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Effect of *Coleus amboinicus* plant extracts in ruminant ration on microbial activity and *in vitro* degradation

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Volatile fatty acids (VFA) are the main energy source for livestock and ammonia is the main protein source for microbial synthesis in the rumen. The low value of VFA and NH_3-N in the rumen can affect the digestibility of feed consumed and animal health will be disrupted which results in low feed efficiency and ultimately low livestock productivity. To enhance the animal productivity, many strategies were applied in the tropic such as supplemented concentrate, feed additive and natural herbal or plant used as feed additive. In this experiment, we focused on plants used as feed additive. One of them is *Coleus amboinicus* L. (CAL) plant.

The CAL plant are identified as semi-succulent perennial plants in the Lamiaceae family. It has a life span of around 3–10 years. Height of plant can reach upto 25 cm above ground level. These plants are widely cultivated and naturalized elsewhere in the tropics, but in Indonesia, these plants are found in all regions with different name and traditionally are used as medicines, herbs ornamental plants and feed additive for animal.

The CAL has various active compounds such as carvacrol or phenol, phytosterol, sphingomyelin and tannin (Kaliappan and Viswanathan 2008) and other phytochemicals which can stimulate increased milk production and increase feed digestibility on goat (Adriani *et al*. 2019). To get good extraction results, it is necessary to use the right solvent. Sun *et al*. (2015) reported that water and ethanol can be used as solvent to extract phenolic compounds of propolis. However, there is no information about the right solvent to extract the active substance contained in CAL on rumen fermentation and ideal level of CAL extract which provide optimum results on VFA value and degradability of feed. Therefore, the objective of this study was to compare the use of water and ethanol to extract active ingredients CAL plant and levels of CAL extract on fermentation characteristic and *in vitro* degradability.

The basal feed used to evaluate the effect of CAL extracted with different solvent and levels CAL extract consisted of 70% forage and 30% concentrate on dry matter basis. The chemical compositions of basal feed are shown in Table 1. Each plant was cut into small pieces and dried naturally under shade (UV plastic house), and ground through a 1.5 mm screen to obtain powder. The powder was extracted with water and 96% ethanol. The extraction CAL with water was done by boiling at 90°C with a ratio of 1:5 for CAL powder and water respectively. The extract was store at 4°C for further use. The extraction with ethanol was done by soaking the CAL powder with ethanol (96%) at room temperature for 3 days. After soaking, the solution was filtered and the solvent removed using rotary evaporator and stored at 4°C for further used. Each extract of CAL was analysed for total tannin and phenol compound according to Makkar (2003). The composition of tannin and phenol compound are shown in Table 2.

Table 1. Chemical composition of basal feed

Parameter	Composition (%)
Dry matter	88.44
Organic matter	89.15
Crude protein	14.10
Crude fibre	21.07
Ether extract	2.57
Ash	10.85
Ca	0.83
P	0.49
Energy (Kcal/g)	3.73

Filtered rumen fluid was mixed with buffer solution in ratio of 4 : 1 for rumen fluid and buffer solution respectively. Fifty millilitres of this mixture was transferred into a serum bottle (100 ml capacity) containing 1 g of feed sample and added CAL extract which was extracted with water and

Table 2. Tannin and phenol compound in CAL extraction with water and ethanol

Extraction solvent	Tannin (%)	Phenol (%)
A1 (Water)	1.27	3.34
A2 (Ethanol 96%)	0.56	1.77

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ethanol (96%) at levels of 2, 4 and 6%, respectively. The bottle was sealed with rubber stopper and incubated for 48 h at 40°C. Incubation was done in triplicate. At the end of incubation period, the *p*H was measured immediately by using a portable electrode *p*H meter. The incubated inoculum was subsampled for analysis of volatile fatty acid (VFA), ammonia and protozoa population. The precipitates were analysed for dry matter (DM), organic matter (OM), crude protein (CP) and crude fibre (CF). The VFA (acetate, propionate and butyrate) were determined by Gas Chromatography (Shimadzu GC-14A). Ammonia concentration was determined by micro diffusion Conway technique. The chemical composition of precipitates (DM, OM, CP and CF) were analysed according to AOAC (2005). Protozoa population were determined by using a levy-Hauser-Neubauer counting chamber observed under the light microscope. Degradability was calculated by comparing the difference in the amount of nutrients before and after incubation with the amount of nutrients before incubation.

Effect of extraction solvent and levels of CAL extract on *p*H, ammonia, VFAs concentration, protozoal population and degradability of DM, OM, CP, CF were analysed by two-way analysis of variance using SAS program (SAS Institute Inc., Railg, North Carolina, 2004). Differences among treatments were compared using Duncan's range test.

The values of *p*H, concentrations of ammonia, acetate, propionate, butyrate, VFAs and protozoal population and degradability are presented in Table 3. The result showed that there was a significant (P<0.01) interaction between extraction solvent and levels of CAL extract on *p*H and ammonia concentration. In ethanol solvent, the value of *p*H and ammonia concentration increased significantly (P<0.001) at 2% CAL extract but there were no significant difference between levels of 2, 4 and 6%. In water solvent, the increasing *p*H value and ammonia were seen at level of 4% and there were no significant differences between level of 4 and 6% on *p*H. However, the concentration of ammonia decreased (P<0.05) drastically, if the level of CAL extracted was increased to 6%. A significant difference in *p*H and ammonia concentration between ethanol solvent and water solvent was observed at level of 2, 4 and 6%. Where the *p*H and ammonia in ethanol solvent was higher than in water solvent. The *p*H value in the present study was still in the range for optimal microbial activity (6.35–6.87). The *p*H for optimum activity of rumen microbes were within the range of 6–6.9 (Kamra *et al*. 2005). The results showed that the *p*H value was related to ammonia concentration. When the ammonia concentration increases, the *p*H value also increases. Likewise, it has been established by various researcher that increase ammonia concentration result in increase of *p*H value (Comacho *et al*. 2019).

The results showed that the concentrations of ammonia are in the range of 58.5–143.3 mg N/L. Although the quantity of protein content in the basal ration was the same in the present study, the ammonia concentration was different for each extraction solvent and levels of CAL extract. The CAL extracted with ethanol had higher ammonia concentration (114.6%) compared to CAL extracted with water (59.5%) at level of 4% of dry matter basal feed. The difference in result in the present study might be due to presence of phytochemical compound of CAL extract such as tannin and phenol compound, which was higher in CAL extracted with water than ethanol (Table 3). Similar result was reported by Sarnataro and Spanghero (2020) that the tannin compound of *Stevia rebaudiana* Bertoni can reduce ammonia concentration *in vitro.* Tannin are a group of phenolic compounds that are anti-microbial (Huang *et al*. 2018). The tannin and phenol compound can

Table 3. Effect of levels of CAL extraction with water (A1) and ethanol (A2) on *p*H, ammonia (mg N/L), VFAs concentrations (mM), protozoa population (×10^4/ml) and degradability of dry matter (DM), organic matter (OM), crude protein (CP) and crude fibre (CF) (%) *in vitro*

Parameter	A1				A2				A×B	Main effect		
	B0	B1	B2	B3	B0	B1	B2	B3		A	B	SEM
*p*H	6.4^{b}	6.4^{b}	6.6^{a}	6.6^{a}	6.6^{b}	6.9^{a}	6.8^{a}	6.9^{a}	**	**	**	0.1
Ammonia	71.7^{bc}	85.6^{b}	114.4^{a}	58.9^{c}	60.1^{b}	138.7^{a}	128.9^{a}	143.3^{a}	**	**	**	13.2
Acetate	29.9	26.6	26.4	24.2	23.5	22.2	22.2	20.8	N	**	NS	2.7
Propionate	15.2	15.7	16.8	18.9	15.6	16.4	17.6	16.1	NS	NS	*	2.5
Butyrate	8.7	8.3	10.7	10.2	7.6^{b}	8.1^{a}	7.1^{c}	5.8^{d}	NS	**	NS	1.4
Total VFA	64.4	59.4	64.3	63.3	59.8^{a}	55.4^{ab}	57.7^{ab}	50.6^{b}	NS	*	NS	6.9
Protozoa	1.3	1.2	1.3	1.5	1.4^{b}	1.9^{a}	1.9^{a}	1.8^{ab}	*	**	*	0.2
Degradability												
DM	48.8^{b}	56.8^{a}	56.5^{a}	53.4^{ab}	48.6^{a}	39.7^{b}	33.7^{c}	26.3^{d}	**	**	**	3.7
OM	59.5^{b}	62.9^{ab}	66.7^{a}	64.2^{ab}	59.3^{a}	49.3^{b}	43.6^{c}	35.7^{d}	**	**	**	3.7
CP	54.9^{b}	58.4^{b}	64.8^{a}	55.1^{b}	52.5^{a}	36.7^{bc}	44.3^{ab}	33.9^{c}	**	**	**	4.2
CF	41.5	41.2	42.7	46.3	42.8^{a}	28.1^{b}	21.8^{b}	23.2^{b}	**	**	**	4.4

Means with different superscript in each extraction solvent are significantly different (P<0.05). A1, CAL extraction with water; A2, CAL extraction with ethanol; B0, level of 0%; B1, 2%; B3, 4%; B4, 6%; A×B, interaction of levels by extraction solvent. Four replicates for each extraction solvent.

inhibit the growth of microorganisms or enzyme activity. The higher ammonia concentration in the CAL extracted with ethanol may also be the result of higher population of protozoa in CAL extracted with ethanol (Table 3). Protozoa are actively involved in the degradation of dietary and microbial proteins in the rumen. Protozoa actively deaminate amino acid to form ammonia (Guoyou 2018).

A significant (P<0.05) difference in acetate, butyrate, total VFA and protozoal population between CAL extracted with water and ethanol were observed. CAL extraction with water yielded higher acetate, butyrate and total production of VFA concentration than CAL extraction with ethanol but the protozoal population was significantly (P<0.05) higher in CAL extracted with ethanol than those in CAL extracted with water. Levels of CAL extract were significantly affected only on propionate. Propionate concentration at level of 0% was significantly (P<0.05) lower than those at levels of 4% and 6% but the difference in propionate between level of 2%, 4% and 6% and between levels of 0% and 2% were not significant.

There are several factors that influence the concentration of VFA, namely phytochemicals contained in feed ingredients such as tannin (Tseu *et al.* 2020), saponin (Hundal *et al*. 2020) and phenolic compound (Wei *et al*. 2019). These compounds can inhibit the rumen microbial activity to ferment the feed ingredients which in turn affects the production of VFAs. Although, the tannin and phenol content were higher in CAL extracted with water (0.76 mg/g sample and 2 mg/g, respectively) than CAL extracted with ethanol (0.56 mg/g and 1.77 mg/g, respectively). However, the higher tannin and phenol compound in CAL extract did not result in lower VFAs concentration. This is probably because tannin and phenol are still in limit allowed in the basal ration. Tannin and phenol compound can function as antioxidant to animal. However, too high concentration in the ration can lead to a number of negative effect as a result of toxicity or inhibit growth of cellulolytic bacteria (Margedus *et al*. 2020). Jayanegara *et al*. (2015) reported that the addition of tannin @ 1 mg/ml *in vitro* reduce VFAs concentration. Benchaar (2021) reported administration of essential oils that contain high levels of phenolic compounds at a dose of 50 mg/kg DM had no effect on VFA concentrations in the rumen. An increase in the level of CAL extracted with ethanol up to 6% can result in a decrease in the concentrations of butyrate and total VFA concentration. The reason for decrease of the VFAs concentration in CAL extracted with ethanol was not clear but the result of protozoal population in CAL extract with ethanol showed significant increase from control (Table 3). This might be due to the high protozoal population in CAL extracted with ethanol.

Effect of extraction solvent and levels of CAL extract on the degradability of dry matter (DM), organic matter (OM), crude protein (CP) and crude fibre (CF) of ration are shown in Table 3. A significant (P<0.01) interaction between extraction solvent and levels of CAL extract was found in all parameters of degradability. In ethanol solvent, increasing levels of CAL extract significantly (P<0.05) decreased DM, OM, CP and CF. Otherwise, increasing levels in CAL extraction with water significantly (P<0.05) increased degradability of DM, OM and CP, but CF degradability had no significant correlation with level of CAL extract. The degradability of DM significantly increased at level of 2% and there was no significant difference between level of 2%, 4% and 6%. The degradabilities of OM and CP significantly increased at level of 4% and CP degradability decreased at level of 6%. The difference in degradability between ethanol solvent and water solvent was significant (P<0.05) at level of 2%, 4% and 6%, whereas the degradability in all parameters was lower in CAL extracted with ethanol than with water. The results in this study showed that greater protozoal population are associated with lower degradability. According to Patel and Ambalam (2018), that protozoa can consume bacteria, which causes the number of bacteria that ferment the feed to be low. Afdal *et al*. (2020) reported that the extraction of CAL with ethanol results in low total gas production. The low gas production indicated the low fermentation that occurs in feed, which in turn reduces degradation of feed.

SUMMARY

This study was conducted to compare the use of water and ethanol as solvents to extract *Coleus amboinicus* L. (CAL) plant and their effect on rumen fermentation and digestion *in vitro*. The experimental design was a 2 × 4 × 4 Factorial arrangement in Complete Random Design. The main effects were extraction solvent (water and ethanol 96%) and levels of CAL extract (0%, 2%, 4% and 6% of amount of sample incubated). The results showed that the addition of CAL extract either extracted with water or ethanol provides an ideal *p*H value for rumen microbial activity (6.35–6.87). The use of water as a solvent for extracting CAL plant provides a lower concentration of ammonia and protozoa population as compared to using ethanol as a solvent. The VFA values, especially acetate, butyrate and all degradation parameters were higher in water solvent. Increasing the level of use of CAL extract to 6% tends to increase the population of protozoa and reduce the concentration of acetate and all degradation parameters except for the concentration of propionate, butyrate and total VFA which did not respond to increasing CAL levels of the extract. It could be concluded that the water solvent gives better results on the fermentation and degradation of feed with a limit of 2% usage in ruminant ration.

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