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Enhancing protein protection and ruminal *in vitro* fermentation using cinnamon leaf powder (*Burmese cinnamon* ness ex bi.) as a cinnamaldehyde source

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ABSTRACT

This study aimed to assess the impact of adding cinnamon leaf flour (*Burmese cinnamon* Ness ex Bl.) to rations on rumen fermentation parameters using an *in vitro* method. Cinnamon leaf flour was added at levels of 0%, 1%, 2%, 3%, and 4%, corresponding to 0, 16, 32, 48, and 64 mg/kg of feed dry matter (DM), with each treatment repeated three times. Data from the study were analyzed for variance with Duncan's multiple range test (DMRT), which was tested using one-way pattern first. The results showed that the addition of cinnamon leaf flour at level of 2% to 4% reduced pH to a range of 6.72 in the rumen. A significant decrease was observed in NH_3 concentration with the addition of cinnamon leaf flour at 4% by 19.53%. Compared to the control, there was a decrease in microbial protein concentration, with 2% level showing the highest reduction of 16.67%. The addition of 1% level decreased the population of protozoa by 13.56%. VFA production increased with the addition of 3% cinnamon leaf meal in the ration, which included total VFA of 81.66 mM, acetic acid of 38.48 mM, propionic acid of 24.20 mM, butyric acid at 19.08 mM, and the acetate-to-propionate ratio of 1.60. This study showed that adding 3% cinnamon leaf flour, which was similar to 48 mg/kg DM of cinnamaldehyde, significantly ($P<0,05$) reduced NH_3 and number of protozoa, maintained protein from microbes, and increased VFA production in the rumen *in vitro*.

Introduction

Livestock feed quality and quantity are factors contributing to an increase in livestock productivity. Good feed quality is related to the nutrient content, including energy, protein, minerals, vitamins, and anti-nutritional compounds such as tannins and lignin. According to Beski *et al*. (2015), protein is a vital nutrient in animal feed formulations due to its essential roles in tissue formation and various metabolic processes, such as enzyme, hormone production, and immune functions. In ruminant livestock and rumen microbes, there is a symbiotic relationship that helps meet nutritional needs, allowing biological processes to function effectively. This livestock provides a suitable substrate and environment for the microbes, leading to a corresponding supply of protein and energy. However, the activity of rumen microbes can decrease the amount of feed protein available for absorption by livestock.

The protein source feed ingredient that is often used in ruminant rations is soybean meal. Protein in ruminant livestock fulfills three critical functions by supplying essential protein for rumen microbes, supporting maintenance, health, and reproduction, and providing amino acids necessary for enhanced productivity (Tedeschi *et al*., 2015). Moran (2005) stated that 40% to 70% of feed protein broken down mostly inside the rumen became amino acids and peptides, changing to NH_3 and α-keto acids. Generally, the significant degradation of soybean meal in rumen is a loss due to the reduction in amino acids for livestock. In this context, protein plays an essential role in the metabolism of livestock, making feed with high protein more expensive. Protein-rich feed ingredients for rations are relatively expensive, necessitating careful consideration of their use as components in livestock diets. Due to the relatively expensive nature, consideration is required when using protein as components of livestock rations.

The exploration of natural protein protection strategies, such as cinnamaldehyde is essential to improve the efficiency and safety of protein feed. Cinnamaldehyde is a secondary metabolite naturally derived from cinnamon plants, with the potential to protect protein. This process is carried out by forming complexes resistant to protease activity, thereby decreasing protein degradation in the rumen. Calsamiglia *et al.* (2007) indicated that the use of essential oil from cinnamon, with cinnamaldehyde as the primary active compound, can manipulate nitrogen (N) metabolism in the rumen by reducing peptidolysis, without affecting VFA concentrations, microbial protein synthesis, or enzymatic activity within the rumen. The improvement showed that overall energy assimilated from feed could be correlated with volatile fatty acids (VFA), serving as the primary energy source. VFA also provides carbon skeletons for microbial protein synthesis during rumen fermentation, thereby showing cinnamaldehyde's potential as a protein-protecting ingredient to reduce rumen protein degradation. Therefore, this study aimed to evaluate impacts of adding cinnamon leaf flour at various levels as source of cinnamaldehyde on rumen fermentation parameters. The parameters examined were pH levels, NH_3 concentration, microbial protein, protozoa population, and VFA production.

Materials and methods

Study location and ethical clearance

The experiment was conducted at the Faculty of Animal Science, Universitas Gadjah Mada, Special Region of Yogyakarta Province, Indonesia and obtained prior consent from the Research Ethics Committee, Faculty of Veterinary Medicine, Gadjah Mada University, under the reference number 053/EC-FKH/Eks./2023.

Study Design and Analysis of Treatment Nutrient Content

This study was carried out using completely randomized design

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(CRD) with a single-factor pattern, adding five treatment groups. Subsequently, treatment groups were characterized by the inclusion of cinnamon leaf flour at levels of 0%, 1%, 2%, 3%, and 4% according to the dry matter (DM). This corresponded to cinnamaldehyde concentrations of 0, 16, 32, 48, and 64 mg/kg DM, with cinnamaldehyde content being 64.38% in 0.25% essential oil. A basal diet was also used consisting of elephant grass as forage wheat bran pollard and soybean meal applied as concentrate. The ratio between forage and concentrate was 60:40 (% DM) with 90:10 (% DM) ratio of wheat bran pollard and soybean meal as a concentrate feed. This study was carried out by repeating three times with each repetition conducted in duplicate.

The fresh Pennisetum purpureum was cut into ± 5 cm pieces and dried in a 55°C oven for three days. This was followed by the preparation of cinnamon leaves, wheat bran pollard, and dry soybean meal, which was ground to obtain a sample with a size of one mesh. The nutrient content of samples was analyzed proximately with the procedure published by AOAC (2005). This included DM, organic matter (OM), crude protein (CP), crude fiber (CF), and ether extract (EE). NFE was calculated according to the formula by Hartadi *et al.* (1997). The chemical composition of feed ingredients then were used for calculating the chemical composition on each ration (Table 1).

Table 1. Chemical composition in rations.

Chemical composition (%DM)	Addition of cinnamon leaf powder (%DM of feed)				
	0	1	2	3	4
Dry matter	91.17	89.56	89.35	89.89	92.6
Organic matter	85.69	85.6	85.72	85.58	85.75
Ash	14.31	14.39	14.28	14.42	14.26
Crude protein	14.77	15.16	14.96	15.44	16.32
Ether extract	3.1	3.23	2.49	2.44	1.99
Crude fiber	24.45	23.45	25.01	24.77	24.53
NFE	43.37	43.76	43.24	42.94	42.91

Adaptation of Donor Animals and Data Collection

A total of two Bali fistula cows, each weighing approximately 300 kg, were adapted by feeding twice daily, in the morning and evening, with DM amounting to 3% of their body weight. Feeding according to the cows' needs followed Kearl (1982) with a feed composition of 60% forage and 40% concentrate. Meanwhile, drinking water was given with ad libitum method for one week to be used as rumen fluid donor livestock.

Rumen fluid for the *in vitro* gas test was collected from Bali cows with fistulas in the morning before feeding. The fluid was obtained using thermos at temperature of 39°C and poured into an empty thermos after being emptied of water. The sample collected was carried to the laboratory, processed with filtration using gauze (five layers) to remove feed particles, and placed into an Erlenmeyer flask. CO_2 gas was flowed into the flask to maintain anaerobic conditions.

pH measurement

In this study, pH measurement was carried out using filtered fermented liquid with pH meter (Hanna brand) calibrated at 4.0 and 7.0.

NH_3 concentration

The fermentation filtrate was centrifuged at 3000 rpm to obtain the top layer, namely supernatant. NH_3 concentration in the filtrate was measured using a method based on the catalyzed indophenol reaction, which produced a stable blue compound. Subsequently, the absorbance was measured using spectrophotometer at wavelength of 630 nm (Chaney and Marbach, 1962).

Volatile fatty acids (VFA) concentration

Gas chromatography (GC) with Shimadzu GC-8A series instrument was used to quantify VFA, as described by Filipek and Dvorak (2009). The detector used was a Flame Ionization Detector (FID) with a temperature of 260ºC and injector was set at 250ºC. The column used was a CP FFAP CB/CP7485, with a temperature of 60ºC, a length of 25 meters, and an inner diameter of 0.32 millimeters. Subsequently, helium gas was adjusted by adding 3 ml/minute as a carrier gas with a split ratio of 27.5.

Microbial proteins

A total of 3 ml of supernatant from the initial centrifugation was subjected to a second centrifugation at 10,000 rpm. The resultant sediment comprising rumen microbes, was analyzed for protein content using the Lowry method (Plummer, 1987). Bovine Serum Albumin (BSA) was used as the standard to establish a curve of protein standard.

Number of protozoa

Protozoa population calculations were carried out using a counting chamber with formalin salt solution (formalin saline) made from a mixture of formalin with 0.9% physiological NaCl in 100 ml of solution (Diaz *et al*., 1993).

"Protozoa population=" "1" /"0.1 × 0.0625 × 16 × 5" "×1000×C×Fp"

Information:

C= Number of colonies counted

Fp= Dilution factor

Data analysis

This study obtained results analyzed by CRD with one-way ANOVA. According to Steel and Torrie (1993), an assessment with Duncan's multiple range test (DMRT) was needed to determine average differences for each treatment. The analysis was carried out using software named Statistical Product and Service Solutions (SPSS) with version 25.

Results

pH value

The statistical analysis data presented in Table 2 showed cinnamon leaf flour addition at 4% levels in ration significantly affected the pH value ($P<0.05$) of rumen fluid *in vitro*. pH values varied from 6.5 (control) to 6.85 (2% cinnamon leaf flour), remaining within the normal range for rumen conditions.

Table 2. Effect of adding cinnamon leaf flour on rumen fermentation parameters

Ruminal fermentation characteristics	Cinnamon Leaf Flour addition levels (%)					SEM	P value
	0	1	2	3	4		
pH	6.66^{c}	6.63^{d}	6.85^{a}	6.81^{b}	6.59^{cd}	0.03	0
NH_3(mg/100 ml)	42.34^{b}	37.98^{ab}	41.41^{b}	36.01^{a}	34.07^{a}	1.02	0.02
Microbial proteins (mg/ml)	0.07^{a}	0.09^{abc}	0.10^{c}	0.07^{ab}	0.09^{bc}	0	0.02
Protozoal counts (*10^5/ml)	1.77^{b}	1.53^{a}	2.13^{c}	1.85^{b}	1.76^{b}	0.05	0

[a-c]Means within the same row with varying superscripts differ significantly ($p < 0.05$).

NH_3 concentration

The statistical analysis showed that adding cinnamon leaf flour at level of 4% in the ration significantly reduced the density of NH_3 contained in the fluid of rumen ($P<0.05$). As shown in Table 2, NH_3 concentration given by cinnamon leaf flour with a treatment level of 3% was lower than the control. The low concentration by administering 4% cinnamon leaf

flour reached 34.07 mg/100ml. Meanwhile, the high concentration of NH_3 reaching 42.34 mg/100ml in the control treatment was not significantly different from 1% and 2% cinnamon leaf flour.

Microbial Proteins

The statistical analysis data in Table 2 showed that cinnamon leaf flour addition as cinnamaldehyde source significantly affected the microbial protein concentrations in fermented rumen fluids ($P<0.05$). The study found that the control had the highest microbial protein concentration, which was not significantly different from the 1% and 3% treatment levels. Moreover, concentrations ranging from 0.07 to 0.12 (mg/ml) showed that these conditions supported the synthesis of microbial protein in rumen.

Number of Protozoa

Statistical analysis data in Table 2 showed that the addition of cinnamon leaf flour as a source of cinnamaldehyde significantly affected the number of protozoa in rumen fluid from *in vitro* fermentation ($P<0.05$). The range of protozoa was 2.13 x 10^5 cells/ml to 1.53 x 10^5 cells/ml. Based on the results, the treatment level of 1% had a significant difference in reducing the number of protozoa compared to others.

Total Volatile Fatty Acid (VFA)

The statistical analysis data in Table 3 showed that the addition of cinnamon leaf flour significantly influenced ($P<0.05$) the total VFA. According to the results, range of total VFA obtained was 54.42 mM to 81.66 mM. The highest value of 81.66 mM was obtained at 3% level of treatment. At levels of 2% to 3% cinnamon leaf flour, there was a significant ($P<0.05$) increase in the proportion of acetate in fluid of the rumen. Acetate proportion ranged from 21.27 mM to 38.48 mM, while the propionate varied between 17.50 mM and 24.20 mM. An increase in concentration also occurred in butyric acid with the same level of cinnamon leaf flour, namely 14.37 to 19.08 mM from the control of 13.60 mM. Finally, it was observed that the ratio of acetic to propionic acid increased, ranging from 1.24 to 1.60 mM.

Table 3. Effect of adding cinnamon leaf flour on rumen volatile fatty acids (VFA)

Volatile fatty acids (VFA) production	Cinnamon Leaf Flour addition levels (%)					SEM	P-value
	0	1	2	3	4		
Total VFAs (mM)	65.06^{bc}	54.42^{c}	70.37^{ab}	81.66^{a}	72.28^{ab}	2.82	0.01
Acetic acid (mM)	23.74^{b}	21.27^{b}	25.51^{b}	38.48^{a}	38.11^{a}	2.04	0
Propionic acid (mM)	18.41^{b}	17.50^{b}	19.06^{b}	24.20^{a}	23.89^{a}	0.78	0
Butyric acid (mM)	1.29^{c}	1.24^{c}	1.48^{b}	1.60^{a}	1.60^{a}	0.73	0

$^{a-c}$Means within the same row with varying superscripts differ significantly ($p < 0.05$).

Discussion

The addition of cinnamon leaf flour as a source of cinnamaldehyde at 3% DM protected feed protein in the rumen by providing optimal conditions to facilitate the fermentation process. Adaptations of microorganisms in the rumen were the differences between the feed factors and their counterparts. This was because fermentation process occurred intensively in the rumen reticule, which allowed feed to be changed and presented in an easily absorbed form.

The primary objective of providing rumen nutrition was to support microbes and protein degradation for cell development. Mosoni *et al.* (2011) stated that good rumen digestion process depends on the type and population of microbes that lived in it. However, the main limiting factor in the use of protein inside the rumen was OM boiled down in the rumen.

Microbial protein synthesis factors in the rumen included the percentage of forage to concentrate, intake of DM, protein rates, carbohydrate degradation, feed digestibility, the balance of protein and energy supply, vitamins, as well as minerals. Flythe *et al.* (2017) observed that high NH_3 in rumen is needed by bacteria to support their growth. This was because NH_3 acted as a primary precursor for microbial cell formation, thereby improving the availability of a balanced amino acid profile to meet the protein requirements of host livestock.

According to Choudhury *et al.* (2015), the physiological pH range of rumen fluid is 5.5 to 6.9. The presence of protozoa can influence the number and types of bacteria, the proportion of volatile fatty acids, rumen pH, and ammonia concentration (McDonald *et al.*, 2011). Protozoa contribution inside the rumen reached 60% of the total rumen biomass with a population range of around 105 to 106 cells/ml of rumen fluid (McDonald *et al.*, 2002). Millen *et al.* (2016) stated that bacteria and protozoa in the rumen could be influenced by feed composition.

Several studies have shown that cinnamaldehyde could interact with protein, leading to protein cross-linking (Balaguer *et al.*, 2011). According to Chen *et al.* (2017), cinnamaldehyde contains an unbound carbonyl group capable of reacting with unlinked amino groups on protein molecules through the formation of Schiff base. Meanwhile, Li *et al.* (2013) stated the reaction with unlinked amino groups could be influenced by the pH value.

The buffer system in neutralizing the acidic conditions of the feed consumed by livestock was assisted by the presence of saliva. This was due to the sodium bicarbonate content which stabilized the pH value in the rumen and provided stable conditions for VFA production as an effect of carbohydrate fermentation by bacteria in the rumen.

Medjekal *et al.* (2023) stated that VFA contributed approximately 70% of the primary energy for ruminant livestock. The range for total VFA in the rumen was between 70 and 150 mM (Bergman, 1990). The observed normal range of fermentation parameters suggested that the addition of 3% cinnamon leaf flour, equivalent to 14.211 mg/kg DM of cinnamaldehyde, served as an effective alternative towards feed protein protection in ruminant.

Conclusion

This study showed that the addition of 3% cinnamon leaf flour was equivalent to cinnamaldehyde 48 mg/kg DM in the overall ratio *in vitro*. The addition of 3% level successfully maintained pH and microbial protein, reduced NH_3 and protozoa population, as well as improved VFA production in the rumen.

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Conflict of interest

The authors declare that they have no competing interests.

References

Balaguer, M.P., Gómez-Estac, J., Gavara, R., Hernandez-Munoz, P., 2011. Biochemical properties of bioplastics made from wheat gliadins cross-linked with cinnamaldehyde. Journal of Agricultural and Food Chemistry 59, 13212-13220.

Bergman, E.N., 1990. Energy contribution of VFA from the gastrointestinal tract in various species. Physiol. 70, 567-590.

Beski, S.S., Swick, R.A., Iji, P.A., 2015. Specialized protein products in broiler chicken nutrition: A review. Animal Nutrition 1, 47-53.

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L., Ferret, A., 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci. 90, 2580-2595.

Chaney, A.L., Marbach, E.P., 1962. Modified reagents for determination of urea and

ammonia. Clinical Chemistry 8, 130-132.
Chen, BJ., Fu, CS., Li, GH., Wang, XN., Lou, HX., Ren, DM., Shen, T., 2017. Cinnamaldehyde analogues as potential therapeutic agents. Mini Reviews in Medicinal Chemistry. 17, 33-43.
Choudhury, P.K., Salem, A.Z.M., Jena, R., Kumar, S., Singh, R., Puniya, A.K., 2015. Rumen Microbiology: An Overview. Pages 3-16 In Rumen Microbiology: From Evolution to Revolution.. A. K. Puniya, R. Singh, and D. N. Kamra, ed. Springer, New Delhi.
Diaz, A., Avendro, M., Escobar, M., 1993. Evaluation of sapindus saponaria as a defaunating agent and its effects on different ruminal digestion parameters. Jurnal Livestock Research for Rural Development 5, 1-6.
Filipek, J., Dvorak, R., 2009. Determination of the volatile fatty acid content in the rumen liquid: comparison of gas chromatography and capillary isotachophoresis. Acta Veterinaria Brno 78, 627-633.
Flythe, M.D., Harlow, B.E., Aiken, G.E., Gellin, G.L., Kagan, I.A., Pappas, J., 2017. Inhibition of growth and ammonia production of ruminal hyper ammonia-producing bacteria by Chinook or Galena hops after long-term storage. Fermentation 3, 1-9.
Hartadi, H.S., Reksohadiprojo, S., Tilman, A.D., 1997. Tabel Komposisi Pakan untuk Indonesia. Cetakan Keempat. Universitas Gadjah Mada Press, Yogyakarta.
Kearl, LC., 1982. Nutrient Requirements of Ruminant. Pages 82 in Developing Countries. International Feedstuff Institute, Utah State University, Logan, Utah.
Leo, S., Maranatha, G., Oematan, G., 2023. Pengaruh level substitusi rumput (*bothiriochola pertusa*) dengan kangkung terhadap pH, konsentrasi VFA dan amonia cairan rumen ternak kambing kacang. Animal Agricultura 1, 13-23.
Li, X., Shao, T., Shi, Q., Hu, M., 2013. A diaryl Schiff base as a photo-and pH-responsive bifunctional molecule. Royal Society of Chemistry Advances 3, 22877-22881.
McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A., Wilkinson, R.G., 2011. Animal Nutrition. 7th ed. Pearson, UK.
McDonald, P., Edwards, RA., Greenhalgh, J.F.D., Morgan, C.A., 2002. Animal Nutrition (6th ed.). Pearson Education Limited. England.
Medjekal, S., Benbelkhir, FZ., López, S., 2023. Assessment of volatile fatty acids and methane production *in vitro* of opuntia ficus-indica cladodes in an Algerian arid area. Journal of Applied Biological Sciences 17, 46-54.
Millen, D., De Beni, A.M., Lauritano P.R.M., 2016. Rumenology. 1st Ed. Switzerland (CH): Springer International Publishing AG.
Moran, J., 2005. How the rumen works. In: Tropical Dairy Farming: Feeding Management for Small Holder Dairy Farmers in the Humid Tropics. Landlinks Press. Victoria. pp. 41-49.
Mosoni, P., Martin, C., Forano, E, Morgavi, D.P., 2011. Long-term defaunation increases the abundance of cellulolytic ruminococci and methanogens but does not affect the bacterial and methanogen diversity in the rumen of sheep1. Journal of Animal Science 89, 783–791.
Plummer, D.T., 1987. An Introduction to Practical Biochemistry. 3rd Edition. Mc. Graw-Hill Book Company Publ. New Delhi.
Steel, R.G.D, Torrie J.H., 1993. Prinsip dan Prosedur Statistika: Suatu Pendekatan Biometrik. Gramedia Pustaka Utama, Jakarta.
Tedeschi, L.O., Fox, D.G., Fonseca, M.A., Cavalcanti, L.F.L., 2015. Models of protein and amino acid requirements for cattle. Revista Brasileira de Zootecnia. 44, 109–132.