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Study of cinnamon leaf powder (*Cinnamomum burmannii* Ness ex. Bl) as a source of cinnamaldehyde on *In vitro* feed digestibility

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ABSTRACT

This study aimed to determine the effect of adding cinnamon leaf powder (*Cinnamomum burmannii* Ness ex. Bl) as a source of cinnamaldehyde at different levels on *In vitro* nutrient digestibility, namely dry matter (DM), organic matter (OM), crude protein (CP), and crude fiber (CF) in the rumen, post-rumen, as well as total digestibility. The treatments consisted of a control diet (without cinnamon leaf powder) and rations supplemented with 1, 2, 3, and 4% DM feed, equivalent to cinnamaldehyde 16, 32, 48, and 64 mg/kg DM feed. A two-stage *In vitro* method was used for 48 hours to determine rumen digestibility and 96 hours for total digestibility, with three replications. The variables observed were dry matter digestibility, organic matter digestibility, crude protein digestibility, and crude fiber digestibility in the rumen, post-rumen, and total. Data were analyzed using a completely randomized design followed by Duncan's New Multiple Range Test (DMRT). The results showed that increasing the level of cinnamon leaf powder up to 3% increased the digestibility of DM in the rumen, CP in post-rumen, and DM and CP in total, but if the level was added to 4% it would decrease the level of digestibility in the rumen and total digestibility. It can be concluded that adding of 3% cinnamon leaf powder, equivalent to cinnamaldehyde 48 mg/kg of feed DM, increase total DM and CP digestibility and protect feed protein from microbial degradation in the rumen.

Introduction

The nutrient content of feed that can meet livestock needs is an important factor in increasing livestock productivity. One of the main nutrients in feed needed by livestock is protein which is used for growth and repair of body tissues. Feed protein in the rumen of ruminant animals will be degraded into peptides and amino acids, then deaminated to produce ammonia (NH_3) which is needed for microbial protein synthesis (Kamalak *et al*., 2005). Undigested feed protein in the rumen will be absorbed in the small intestine, while the remaining NH3 in the protein decomposition process will accumulate in the rumen fluid and then be carried to the liver to be processed into urea and will be excreted in urine (Kumari and Kumar, 2015).

One way of manipulation to reduce protein degradation in the rumen is to carry out protein protection. Feed protein protection aims to reduce feed degradation in the rumen and ammonia emissions and increase protein bypass in ruminant livestock so that the biological value of feed protein is maintained (Tunkala *et al*., 2023). Cinnamaldehyde is one of the compounds that can be used as a protein protection agent. Cinnamaldehyde is an essential oil of the aldehyde group contained in cinnamon plants (Silva *et al*., 2024). Cinnamaldehyde as a protein protection agent can react with proteins due to addition or condensation reactions by forming bonds between the active side that makes up amino acids (S-H, N-H) with the carbonyl group (-C=O) of cinnamaldehyde (Weibel and Hansen, 1989). The reaction between the carbonyl group and the amino group through the Schiff base reaction which is influenced by the degree of acidity (Li *et al*., 2013). Protein protection is stable at pH 3.5 to 7 and the bond will be released in the abomasum or at pH less than 3.5 (Kamalak *et al*., 2005).

Cinnamaldehyde as a protein protection agent can also modify the growth of rumen proteolytic bacteria so that the proteolytic and peptidolytic processes are reduced. Microbial enzyme activity can also be inhibited by cinnamaldehyde so that protein degradation in the rumen decreases (Antoniewicz *et al*., 1992). The addition of cinnamaldehyde compounds in livestock feed rations can reduce protein digestibility in the rumen and increase post-rumen protein digestibility so that protein in the feed can be optimally used by livestock. The aim of this study was to determine the effect of adding cinnamon leaf powder at different levels on *In vitro* nutrient digestibility, namely dry matter (DM), organic matter (OM), crude protein (CP), and crude fiber (CF) in the rumen, post-rumen, as well as total digestibility.

Materials and methods

Ethical clearance

The inclusion of living animals in this study was under check and approval by the Research Ethics Committee, Faculty of Veterinary Medicine, Gadjah Mada University, with the number 053/EC-FKH/Eks./2023.

Study design and analysis of nutrient content in treatments

The method used in this study was an experimental approach employing a completely randomized design (CRD) with a one-way pattern, consisting of five treatments and three replications, with each replication performed in duplicate. The *In vitro* test, based on the method by Tilley and Terry (1963), was conducted in two phases: a one-stage *In vitro* (48-hour incubation) and a two-stage *In vitro* (96-hour incubation).

This study utilized cinnamon leaf powder supplementation at levels of 0, 1, 2, 3, and 4% of the feed dry matter (DM), which corresponds to cinnamaldehyde levels of 0, 16, 32, 48, and 64 mg/kg DM, based on a cinnamaldehyde content of 64.38% in the cinnamon leaf powder (with essential oil content of 0.25%). The basal diet used in this study consisted of forage and concentrate at a ratio of 60:40. The forage used was elephant grass (Pennisetum purpureum), while the concentrate consisted of 90% wheat bran pollard and 10% soybean meal as the protein source.

The forage material, in the form of fresh elephant grass, was chopped into about 5 cm pieces and dried in an oven at 55°C for three days. The next step involved grinding both the elephant grass and cinnamon leaves into powder form. The resulting cinnamon leaf powder was then analyzed for its bioactive compound contents, including total phenols, saponins, total tannins, and total flavonoids. Total phenol, flavonoid, and tannin

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contents were analyzed using UV-Vis spectrophotometry, while saponin content was analyzed using UV-Vis spectrophotometry with Quillaja bark saponin as the standard.

The soybean meal was ground before being analyzed and used as a substrate. The powders of elephant grass, cinnamon leaf, soybean meal, and wheat bran pollard were analyzed for their nutrient contents. The analyzed nutrient components included dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), and ether extract (EE), using proximate analysis based on AOAC (2005) methods. Subsequently, nitrogen-free extract (NFE) and total digestible nutrients (TDN) were calculated using formulas described by Hartadi *et al*. (1980).

Adaptation of donor livestock

Two fistulated Bali cattle, each weighing approximately 300 kg, were adapted by providing feed twice daily (morning and evening) at a dry matter (DM) intake of 3% of body weight for 7 to 14 days. The feed was provided according to the cattle's needs, following Kearl (1982), with a composition of 60% forage and 40% concentrate. Meanwhile, the donor cattle were given water ad libitum.

Rumen fluid for In vitro testing

Rumen fluid for *In vitro* testing was obtained from two fistulated Bali cattle, each weighing approximately 300 kg. The rumen fluid was collected in the morning before feeding using a thermos pre-filled with water at 39°C. The rumen fluid was poured into the thermos until full, after first emptying all the water. The rumen fluid was then transported to the laboratory and filtered through a layered gauze to separate feed particles, while CO_2 gas was bubbled through to maintain anaerobic conditions.

Substrate preparation

The feed composition used was 60% forage and 40% concentrate. The forage used was elephant grass, and the concentrate consisted of wheat bran pollard and soybean meal. Test tubes with a capacity of 25 mL were used for dry matter digestibility (DMD) and organic matter digestibility (OMD) samples, while 50 mL capacity test tubes were used for crude protein digestibility (CPD) and crude fiber digestibility (CFD) samples. DMD and OMD test tubes were filled with 250 mg of the feed mixture, while CPD and CFD test tubes contained 500 mg. Cinnamon leaf powder was added at concentrations of 0, 1, 2, 3, and 4% of dry matter. The blank tubes were filled with no sample, and the standard used was pangola grass powder.

Buffer preparation

An amount of 1000 mL McDougall solution was prepared, consisting of 9.8 g Na_2HCO_3, 4.62 g $Na_2HPO_4.2H_2O$, 0.57 g KCl, 0.47 g NaCl, 0.12 g $MgSO_4.7H_2O$, and 0.06 g $CaCl_2$. All ingredients were mixed and dissolved in 1000 mL of distilled water, except for $CaCl_2$. The solution was stirred until homogeneous using a magnetic stirrer. $CaCl_2$ was added after homogenization, then dissolved and flushed with CO_2.

In vitro testing Tilley and Terry (One-stage incubation for 48 hours)

Nutrient digestibility was measured using the *In vitro* method of Tilley and Terry (1963). The fermentation process was conducted in one stage for 48 hours of incubation using a medium consisting of rumen fluid and McDougall solution in a 1:4 ratio, which was added to the test tubes containing the feed substrate. Blank tubes contained only buffer solution and rumen fluid. The test tubes were flushed with CO_2 and sealed with rubber stoppers to maintain anaerobic conditions. The tubes were placed in a water bath at 39°C and agitated every 8 hours. After 48 hours of fermentation, the contents of the test tubes were filtered to separate the filtrate from the fermentation residue using a crucible with a pre-weighed glass wool filter. The feed residue was then analyzed for its nutrient content to determine the digestibility of crude protein (CP), crude fiber (CF), organic matter (OM), and dry matter (DM) during the 48-hour one-stage *In vitro* incubation.

In vitro testing Tilley and Terry (Two-Stage incubation for 96 hours)

Nutrient digestibility was measured using the *In vitro* method of Tilley and Terry (1963). The two-stage *In vitro* test was performed with a 96-hour incubation period. The procedure was continued from the 48-hour incubation to the full 96-hour incubation. At hour 48, each test tube was added with 20% HCl and 5% pepsin in a 3:1 ratio. The tubes were tightly sealed and further incubated for 48 hours in a water bath at 39°C, with agitation every 8 hours. After the 96-hour two-stage fermentation, the contents were filtered as in the one-stage *In vitro* test (48 hours). The feed residue was then analyzed for its nutrient content to determine the digestibility of crude protein (CP), crude fiber (CF), organic matter (OM), and dry matter (DM) during the 96-hour two-stage *In vitro* incubation.

Observed variables

Dry Matter Digestibility (DMD). The *In vitro* digestibility residue was filtered using a crucible containing glass wool, then dried in an oven at 105ºC for 12 hours, placed in a desiccator for one hour, and then weighed to determine the undigested dry matter (residual DM). The DMD can be calculated using the following equation:

Initial DM weight (g)- Residual DM weight (g))/Initial DM weight (g)×100%

Initial DM weight= DM content of the sample × sample weight

Residual DM weight= (Weight of crucible + residue) - Weight of crucible

Organic Matter Digestibility (OMD). The feed residue after drying for DM content determination was used to determine organic matter (OM) content by ashing in a furnace at 600ºC for two hours, then placing it in a desiccator for one hour and weighing it to determine the undigested organic matter (residual OM). The OMD can be calculated using the following equation:

Initial OM weight(g)- Residual OM weight (g)/Initial OM weight (g)×100%

Initial OM weight= OM content of the sample × sample weight

Residual OM weight= (Weight of crucible+residue at 105ºC)-(Weight of crucible+residue at 600ºC)

Crude Protein Digestibility (CPD). The residue from filtration with filter paper was left to dry. The dried precipitate was then placed into a tube for the destruction process. The resulting solution was distilled, and the distillate was titrated with 0.1 N HCl until the color changed from green to purple. A blank was prepared and processed in the same manner. The method for determining crude protein content follows AOAC (2005). The results obtained from determining the undigested crude protein residue were used to calculate the CPD. The CPD can be calculated using the following equation:

Initial CP weight (g)-Residual CP weight (g)/Initial CP weight (g)×100%

Initial CP weight = CP content of the sample × sample weight

Residual protein is calculated by:

(Blank titration −Sample titration) × NHCl ×0.014×6.25

Crude Fiber Digestibility (CFD). The residue from the Tilley and Terry (1963) filtration process was placed in a crucible containing glass wool. The residue was boiled with 1.25% H_2SO_4 and 1.25% NaOH for 30 minutes after boiling. It was then filtered through a crucible covered with glass wool. The residue was washed with alcohol, placed in an oven at 105 ºC for 12 hours, and then ashed in a furnace at 600ºC for two hours. After that, it was placed in a desiccator for one hour and weighed (AOAC, 2005). The CF weight after *In vitro* is the difference between the weight after oven drying and ashing. The results obtained from determining the undigested crude fiber residue were used to calculate the CFD. The CFD

can be calculated using the following equation:
Initial CF weight (g)-Residual CF weight (g) /Initial CF weight (g)×100%
Initial CF weight= CF content of the sample×sample weight
Residual CF weight= (Weight of crucible+residue at 105ºC)-(Weight of crucible+residue at 600ºC).

Data analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) for a completely randomized design (CRD). If there are differences among treatments, further analysis using Duncan's New Multiple Range Test (DMRT) will be conducted to determine the significant differences in the means of each treatment (Rosner, 1990).

Results

The results of the analysis of *In vitro* nutrient digestibility in the rumen with the addition of cinnamon leaf powder as a source of cinnamaldehyde on dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), and crude fiber digestibility (CFD) are presented in Table 1. Statistical analysis showed that the addition of cinnamon leaf powder as a source of cinnamaldehyde in the ration had a significant effect (P<0.05) on the *In vitro* digestibility of dry matter and crude protein in the rumen. Based on the research that has been conducted, the *In vitro* digestibility of dry matter in the rumen given cinnamon leaf powder at a level of 3% was higher compared to the control treatment. The addition of cinnamon leaf powder as a source of cinnamaldehyde up to a level of 4% reduced protein digestibility in the rumen compared to the control. The addition of cinnamon leaf powder as a source of cinnamaldehyde in the ration did not significantly affect (P>0.05) the *In vitro* digestibility of organic matter and crude fiber in the rumen.

Table 1. Effect of adding cinnamon leaf powder on *In vitro* rumen digestibility of feed nutrients

Digestibility (%)	Addition of cinnamon powder (%DM Feed)				
	0	1	2	3	4
DM	62.10^{a}±0.07	60.43^{a}±0.09	61.83^{a}±1.19	64.99^{b}±0.43	61.28^{a}±1.21
OM^{ns}	59.18±0.92	57.60±1.58	59.04±0.84	57.06±0.48	58.69±1.00
CP	56.21^{c}±2.10	42.69^{a}±1.37	54,07^{c}±2.27	53.00^{c}±1.35	48.18^{b}±1.32
CF^{ns}	51.03±1.85	49.54±2.31	51.49±1.42	50.84±3.82	47.28±0.93

^{ns} not significantly different (P>0.05)
^{a,b,c} different superscripts on the same line indicate significant differences. (P<0.05)
DM= dry matter, OM= organic matter, CP= crude protein, CF=crude fiber

The results of the analysis of post-rumen *In vitro* nutrient digestibility with the addition of cinnamon leaf powder as a source of cinnamaldehyde on dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), and crude fiber digestibility (CFD) are presented in Table 2. The data from the statistical analysis showed that the addition of cinnamon leaf powder as a source of cinnamaldehyde did not significantly affect (P>0.05) the digestibility of dry matter, organic matter, and crude fiber post-rumen. The addition of cinnamon leaf powder as a source of cinnamaldehyde in the ration can affect the *In vitro* digestibility of crude protein (P<0.05) post-rumen. The addition of cinnamon leaf powder increased the digestibility of crude protein in the post-rumen compared to the control treatment.

The results of the analysis of total *In vitro* nutrient digestibility with the addition of cinnamon leaf powder as a source of cinnamaldehyde on dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), and crude fiber digestibility (CFD) are presented in Table 3. The data from the statistical analysis of this study showed that the addition of cinnamon leaf powder as a source of cinnamaldehyde in the ration can affect the *In vitro* digestibility of dry matter and crude protein (P<0.05). Based on the research that has been done, the *In vitro* digestibility of total dry matter given cinnamon leaf powder at a level of 3% is higher compared to the control treatment. The addition of cinnamon leaf powder up to a level of 4% can increase the digestibility of total crude protein compared to the control treatment. The addition of cinnamon leaf powder as a source of cinnamaldehyde in the ration did not have a significant effect (P>0.05) on the *In vitro* digestibility of organic matter and total crude fiber.

Table 2. Effect of adding cinnamon leaf powder on *In vitro* post-rumen digestibility of feed nutrients.

Digestibility (%)	Addition of cinnamon powder (%DM Feed)				
	0	1	2	3	4
DM^{ns}	4.21±0.13	3.68±0.70	2.53±0.52	3.67±0.16	3.29±0.72
OM^{ns}	3.67±0.30	3.24±0.23	2.18±0.20	3.01±1.47	2.54±0.43
CP	11.11^{a}±1.37	25.46^{c}±0.77	11.56^{a}±0.21	18.98^{b}±0.46	26.00^{c}±1.17
CF^{ns}	1.84±0.45	2.03±0.92	2.64±1.41	3.63±1.65	3.99±0.64

^{ns} not significantly different (P>0.05)
^{a,b,c} different superscripts on the same line indicate significant differences. (P<0.05)
DM= dry matter, OM= organic matter, CP= crude protein, CF=crude fiber

Table 3. Effect of adding cinnamon leaf powder on *In vitro* total digestibility of feed nutrients.

Digestibility (%)	Addition of cinnamon powder (%DM Feed)				
	0	1	2	3	4
DM	66.31^{b}±0.06	64.11^{a}±0.79	64.35^{a}±0.86	68.66^{c}±0.58	64.57^{a}±0.50
OM^{ns}	62.85 ±0.89	61.73 ±0.51	61.59±0.79	62.54 ±1.54	61.23±0.91
CP	67.31^{ab}±3.40	68.15^{ab}±0.59	65.63^{a}±2.07	71.98^{bc}±1.78	74.18^{c}±0.13
CF^{ns}	52.87 ±1.68	51.58 ±3.23	54.13±0.01	54.47 ±2.17	51.27±1.58

^{ns} not significantly different (P>0.05)
^{a,b,c} different superscripts on the same line indicate significant differences. (P<0.05)
DM= dry matter, OM= organic matter, CP= crude protein, CF=crude fiber

Discussion

The addition of cinnamon leaf powder as a source of cinnamaldehyde affects the digestibility of dry matter in the rumen and total. Based on the research that has been done, the *In vitro* digestibility of dry matter in the rumen and total given cinnamon leaf powder at a level of 3% is higher compared to the control treatment. Cinnamaldehyde is antimicrobial against pathogenic bacteria so that it can optimize the digestibility of dry matter in the rumen. Liefferinge *et al.* (2022) stated that cinnamaldehyde has effective microbial activity so that it can maintain the balance of microbes in the rumen and the fermentation process can run optimally. The effect of adding cinnamon leaf powder as a source of cinnamaldehyde on the digestibility of dry matter is related to the activity of rumen microbes. Suardin *et al.* (2014) stated that microbial activity is a factor that affects the digestibility of dry matter in the rumen. A similar study conducted by Khairani (2024) stated that the addition of cinnamon leaf powder as a source of cinnamaldehyde had a significant effect (P <0.05) on the concentration of microbial protein in the rumen.

The addition of cinnamon leaf powder at a level of 4% resulted in lower total dry matter digestibility than the control treatment, indicating that at that level cinnamaldehyde interferes with the activity of total dry matter digestion. Hadianto *et al.* (2019) stated that cinnamaldehyde can have a negative effect on the livestock digestion process, namely by providing an inhibitory effect on enzymes that play a role in nutrient degradation in the digestive tract. Phesatcha *et al.* (2021) stated that the level of supplementation is an important factor in increasing the positive effect on feed nutrient degradation.

The addition of cinnamon leaf powder as a source of cinnamaldehyde in the ration had a significant effect (P <0.05) on the *In vitro* digestibility of crude protein in the rumen, post-rumen, and total. The addition

of cinnamon leaf powder as a source of cinnamaldehyde up to a level of 4% reduced protein digestibility in the rumen compared to the control. Kurniawati *et al*. (2019) stated that the protein digestibility of the ration consisting of greens and concentrates was 56.02%. The decrease in protein degradation in the rumen occurs due to a decrease in proteolytic activity in the rumen by cinnamaldehyde. Ishak *et al*. (2015) stated that the decrease in protein degradation in the rumen occurs due to the interaction between cinnamaldehyde and protein that can bind so that it is resistant to proteolytic enzymes. The decrease in protein digestibility in the rumen is beneficial for livestock because it will increase the feed protein that goes to the post-rumen to be digested and absorbed by livestock. Tager and Krause (2010) stated that the addition of cinnamon oil can reduce protein metabolism in the rumen so that there is an increase in post-rumen protein digestibility that can be optimally absorbed by livestock.

The digestibility of crude protein in the post-rumen with the addition of cinnamon leaf powder was higher than the control treatment. The increase in protein digestibility in the post-rumen occurred because the protein that had not been degraded in the rumen would be digested in the abomasum and small intestine enzymatically. The addition of cinnamaldehyde increased protein digestibility in the post-rumen because the protein that was initially protected at a stable pH of 3.5 to 7 would be released at a pH below 3.5, namely in the abomasum, so that it could be degraded. Idowu *et al*. (2024) stated that providing feed containing bypass protein can increase livestock productivity and efficiency of use because feed protein can be used optimally.

The addition of cinnamon leaf powder up to 4% can increase the total digestibility of crude protein compared to the control treatment. Shinkai *et al*. (2012) stated that the total digestibility of crude protein was 68.2%. Hadianto *et al*. (2019) stated that there was an increase in the total digestibility of crude protein with the addition of cinnamon bark as a source of cinnamaldehyde. The addition of cinnamaldehyde will reduce the process of protein digestion in the rumen but increase post-rumen digestibility. Yang *et al*. (2010) stated that protein supplementation with cinnamaldehyde can reduce the degradation of crude protein in the rumen so that it will provide more protein that is degraded in the post-rumen.

The addition of cinnamon leaf powder as a source of cinnamaldehyde in the ration did not significantly affect the digestibility of organic matter and crude fiber in the rumen, post-rumen, and total. The addition of cinnamon leaf powder as a source of cinnamaldehyde did not interfere with the *In vitro* digestibility of organic matter. Tager and Krause (2011) stated that the total digestibility of organic matter was not affected by the addition of cinnamaldehyde. Cinnamaldehyde does not interfere with microbial activity in fermenting feed in the rumen and the enzymatic digestion process in the post-rumen. Liefferinge *et al*. (2022) stated that cinnamaldehyde is a selective antimicrobial against pathogenic microbes in the digestive tract.

The digestibility of crude fiber in the rumen is influenced by the activity of the CMCase enzyme in the rumen. The CMCase enzyme is secreted by cellulolytic bacteria which play a role in hydrolyzing the components of feed fiber. The addition of cinnamon leaf powder did not affect the digestibility of crude fiber in the rumen, indicating that it did not interfere with the activity of the CMCase enzyme in hydrolyzing cellulose in the rumen. Hadianto (2020) stated that cinnamaldehyde probably does not bind to the CMCase enzyme protein so that it does not interfere with the structure and stability of the enzyme in degrading cellulose optimally. Benchaar*et al*. (2007) stated that the effect of essential oils on rumen microbial activity is influenced by the amount and compounds contained in the essential oil.

Conclusion

Based on the results of this study, it can be concluded that the addition of cinnamon leaf powder up to a level of 3%, equivalent to 48 mg cinnamaldehyde/kg dry matter (DM) of feed, able to increase the total *In vitro* dry matter and crude protein digestibility. The addition of cinnamon leaf powder as a natural source of cinnamaldehyde can protect feed protein from microbial degradation in the rumen.

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Conflict of interest

The authors have no conflict of interest to declare.

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