Jurnal Sain Peternakan Indonesia
Available at https://ejournal.unib.ac.id/index.php/jspi/index
DOI: https://doi.org/10.31186/jspi.id.18.3.185-192
P-ISSN 1978-3000
E-ISSN 2528-7109
Volume 18 Issue 3 July-September 2023

The Effects of Papaya Leaf Extract and Turmeric Extract Inclusion on Total Apparent Digestibility of Nutrients and Methane Gas Production in Bali Cattle

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Revised: 2023-09-25, Accepted: 2023-09-27, Publish: 2023-09-30

ABSTRACT

This research evaluated the effects of papaya leaf and turmeric extract supplementation on the total apparent digestibility of nutrients and methane gas production in Bali cattle. The livestock used were 18 Bali cattle that were distributed into treatment without papaya leaf and turmeric extract supplementation (P0), Supplementation of 0075% turmeric extract g/kg live weight/day (P1), and supplementation of 0.00375% turmeric extract per kg live weight per day + 0.00375% papaya leaf extract per kg live weight per day (P2). The basal diets were 60% elephant grass cv. Taiwan (*Pennisetum purpureum cv. Taiwan*) and 40% concentrate. The ration provision was three per cent dry matter of body weight. The observed parameters included feed consumption, feces production, dry matter digestibility, organic matter digestibility, crude protein digestibility, crude fat digestibility, crude fibre digestibility, nitrogen-free extract digestibility, and methane gas production. The research results indicated that the treatment did not significantly affect feed consumption ($P>0.05$) but did affect crude protein digestibility, crude fibre digestibility, nitrogen-free extract digestibility, and methane gas production. This study concludes that the feeding of elephant grass cv. Taiwan *(Pennisetum purpureum cv. Taiwan),* with the addition of papaya leaf extract and turmeric extract, does not positively affect feed consumption and feed digestibility; thus, it cannot suppress methane gas production in livestock.

Keywords: Papaya leaf extract, turmeric extract, feed digestibility, methane gas production, bali cattle

INTRODUCTION

Greenhouse gas emissions are one of the triggers for global warming, which impacts rising temperatures on Earth and climate change. Global warming occurs due to the greenhouse effect in the form of carbon dioxide (CO_2), methane (CH_4), dinitrogen oxide (N_2O), and *chlorofluorocarbons* (CFCs) in the Earth's atmosphere. The livestock sector produces methane and nitrogen oxide emissions. Methane gas emissions come from livestock enteric fermentation and manure management, while nitrous oxide emissions come from livestock manure management (Ratnia, 2018).

Apart from contributing to global warming, methane gas can also be detrimental to farmers because around 6-10% of the gross energy of feed consumed, which should be used to support productivity by livestock, will be lost as methane gas (Jayanegara et al., 2009). Reducing methane gas production means reducing the greenhouse effect and increasing feed efficiency.

Previous researchers have done several experiments to reduce methane gas production, namely through concentrate supplementation (Lovett et al*.,* 2005), lipid (Ungerfeld et al*.,* 2005), forage supplementation containing tannins (Jayanegara et al*.*, 2009), use of natural additives (Jayanegara, 2011). Studies on using natural compounds, such as tannins, saponins, and essential oils, are increasingly being carried out in line with the banning of antibiotics (Yuliana et al*.*, 2019).

Papaya leaves are antibacterial and antioxidant, containing vitamins C and E, papain enzymes, and B-carotene. Apart from that, papaya leaves contain alkaloid compounds, calpain, saponins, flavonoids, and tannins. The content of saponins functions as a defaunation agent to reduce the protozoa population by breaking down the protozoa membrane so that protozoa cells lyse and cause protozoa death (Wahyuni et al*.,* 2014). By decreasing the protozoa population, bacterial cells will increase, especially cellulolytic bacteria, allowing the feed to be decomposed effectively (Ichwani et al., 2013).

Turmeric is a plant material often used as a raw material for traditional medicine. It contains 5% essential oil, 60% curcumin, 25% essential oil, 28% *glucose*, 12% *fructose*, and 8% protein and vitamins. The content of compounds contained in turmeric is expected to increase the digestibility of feed; thus, the absorption of nutrients increases.

These two herbal ingredients can potentially increase feed fermentability due to the presence of active compounds as defaunation agents. Increasing feed fermentability is expected to reduce methane gas production in ruminants. This study aims to evaluate the effect of papaya leaf extract and turmeric extract on digestibility and methane gas production in Bali cattle.

RESEARCH METHODS

The materials used were 18 Bali cattle with an average body weight of 190-197 kg. The cattle were housed individually in a 1.5 x 3 m pen. The forage given is elephant grass cv. Taiwan (*Pennisetum purpureum cv. Taiwan*). The herbal ingredients used are papaya leaf extract and turmeric extract. Concentrate feed consisting of rice bran, cassava flour, ground corn, coffee husk, mineral premix, salt, and drinking water is provided. The solvent used was 96% ethanol. The ration was given twice daily at 7 a.m. and 15 a.m. based on 3% body weight dry matter, and the refusals were recorded daily.

Feed adaptation was carried out for ten days. The feed formulation used and the nutritional content of the feed are presented in Table 1. The feed formulation and the nutritional composition of each treatment are presented in Table 2. The results of the phytochemical tests of papaya leaf extract and turmeric extract are presented in Table 3.

Table 1. The Nutritional content of feed ingredients

Rations Material	Dry matter (%)	Crude protein (%)	Crude Fibre (%)	Total digestible nutrient (%)	Ash (%)	P (%)
Rice bran	86	8.50	17	57.4	0.09	1.09
Cassava flour	85.20	3.30	2.8	73.49	0.84	1.25
Corn flour	90	22	8	75	0.05	0.50
Coffee husk	30	3	2.14	17.16	0.68	0.20
Mineral premix	0	0	0	0	32	10
Salt	0	0	0	0	0	0
Elephant grass	20.29	10.00	32.60	46.00	0.12	0.18

Table 2. The feed formulation and the nutritional composition for each treatment

Feed Ingredients	Feed nutritional composition		
	P0	P1	P2
Rice bran (%)	8	8	8
Cassava flour (%)	8	8	8
Corn flour (%)	20	20	20
Coffee husk (%)	3	3	3
Mineral premix (%)	0.5	0.5	0.5
Salt (%)	0.5	0.5	0.5
Elephant grass (%)	60	60	60
Turmeric extract (% of body weight)	-	0.0075	0.00375
Papaya leaf extract (% of body weight)	-	-	0.00375
Total	100	100.0075	100.0075
Concentrate nutritional content			
Dry matter (%)	33.54	33.54	33.54
Crude protein (%)	5.43	5.43	5.43
Crude fibre (%)	3.25	3.25	3.25
Total digestible nutrient (%)	26.39	26.39	26.39
Ash (%)	0.21	0.21	0.21
P (%)	0.38	0.38	0.38
Elephant grass's nutritional content			
Dry matter (%)	12.17	12.17	12.17
Crude protein (%)	6.12	6.12	6.12
Crude fibre (%)	19.56	19.56	19.56
Total digestible nutrient (%)	27.60	27.60	27.60
Ash (%)	0.30	0.30	0.30
P (%)	0.12	0.12	0.12

Research design

The papaya and turmeric leaf extract added to the ration was 0.0075% of the animal's body weight. The percentage of forage, concentrate feed, and herbal additives used is as follows:

P0 = 60% forage + 40% concentrate (without adding herbal extracts)

P1 = 60% forage + 40% concentrate containing 0.0075% of the body weight of cattle turmeric extract

P2 = 60% forage + 40% concentrate containing 0.00375% body weight of papaya leaf extract + 0.00375% body weight of turmeric extract.

Table 3. Phytochemical test results of papaya leaf extract and turmeric extract

Active compound	Turmeric	Papaya leaf
	---------------%------------	
Saponin*	3.73	3.42
Alkaloid*	0.24	0.56
Steroid*	1.55	2.48
Flavonoid*	1.92	4.69
Tannin*	41.33	53.98
Phenol*	1.71	3.62
Essential oil**	1.81	0
Curcumin***	10.92	0

*Liizza (2018)

** Sundari (2016)

*** Research Center for Medicinal and Aromatic Plants

Parameters

The parameters observed in this study were feed consumption and digestibility of dry matter, organic matter, crude protein, crude fat, crude fibre and nitrogen-free extract and methane gas production.

Methane Gas Production

Methane gas produced by livestock is measured using the formula according to Jentsch et al. (2007) as follows:

Methane (kj/d) = $1.62 \times d_{CP} - 0.38 \times d_{Cfat} + 3.78 \times d_{CF} + 149 \times d_{FUN} + 1142$ (g/d)

Information :

d_{CP} = digestibility of crude protein

d_{Cfat}= digestibility of crude fat

d_{CF} = digestibility of crude fibre

d_{FUN} = Nitrogen-free extract digestibility

Data Analysis

The research data were analyzed using test analysis of variance (ANOVA). The significance level in this study was 5%. If the results show a significant effect, proceed with the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Feed Consumption

Data on the effect of herbal extracts on feed consumption during the study is presented in Table 4. Feed consumption showed no difference among treatment groups (P>0.05). Even though the turmeric extract has a distinctive aroma and a bitter taste (Duraisankar and Ravindran, 2015). The supplementation did not negatively affect feed intake in this research. Choubey et al. (2016) explained that phytogenic feed additives enhance feed utilization without harming dry matter consumption. Table 5 presents the effect of treatment on dry matter consumption, organic matter consumption, crude fibre consumption, crude fat consumption, and nitrogen-free extract consumption.

Adding only turmeric extract to the concentrate and turmeric plus papaya leaf extract did not affect (P>0.05) dry matter consumption, organic matter consumption, crude fibre consumption, crude fat consumption, and nitrogen-free extract consumption. A higher dosage of turmeric addition of 0.1 and 0.2 curcumin to the concentrate showed similar results on feed intake (Vorlaphim et al., 2011). However, a higher dose of turmeric supplementation may effectively support a healthy rumen and feed efficiency (Budiari et al., 2020). It is worth noting that the effects can vary depending on the species, formulation and dosage.

Table 4. Feed consumption

Parameters	Consumption (kg/head/day)			P
	P0	P1	P2	
2nd-week average consumption	13.82 ± 1.35	14.45 ± 3.84	15.32 ± 5.51	0.807
4th-week average consumption	14.26 ± 1.61	14.35 ± 3.97	15.25 ± 5.42	0.893
Average total consumption for one month	14.04 ± 1.47	14.40 ± 3.90	15.29 ± 5.46	0.856

Table 5. Average daily feed consumption for Bali cattle during the study (dry matter basis)

Parameter	Nutrient consumption (kg/head/day)			P
	P0	P1	P2	
Dry matter				
2nd-week average consumption	5.18 ± 0.46	5.29 ± 1.37	5.58 ± 1.89	0.874
4th-week average consumption	5.29 ± 0.53	5.27 ± 1.41	5.56 ± 1.86	0.920
Average total consumption 1month	5.23 ± 0.49	5.28 ± 1.39	5.57 ± 1.87	0.901
Organic matter				
2nd-week average consumption	4.73 ± 0.42	4.85 ± 1.26	5.11 ± 1.74	0.870
4th-week average consumption	4.84 ± 0.48	4.82 ± 1.29	5.09 ± 1.71	0.918
Average total consumption 1month	4.79 ± 0.45	4.83 ± 1.27	5.10 ± 1.73	0.899
Crude Protein				
2nd-week average consumption	0.29 ± 0.02	0.29 ± 0.08	0.31 ± 0.11	0.865
4th-week average consumption	0.29 ± 0.03	0.29 ± 0.08	0.31 ± 0.10	0.920
Average total consumption 1month	0.29 ± 0.03	0.29 ± 0.07	0.31 ± 0.10	0.881
Crude Fat				
2nd-week average consumption	0.09 ± 0.01	0.09 ± 0.02	0.09 ± 0.03	0.967
4th-week average consumption	0.09 ± 0.01	0.09 ± 0.02	0.09 ± 0.03	1.000
Average total consumption 1month	0.09 ± 0.01	0.09 ± 0.02	0.09 ± 0.03	1.000
Crude Fibre				
2nd-week average consumption	1.36 ± 0.12	1.40 ± 0.37	1.48 ± 0.52	0.852
4th-week average consumption	1.39 ± 0.15	1.39 ± 0.38	1.48 ± 0.51	0.904
Average total consumption 1month	1.37 ± 0.14	1.40 ± 0.37	1.48 ± 0.51	0.883
Nitrogen-free Extract				
2nd-week average consumption	3.01 ± 0.26	3.07 ± 0.79	3.23 ± 1.08	0.880
4th-week average consumption	3.07 ± 0.30	3.05 ± 0.81	3.22 ± 1.07	0.923
Average total consumption 1month	3.04 ± 0.28	3.06 ± 0.80	3.23 ± 1.08	0.906

Feed Digestibility

The average nutrient digestibility of feed can be seen in Table 6.

Dry Matter Digestibility (DMD)

Dry matter digestibility indicates the amount of digestible nutrients in the rumen. (Suardin et al., 2014). Adding herbal extracts did not increase the value of dry matter digestibility. The average DMD value in this study was lower than the in vitro study's result. Liizza's study (2018) stated that an in vitro study on the DMD value in dairy cattle was significantly higher in papaya leaf extract and turmeric supplementation. According to Wahyuni et al. (2014), 1% tannin and 0.6% saponin administration inhibited the protozoa population, resulting in increased bacterial growth and feed digestibility. It shows that adding herbal extracts containing tannins and saponins does not interfere with feed digestibility or increase digestibility.

The higher the DMD value, the higher the nutritional opportunities livestock can use for growth and development (Aka and Sandiah, 2014). Factors that affect DMD are the activity of microorganisms found in the rumen, especially cellulolytic bacteria, which play a role in feed fermentation. In contrast, the activity of rumen microorganisms is influenced by the content of substances contained in feed ingredients (Suardin et al., 2014).

Organic Matter Digestibility (OMD)

The digestibility of organic matter indicates the availability of nutrients in the feed. The digestibility of organic matter is closely related to the digestibility of dry matter because some of the dry matter is organic matter consisting of crude protein, crude fat, crude fibre and nitrogen-free extract. The digestibility of organic matter describes the amount of fat, carbohydrate and protein nutrients in the feed ingredients (Riswandi et al., 2015). The digestibility value of organic matter in this study had no significant effect ($P>0.05$) on the treatment. The digestibility value of the organic matter in Liizza's study (2018), which used the addition of the same herbal ingredients, showed a lower value, namely 52.63-60.26%.

Table 6. The average nutrient digestibility of Bali cattle feed during the study

Parameter	Digestibility (%)			P
	P0	P1	P2	
Dry matter digestibility (DMD)				
2nd-week average	46.96 ± 10.01	60.17 ± 7.61	50.97 ± 11.27	0.087
4th-week average	51.71 ± 5.16	59.13 ± 9.96	50.72 ± 11.26	0.254
Average total 1month	49.37 ± 7.38	59.66 ± 8.55	50.84 ± 10.87	0.138
Organic matter digestibility (OMD)				
2nd-week average	55.60 ± 8.05	66.72 ± 6.62	58.00 ± 9.28	0.071
4th-week average	59.57 ± 3.95	65.88 ± 8.43	57.71 ± 9.58	0.192
Average total 1month	57.62 ± 5.82	66.31 ± 7.31	57.85 ± 9.08	0.107
Crude protein digestibility (CPD)				
2nd-week average	59.09 ± 7.38[ab]	72.54 ± 8.30[b]	50.06 ± 16.67[a]	0.014
4th-week average	62.81 ± 3.61[b]	72.24 ± 7.82[b]	50.20 ± 14.84[a]	0.006
Average total 1month	60.98 ± 5.30[ab]	72.40 ± 7.79[b]	50.13 ± 15.53[a]	0.008
Crude fat digestibility (CFD)				
2nd-week average	58.16 ± 23.06	72.14 ± 5.58	61.41 ± 11.23	0.278
4th-week average	61.88 ± 18.97	71.82 ± 4.98	60.32 ± 15.02	0.344
Average total 1month	60.04 ± 20.94	71.99 ± 4.97	60.86 ± 13.00	0.310
Crude fibre digestibility (CFrD)				
2nd-week average	67.16 ± 6.79[b]	67.03 ± 19.93[b]	40.33 ± 17.69[a]	0.015
4th-week average	70.28 ± 3.46[b]	65.46 ± 24.66[b]	39.90 ± 19.67[a]	0.025
Average total 1month	68.75 ± 5.02[b]	66.27 ± 22.12[b]	40.10 ± 18.29[a]	0.018
Nitrogen-free extract digestibility (NFEtD)				
2nd-week average	49.99 ± 8.99[a]	65.84 ± 10.45[b]	66.70 ± 10.59[b]	0.018
4th-week average	54.35 ± 6.76	65.22 ± 10.80	66.48 ± 10.75	0.072
Average total 1month	52.20 ± 6.63[a]	65.54 ± 10.53[b]	66.59 ± 10.50[b]	0.032

According to Suardin et al. (2013), saponin compounds contained in papaya leaf extract and turmeric extract are thought to increase the bacterial population, thereby triggering an increase in the rumen's digested organic matter. An increase in the organic matter digestibility value was due to the organic matter contained in the dry matter, so the high digestible dry matter content also resulted in high digestible organic matter content. But this is not in line with the results found in this study, where concentrates added with herbal extracts or without additional herbal extracts have relatively the same organic matter digestibility value, meaning that the saponin compounds found in papaya leaf extract and turmeric extract have not been able to increase the content of digested organic matter in the rumen.

Crude Protein Digestibility (CPD)

Digestibility of the crude protein showed significantly different results ($P<0.05$) between P1 and P2, but P0 was not significantly different ($P>0.05$) between P1 and P2.

P2 has the lowest CPD value compared to P0 and P1. The level of protein digestibility is closely related to the nutrient content contained in the feed ingredients (Prawitasari et al., 2012). It means that the presence or absence of additional turmeric extract has the same sound effect on CPD. The content of natural compounds contained in turmeric extract, which is believed to have the potential to increase feed fermentability, can increase CPD. However, adding papaya leaf extract and turmeric extract decreases CPD. It is presumably because the dose of turmeric extract added to P1 can increase the number of bacteria in the rumen. Vorlaphim et al. (2011) reported that including 0.1% and 0.2% curcumin increased the number of bacteria and reduced the protozoa population in the rumen. The low CPD in P2 was due to tannin compounds in this treatment. Tseu et al. (2020) mentioned that tannins can linearly reduce the digestibility of crude protein.

Crude Fat Digestibility (CFD)

The crude fat digestibility of the feed supplemented with turmeric extract (P1) was higher than that of the feed not supplemented and supplemented with a mixture of turmeric extract and papaya leaf extract. According to Sastrawan (2009), the digestibility of a feed depends on the quality of the nutrients contained in the feed and will affect the growth of microorganisms. The high digestibility of crude fat is caused by the chemical structure of the fat, which is easily digested by microbes in the rumen (Wiseman, 1990).

Crude Fibre Digestibility (CFrD)

Supplementation of turmeric and papaya leaf extract (P2) decreased crude fibre digestibility ($P<0.05$). However, CFrd of a control group and treatment of merely turmeric extract showed similar results ($P>0.05$). Dietary curcumin significantly and dose-dependently reduced fecal nitrogen excretion. Thus, curcumin feeding increased nitrogen absorption, in absolute terms or as a percentage of consumption. The P2 treatment had a lower digestibility value for crude fibre, presumably because the tannin content present in this treatment was higher, thereby reducing the digestibility of fibre in the rumen. Besides functioning as defaunation agents, tannins also protect feed proteins. In addition to the positive impact given by tannins, tannins also have a weakness in their function as defunct agents because the phenol groups in tannins also have antibacterial properties (Wahyuni, 2014), so in this study, it can reduce the digestibility of crude fibre, namely 40.20%.

Nitrogen Free Extract Digestibility (NFEtD)

Including herbal extracts in concentrate increased NFEtD ($P<0.05$). Although the protein content and NFEtD for each treatment were the same, the results showed that the digestibility of NFEtD-given herbal extracts was higher than that of the control treatment. It is because the saponins contained in herbal extracts can form complexes irreversible with cholesterol (a component in the cell membrane of microorganisms except bacteria); as a result, there is death in protozoa and increased bacterial growth, so that it also increases the digestibility of NFEtD as well (Khoiriyah, 2017).

Production of Methane (CH_4) based on Enteric (Digestive)

Increased consumption of crude fibre is related to increased production of methane gas. The higher the crude fibre content consumed by livestock, the higher methane gas production (Philippe and Nicks, 2015). High methane gas production indicates low feed digestibility. The amount of energy wasted from the feed consumed after being fermented in the rumen causes methane gas production to increase. The methane gas production produced during the study is presented in Table 7.

Control treatment with the addition of different herbal extracts had a significant effect ($P<0.05$) on the production of methane gas in the second week and during the study (Table 7). The administration of papaya leaf extract and turmeric extract significantly increased methane gas production. The increase in gas production after adding herbal extracts is thought to be due to some of the saponins being degraded into aglycone and glycone (sugar) components. Then, the components of these sugars are metabolized by microbes into gas (Patra et al*.,* 2010). Apart from originating from the active compounds in the herbal ingredients, the initial protozoa population also determines the defaunation effect (Mastika, 2014). The protozoa in the rumen contribute to methane gas production because protozoa will produce hydrogen when digesting structural carbohydrates (Nguyen et al., 2020). Methane gas is produced from the metabolism of carbohydrates by rumen methanogenic bacteria, which are symbiotic with protozoa (Adawiyah et al., 2007). Methane gas production positively affects the digestibility of organic matter because the two mutually describe the level of feed degradation in the rumen.

Table 7. Bali cattle methane production during the study

Methane gas production average (kj/day)	P0	P1	P2	P
2nd week of methane gas production	8,917.16 ± 1,369.87^{a}	11,295.33 ± 1,519.91^{b}	11,290.17 ± 1,597.53^{b}	0.021
4th week of methane gas production	9,583.52 ± 722.05	11,196.88 ± 1,578.74	11,256.80 ± 1,615.09	0.087
Methane gas production for one month	9,254.76 ± 1,00826^{a}	11,247.12 ± 1,533.06^{b}	10,591.60 ± 1,633.51^{b}	0.037

The results of methane gas production found in this study were higher than Nur et al. (2015), who reported that the production of methane gas in dairy cows with different feeds produced methane gas from 6,014.53 kJ/day to 6,601.90 kJ/day. This difference occurs due to differences in the type of livestock, age, and feed treatment given. A decrease in the proportion of methane gas to total gas during feed fermentation in the rumen is attributable to tannin and saponin compounds Widiawati et al. (2010).

Adding turmeric extract and a mixture of turmeric and papaya leaf extract in this study could not reduce methane gas production. In contrast, pure tannin inclusion at 0.5 mg/ml could reduce methane gas production. Tannins that were easily hydrolyzed were more capable of reducing methane gas production than condensed tannins (Jayanegara et al., 2015). Several possibilities cause differences in the methane gas production from the two studies, such as tannin concentration. Tannins concentration in Jayanegara et al. (2009) is higher. Secondly, the papaya leaf extract and turmeric extract, which contain tannins used in this study, did not undergo a purification process, so the effect of the tannins in these two herbal extracts was influenced by other components contained in the extracts from both of them.

Including more than 5% of tannins is not recommended because it harms the digestibility and performance of livestock (Jayanegara et al., 2009). A 2-5% tannin level in the ration facilitates an optimal result for reducing methane gas production.

CONCLUSIONS

Provision of elephant grass forage cv. Taiwan (*Pennisetum purpureum* cv. Taiwan) with papaya leaf extract and turmeric extract did not positively affect feed consumption and feed digestibility, so they could not suppress methane gas production in livestock.

REFERENCES

Adawiah, T. Sutardi, T. Toharmat, W. Manalu, N. Ramli, dan U.H. Tanuwiria. 2007. Respons terhadap suplementasi sabun mineral dan mineral organik serta kacang kedelai sangrai pada indikator fermentabilitas ransum dalam rumen domba. Media Peternakan. 3(1): 63-70.

Aka, R., dan Sandiah, N. 2014. Kecernaan bahan kering dan bahan organik campuran rumput mulato (*Brachiaria hybrid*. cv. mulato) dengan jenis legum berbeda menggunakan cairan rumen sapi. Jurnal Ilmu dan Teknologi Peternakan Tropis, 1(1): 16-22.

Budiari, N. L. G., Pujiawati, Y., Adijaya, I. N., dan Kertawirawan, I. P. A. 2020. Pengaruh level tepung kunyit pada ransum sapi bali terhadap performans dan pendapatan peternak. In Prosiding Seminar Nasional Teknologi Agribisnis Peternakan (STAP) 7, 618-627).

Choubey, M., Pattanaik, A.K., Baliyan, S., Dutta, N., Jadhav, S.E., and Sharma, A. Kumar, A. Kumar, Dutta, Narayan & Jadhav, Sunil & Kusumakar, Sharma. (2015). Effect of a composite phytochemical feed additive on in vitro substrate degradation and methanogenesis and in vivo rumen fermentation. Animal Nutrition and Feed Technology, 14(3): 523-534.

Duraisankar, M., and Ravindran, A. D. (2015). Identification of Curcuma longa rhizomes by physicochemical and TLC fingerprint analysis. Int. Journal of. Pharm Tech. Research, 2(9): 459-465.

Hidayah, N. 2016. Pemanfaatan senyawa metabolit sekunder tanaman (tanin dan saponin) dalam mengurangi emisi metan ternak ruminansia. Jurnal Sain Peternakan Indonesia, 11(2): 89-98.

Ichwani, F., Rustomo, B., dan Bata, M. 2013. Pengaruh penambahan tepung daun waru (hisbiscus tileaceus) dalam ransum sapi lokal berbasis jerami padi amoniasi terhadap kecernaan bahan kering dan bahan organik. Jurnal Ilmiah Peternakan, 1(2): 554-560.

Jayanegara, A. 2011. Polifenol sebagai aditif alami dalam upaya mitigasi emisi gas metana asal ternak ruminansia. Departemen Ilmu Nutrisi dan Teknologi Pakan, Fakultas Peternakan IPB Jl. Agatis Kampus IPB Darmaga Bogor, 16680: 1-20

Jayanegara, A., dan Sofyan, A. 2008. Penentuan aktivitas biologis tanin beberapa hijauan secara in vitro menggunakan'Hohenheim gas test'dengan polietilen glikol sebagai determinan. Media Peternakan, 31(1): 44-52

Jayanegara, A., Makkar, H. P., & Becker, K. 2009. Emisi metana dan fermentasi rumen in vitro ransum hay yang mengandung tanin murni pada konsentrasi rendah. Media Peternakan, 32(3): 185-195

Jayanegara, A., Sofyan, A., Makkar, H. S., dan Becker, K . 2009. Kinetika produksi gas, kecernaan bahan organik dan produk si gas metana in vitro pada hay dan jerami yang disuplementasi hijauan mengandung tanin. Media Peternakan, 32(2): 120-129

Jentsch, W., Schweigel, M., Weissbach, F., Scholze, H., Pitroff, W., dan Derno, M. 2007. Methane production in cattle calculated by the nutrient composition of the diet. Archives of animal nutrition, 61(1): 10-19.

Kementerian Lingkungan Hidup. 2012. *Pedoman penyelenggaraan inventarisasi gas rumah kaca nasional.* Kementerian Lingkungan Hidup. Jakarta.

Khoiriyah, M., Chuzaemi, S., dan Sudarwati, H. 2017. Effect of flour and papaya leaf extract (Carica papaya l.) addition to feed on gas production, digestibility and energy values in vitro. TERNAK TROPIKA Journal of Tropical Animal Production, 17(2): 74-85.

Liizza, R. M., Harjanti, D. W., dan Muktiani, A. 2018. Pengaruh ekstrak daun pepaya (carica papaya linn) dan kunyit (curcuma domestica) terhadap kecernaan nutrien pada sapi perah secara in vitro. AGROMEDIA: Berkala Ilmiah Ilmu-ilmu Pertanian, 36(1): 114-122

Lovett, D.K., L.J. Stack, S. Lovell, J. Callan, B. Flynn, M. Hawkins., dan F.P. O'Mara. 2005. Manipulating enteric methane emissions and animal performance of late-lactation dairy cows through concentrate supplementation at pasture. Journal of Dairy Science, 88: 2836-2842.

. Yuliana, P., E. Laconi, A. Jayanegara, S. Achmadi, A.A. Samsudin (2019). Modulasi Pola Fermentasi Rumen dan Emisi Gas Metana dari Penambahan Ekstrak Lerak pada Silase Gamal (modulation of rumen fermentation and methane emission by lerak extract addition to gliricidia silage). 20. 2477-5665. 10.19087/jveteriner.2019.20.2.202.

Suardin., N. Sandiah dan R. Aka. 2014. Kecernaan bahan kering dan bahan organik campuran rumput Mulato (*Brachiaria hybrid* cv. mulato) dengan jenis legum berbeda menggunakan cairan rumen sapi. J. Ilmu dan Teknologi Peternakan Tropis. 1 (1): 16 – 22.

Sundari, R. 2016. Pemanfaatan dan efisiensi kurkumin kunyit (Curcuma domestica Val) sebagai indicator titrasi asam basa. Teknoin, 22 (8): 595-601

Wahyuni, I. M. D., Muktiani, A., dan Christiyanto, M. 2014. Kecernaan bahan kering dan bahan organik dan degradabilitas serat pada pakan yang disuplementasi tanin dan saponin. Jurnal Agripet, 14(2): 115-124.

Widiawati, Y., M. Winugraha dan P. Mahyuddin. 2010. Estimasi produksi gas metana dari rumput dan tanaman leguminosa yang diukur secara in vitro. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner. Bogor, 3 – 4 Agustus 2010. Puslitbang Peternakan, Bogor. 131 – 136.

Wiseman, J. 1990. Variability in the nutritive value of fast for ruminant. In: Feedstuff Evaluatrion. Wiseman, J. And D.J.A.Cole (Eds). Butterworths.