary treatments (Table 1). VFA reflects rumen digestion and fermentation, whereas VFA concentration on in vitro fermentation is a direct measure of feed digestion [21]. There were no effects on VFA concentration (total and individual molar proportions) in our study was consistent with no change on in vitro dry matter and organic matter digestibility (data not shown) due to cinnamon bark meal addition. The effect of cinnamon bark meal and its main active component cinnamaldehyde on VFA concentration is agreed with the results in the current study no significant effects on nutrient digestibility and ruminal NH3 concentration were observed. Similar effect was also found by [22] using low doses of cinnamaldehyde in batch culture; and Cardozo et al. [23] who reported that supplementation of cinnamaldehyde (132 or 264 mg/L) and a mixture of 0.18 g/d of cinnamaldehyde and 0.09 g/d of eugenol did not alter total VFA production or molar proportion of individual VFA. These experiments and many of in vitro studies have suggested that the effects of cinnamaldehyde on total VFA concentration seem to be diet and pH-dependent [24,25]. Cardozo et al. [26] observed the effects of cinnamaldehyde and eugenol in vitro using rumen fluid from beef cattle fed a 10:90 forage to concentrate diet. At pH 7.0, all these compounds increased acetate to propionate ratio and decreased total VFA concentration. In contrast, at pH 5.5, total VFA concentration increased, and the acetate: propionate ratio declined with cinnamaldehyde and eugenol supplementation. Juven et al. [27] previously reported that the effect of cinnamaldehyde enhanced as pH decreased from 6.5 to 5.5. The impact of pH on the response of cinnamaldehyde might be related to the dissociated (hydrophilic) or undissociated (hydrophobic) status of the active molecules. The undissociated form of the molecule can interact with feed particles in the rumen [28]. Consequently, cinnamon bark meal and their main
active components cinnamaldehyde have the potential to improve rumen VFA profile at low rumen pH.

As data are shown in Table 1, the addition of cinnamon bark meal as cinnamaldehyde source did not effect on ammonia (NH$_3$) concentration and averaged 52.39 to 54.92 mg/100ml. Cinnamaldehyde had variable impacts on ruminal NH$_3$ concentration in the different studies. Busquet et al. [29] evaluated the effects of increasing doses (0, 3, 30, 300, 3000 mg/L) of cinnamon oil on rumen fermentation in an in vitro batch culture fermentation and reported that at the highest dose (3,000 mg/L), these compounds decreased the ruminal concentration of NH$_3$, but no effects were observed at lower doses. Our results are not consistent with results of Mateos et al. [30] who demonstrated that increasing levels of CIN (540 mg/L) in a batch culture system linearly decreased NH$_3$ concentration in medium-concentrate diet concentrations. Results from different in vitro studies showed that the effects of cinnamaldehyde on rumen NH$_3$ concentration are dose-dependent and that negatively affected fermentation when used at high doses compared with at low doses. Most of the plant metabolites secondary and its main active component modified rumen fermentation by changing NH$_3$ production when fed at high doses [28]. Accordingly, results suggested that the addition of cinnamon bark meal as cinnamaldehyde source in ruminant diets had no detrimental effect on NH$_3$ concentrations at least within the range of the dosage level which used in this study. Although NH$_3$ concentration was not affected by the addition of cinnamon bark meal as cinnamaldehyde source, the reduced the degradability of protein in the rumen suggested that the deamination process was not inhibited. This study showed that the decrease on degradability of protein in rumen indicating that cinnamaldehyde was able to bind protein that is resistant to proteolytic enzymes from rumen microbes. Rumen protected protein could be available in post rumen as a protein bypass for ruminants. It increased the availability of feed proteins for digestion and more amino acids are absorbed in the small intestine [28]. Based on these findings, the addition of cinnamon bark meal up to 4.6% or equivalent to cinnamaldehyde with 800 mg per kg DM basis did not inhibit rumen microbial fermentation.

3.2. Kinetics of gas production

The values for kinetics of gas production parameters are shown in table 2 and total gas production for each of treatments was presented as gas production curve (Figure 1) (ml/ mg DM).

| Variable | T0  | T1  | T2  | T3  | T4  |
|----------|-----|-----|-----|-----|-----|
| Total gas production at 48 h | 65.08 | 64.58 | 64.14 | 63.50 | 62.92 |
| a | -1.93 | -1.15 | -1.36 | -2.12 | -1.89 |
| b | 72.75 | 70.80 | 69.80 | 69.40 | 68.31 |
| a+b | 70.82 | 69.64 | 68.43 | 67.28 | 66.42 |
| c | 0.05 | 0.05 | 0.05 | 0.06 | 0.06 |

ns: Not significant

Total gas production was not affected by the addition of cinnamon bark meal as cinnamaldehyde source, which is consistent with the lack of effects of cinnamon bark meal on rumen fermentation characteristic (Table 1). Total gas production is strongly influenced by the rumen microbial activity to degrade carbohydrate in the rumen to produce VFA. Such result might be related to a view that VFA production was no effect of treatments. The absence of effect of cinnamon bark meal on VFA in our study is consistent with the report by [16] in which there was no change on VFA concentration supplied with 800 mg/day cinnamaldehyde in growing beef heifer diets. Gas production is related to volatile fatty acid (VFA) production following substrate fermentation [31]. These findings suggest that the addition of cinnamon bark meal as cinnamaldehyde source in diet does not have negative effect on carbohydrate fermentation.
Figure 1. The effect of cinnamon bark meal (Cinnamomum burmanni Ness ex Bl) addition as cinnamaldehyde source on cumulative gas production at different times of incubation.

Values for kinetics of gas production did not affect treatment (table 2), might due to unchanged of gas production in cinnamon bark meal addition as well as might in consequence of unchanged of VFA production. The intercept value (a) representing gas production from soluble fraction, gas production from the insoluble fraction (b) and potential extent of gas production was not significantly different among treatments (P>0.05). Negative values were obtained for gas production from the soluble fraction (a), similarly reported by [32] when the same mathematical model was used to fit gas production kinetics. They attributed these findings to a delay in fermentation caused by delaying microbial colonization or occurrence of a lag period after the soluble part of the substrate had been consumed but before fermentation of the cell walls had started [33]. These results showed that the addition of cinnamon bark meal as cinnamaldehyde source did not give any adverse effects on rumen fermentation.

4. Conclusion
Addition of cinnamon bark meal as cinnamaldehyde source to the diet up to 4.6% of DM feed or equivalent to cinnamaldehyde with 800 mg per kg DM feed, did not have any detrimental effects on rumen fermentation.

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