Effect of cinnamon bark meal (Cinnamomum burmanni Ness ex Bl) addition as cinnamaldehyde source on in vitro nutrient digestibility

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Abstract. Protection of high-quality protein from rumen microbe degradation by cinnamaldehyde might improve feed protein utilization for ruminants. The study investigated the effect of cinnamon bark meal as a source of cinnamaldehyde on in vitro nutrient digestibility. This research consisted of the diet without (control) and with cinnamon bark meal at 1.16, 2.3, 3.5, and 4.6% of feed dry matter (DM) equivalent to 200, 400, 600 and 800 mg cinnamaldehyde per kg DM. Feed consisted of 60% elephant grass, 30% wheat bran and 10% soybean meal. The experiment was conducted using a completely randomized design with three replications. Data obtained were analyzed by one-way analysis of variance (ANOVA) continued by DMRT. The results showed that the addition of cinnamon bark meal up to 4.6% did not affect dry matter, organic matter and crude fiber digestibility, but decreased (P<0.05) rumen crude protein digestibility (48 h) and increased total crude protein digestibility (96 h). It was concluded that the cinnamon bark meal addition up to 4.6% of DM feed or equal to cinnamaldehyde with 800 mg per kg DM feed could increase crude protein digestibility (96 h) without any adverse effects on in vitro nutrient digestibility.

1. Introduction
Dietary protein could be a limiting factor of nutrient for ruminants. When dietary rumen degradable protein is more than the required amount by rumen microbes, the protein is degraded to ammonia (NH₃), absorbed, metabolized to urea in the liver, and lost in the urine [1]. Under this condition, manipulation of protein degradation in the rumen is the most effective strategy to reduce dietary protein and amino acid losses with rumen-protected protein [2].

Many plants produce secondary metabolites such as tannin, saponin, and cinnamaldehyde that modify proteolysis and nutrient digestibility in the rumen [3–5]. Plant secondary metabolites are dietary phenolics or polyphenols, which present in the plants [6]. Cinnamaldehyde (CIN), a phenylpropanoid, is the main active component of cinnamon oil on cinnamon bark meal [7]. Cinnamon bark meal as cinnamaldehyde source may modify the activity of rumen microbes. Cinnamaldehyde is thought to inhibit peptidolysis of rumen microbes and reduce deamination [8]. Decreasing ruminal protein degradation with cinnamon bark meal will reduce ruminal NH₃, providing true protein to the duodenum for digestion and absorption. However, their strong beneficial effects on nutrient digestibility have also been considered with a negative impact on the digestion process, where they then can exert inhibitory effects on enzymes involved in the degradation of nutrients. It is
important that procedures do not interfere with ruminal metabolism or post-ruminal digestion, but beneficial in improving the feeding value of protein and reducing wasteful NH$_3$ production in the rumen. This the research was aimed to study the cinnamon bark meal addition as a source of cinnamaldehyde on in vitro nutrient digestibility to increase feed efficiency and animal production.

2. Materials and methods

This research was conducted at the Laboratory of Nutritional Biochemistry, Department of Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia. Rumen fluid was obtained from a rumen of fistulated Bali cattle fed a diet consisting of *Pennisetum purpureum* and wheat pollard 60:40 DM basis containing TDN 62.01% and CP 13.16%. Rumen fluid was collected before morning feeding and squeezed through the polyester cloth into a vacuum flask, and immediately sent to the laboratory.

Feedstuffs and cinnamon bark meal were ground by Wiley mill with 1 mm sieve. The dietary treatments were: T0 (60% elephant grass + 30% wheat bran + 10% soybean meal), T1 (T0+1.16% cinnamon bark meal equal to cinnamaldehyde with 200 mg/kg DM basis) T2 (T0+2.3% cinnamon bark meal equal to cinnamaldehyde with 400 mg/kg DM basis), T3 (T0+3.5 cinnamon bark meal equal to cinnamaldehyde with 600 mg/kg DM basis), T4 (T0+4.5% cinnamon bark meal equal to cinnamaldehyde with 800 mg/kg DM basis). Each treatment was examined in triplicate.

In vitro digestibility was carried out using modified 2-stage in vitro technique of Tilley and Terry [9]. Rumen microbial inoculum consisted of a 1:4 (v/v) mixture of rumen fluid and mineral buffer. The first set of this method filtered the content of glass tube after 48h incubation to estimate DM, organic matter (OM), crude protein (CP), and crude fiber (CF) digestibility in the rumen. On the second set, upon 48h incubation, HCl and pepsin were added to the glass tube, incubated up to 96h to estimate total DM, OM, CP, and CF digestibility. Chemical analysis including DM, OM, CP, and CF according to proximate analysis method [10].

All of the data were analyzed by one-way analysis of variance (ANOVA) using SPSS ver. 16 (IBM, USA) and statistically significant differences between means were determined by Duncan’s Multiple Range Test (DMRT) when the effects of treatment (P<0.05) were detected [11].

3. Result and discussion

The in vitro digestibility of cinnamon bark meal addition as cinnamaldehyde source with different level were shown in Table 1.

In vitro crude protein digestibility (48 h incubation) was affected (P<0.05) by the addition cinnamon bark meal. Compared with the control diet, IVCPD decreased (P<0.05) only with the T3 and T4 diets. The decrease of protein degradation was higher in T4 diets. The reduction in IVCPD averaged 3.09% for T3 diet and 4.85% with T4 diet, respectively. The decrease IVCPD on 48 h incubation may be due to the interaction between cinnamaldehyde and protein. Cinnamaldehyde can bind protein that is resistant to proteolytic enzymes from rumen microbes, which reflected by decreasing ruminal protein degradation [12,13]. Condensed tannin effect of quebracho at 3% of DM decreased ruminal protein degradation [14]. In addition, Cardozo et al. [15] reported that 7.5 mg/kg DM cinnamaldehyde addition reduced peptidolysis. Total crude protein digestibility (96 h) was affected (P<0.05) by the addition cinnamon bark meal. This study observed increases the digestibility of total crude protein due to the addition of cinnamon bark meal at the level of 3.5-4.6%. The increases in IVCPD on 96 h incubation with T3 and T4 diet could be partly due to decrease in deamination of amino acids or low availability of amino acids resulted from the decrease in protein degradation in IVCPD on 48 h incubation. This phenomenon showed the increase of rumen undegraded protein due to binding of this compound by cinnamaldehyde. Cinnamaldehyde could modify the growth of rumen proteolytic bacteria either directly or indirectly by preventing access to the protein, thus, proteolytic and peptidolytic activities were reduced [7]. Kamalak et al. [16] reported that soybean meal protected with formaldehyde was resistant to rumen degradation, but it was digestible in the lower tract. Protected protein is stable over
the pH range of 3.5–7.0, but dissociate in the abomasum (pH below 3.5). Rumen protected protein could be available in post rumen as a protein bypass for a ruminant. It increased the availability of feed proteins for digestion, and more amino acids are absorbed in the small intestine [17].

Table 1. Effect of cinnamon bark meal supplementation as cinnamaldehyde source on in vitro DM, OM, CP, and CF digestibility in the rumen (48 h) and total DM, OM, CP, and CF digestibility (96 h).

| Digestibility1 (%) | Treatments |
|--------------------|------------|
|                    | T0     | T1       | T2       | T3       | T4       |
| IVDMDns            | 48 h   | 50.34±0.83 | 49.90±1.16 | 49.84±1.27 | 49.74±0.66 | 48.61±0.50 |
|                    | 96 h   | 56.95±1.12 | 57.84±0.90 | 58.11±0.11 | 58.30±0.74 | 59.02±0.25 |
| IVOMDns            | 48 h   | 47.30±0.65 | 46.98±0.23 | 46.79±0.51 | 46.06±1.00 | 45.69±0.60 |
|                    | 96 h   | 55.86±0.44 | 56.77±0.45 | 56.98±0.89 | 57.38±1.34 | 57.68±0.97 |
| IVCPCD             | 48 h   | 55.23±0.91a | 54.43±0.70ab | 54.20±0.77ab | 53.52±0.62bc | 52.55±0.62c |
|                    | 96 h   | 59.61±0.81a | 60.23±0.65a | 60.26±0.55ab | 61.22±0.71ab | 61.55±0.73b |
| IVCFCFD            | 48 h   | 51.86±1.38 | 51.34±2.00 | 51.30±1.94 | 50.72±2.24 | 49.95±1.90 |
|                    | 96 h   | 54.83±1.46 | 55.43±1.98 | 56.22±1.86 | 56.19±1.89 | 57.18±1.58 |

a,b,c Means with different superscripts in the same row differ at P<0.05
ns Not significant
1 T0=60% elephant grass + 30% wheat bran + 10% soybean meal), T1 (T0+1.16% cinnamon bark meal), T2 (T0+2.3% cinnamon bark meal), T3 (T0+3.5 cinnamon bark meal), T4 (T0+4.5% cinnamon bark meal)
2 IVDMD = in vitro dry matter digestibility; IVOMD = in vitro organic matter digestibility; IVCPCD = in vitro crude protein digestibility; IVCFCFD = in vitro crude fiber digestibility.

Our study showed that the effects of addition cinnamon bark meal as cinnamaldehyde source depended on dose of addition. At a low dose (1.16-2.3% of cinnamon bark meal), there were no significant improvements on IVCPD digestibility in the rumen and total tract. However, on the contrary, at a high dose (3.5-4.5% of cinnamon bark meal) ruminal digestibility of CP. Decreased ruminal digestibility of CP observed with the high dose resulted in the greater flow of rumen undegraded feed protein to the intestine, which could be beneficial for ruminant with a high requirement for production [18].

In vitro digestibility of DM, OM and CF on 48 h and 96 h incubation were not affected (P>0.05) by the addition of cinnamon bark meal. The result from this study is in agreement with the findings where the addition of cinnamaldehyde did not affect IVDMD, IVOMD, IVCFCFD [12, 19]. The digestibility of DM, OM and CF were not modified by the inclusion of cinnamon bark meal. There appears to be a threshold of cinnamon bark meal contents (approximately 4.6%) which no adverse effect was evident on nutrient digestibility, except protein.

4. Conclusion
Addition of cinnamon bark meal as cinnamaldehyde source decreased digestibility of protein in the rumen but increased total crude protein digestibility as indicator of protein availability in intestine. The optimum level of cinnamon bark meal as cinnamaldehyde source addition at level 4.6% was the most effective protection agent for feed protein.
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