



Effect of Tea Leaves Powder Supplementation on Fermented Oil Palm Fronds on Fermentation Characteristics, Rumen Microbial Profile, and Methane Production *In vitro*

NURHAITA¹, NUR HIDAYAH^{2*}

¹Animal Science Program, Agricultural and Animal Science Faculty, Bengkulu Muhammadiyah University, Kampung Bali, Bengkulu-38119, Indonesia; ²Animal Science Program, Agricultural Faculty, Tidar University, Potrobangsari, North Magelang, Central Java-56116, Indonesia.

Abstract | Tea leaves are types of plant leaves with the potential to be used as an antiprotozoal to increase livestock production. Therefore, this research aimed to evaluate the effect of tea leaves powder supplementation on fermented oil palm fronds (FOPF) on fermentation characteristics, rumen microbial profile, and methane production *in vitro*. Randomized block design with four treatments were used in this research. Treatments were tea leaves powder supplementation at 0, 2, 4, and 6% and four groups as replications. The observed variables were fermentation characteristics, rumen microbial profile, and methane production *in vitro*. The results showed that at 2% supplementation, the substrate had the lowest pH value. Meanwhile, at 4-6%, the supplement decreased NH₃, total VFA, the proportion of propionate, DMD, OMD, protozoa population, and increased A:P ratio. However, there was no effect on bacteria population and methane production. In conclusion, supplementation of tea leaves powder at 2% on fermented oil palm fronds did not influence fermentation characteristics, rumen microbial profile, and methane production *in vitro*. The tea can be used in ration to optimize the utilization of oil palm fronds.

Keywords | Fermentation characteristics, Fermented palm oil fronds, Methane production, Rumen microbial profile, Tea leaves powder

Received | March 12, 2021; **Accepted** | March 17, 2021; **Published** | June 01, 2021

***Correspondence** | Nur Hidayah, Animal Science Program, Agricultural Faculty, Tidar University, Potrobangsari, North Magelang, Central Java-56116, Indonesia; **Email:** nurhidayah@untidar.ac.id

Citation | Nurhaita, Hidayah N (2021). Effect of tea leaves powder supplementation on fermented oil palm fronds on fermentation characteristics, rumen microbial profile, and methane production *in vitro*. *Adv. Anim. Vet. Sci.* 9(7): 971-977.

DOI | <http://dx.doi.org/10.17582/journal.aavs/2021/9.7.971.977>

ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

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INTRODUCTION

Indonesia is the biggest producer of palm oil worldwide, and in 2019/2020 the production volume was recorded at 42.50 million metric tons (Shahbandeh, 2020). Directorate General of Estate Crops (2019) reported that in 2018 there were 14.33 million hectares of oil palm planted area, with private companies controlling 55.09%, smallholders 40.62%, and state-owned companies, 4.29%. Moreover, Poh et al. (2020) stated that 10 tonnes of oil palm fronds (OPF) are produced in one hectare of oil palm plant area, therefore, in Indonesia, there should be 140.33 mil-

lion tons of OPF. In tropical countries, these fronds are used as ruminant feed in the livestock industry (Ng et al., 2011; Ebrahimi et al., 2015). This statement is supported by Azevêdo et al. (2012) which reported that ruminants could convert renewable agro-industrial by-products to high-quality feed.

Nurhaita et al. (2014) reported that oil palm fronds (OPF) are feed from a by-product that has low quality and nutritional value, high fiber content, low palatability and digestibility. Furthermore, the major constraints to the use of OPF as livestock feed are the high crude fiber (CF,

50.94% DM; Nurhayu et al., 2014), neutral detergent fiber (NDF, 74.06% DM; Ebrahimi et al., 2015), lignin (20.5 % DM; Abdul Khalil et al., 2006), acid detergent fiber (ADF, 51.72% DM; Ebrahimi et al., 2015), and low protein content (CP, 3,07 % DM; Nurhayu et al., 2014). Chaves et al. (2006) stated that in an *in vitro* study, feed quality affected methane gas production (CH_4), as low concentrations of non-fibrous carbohydrates in legumes and forages contributed to the increased methane gas production.

The low nutritional and digestibility value of OPF as a by-product could be improved using fermentation technology. This claim is supported by Wizna et al. (2009) which reported that the fermentation process was able to reduce crude fiber content by 32% and increase crude protein content by 360%. Fermented products have better nutritional quality and digestibility than unfermented products (Astuti et al., 2017; Wajiyah et al., 2015). Meanwhile, to improve the quality and modify rumen microbes, fermented OPF (FOPF) products need to be supplemented with bioactive compounds. A study on meta-analysis *in vitro* by Klevenhusen et al. (2012) and *in vivo* by Khiaosa and Zebeli (2013) reported that bioactive compound supplementation is used as a rumen microbial modifying agent, and reducing methane gas production has a positive effect on livestock productivity.

Tea leaves are one of the plant leaves that have the potential to be used as *antimethanogenic* and antiprotozoal agents (Hu et al., 2005; Ramírez-Restrepo et al., 2016) to increase the growth performance of small ruminants (Mao et al., 2010; Zhou et al., 2011). However, there is not much information on the effect of the supplementation of these leaves on FOPF on fermentation characteristics, rumen microbes profile, and methane production. Therefore, this research aimed to evaluate the effect of tea leaves powder supplementation in FOPF on fermentation characteristics, rumen microbes profile, and methane gas production *in vitro*.

MATERIALS AND METHODS

PREPARATION OF TEA LEAVES POWDER

Tea leaves were obtained from the plantation area of Kepahiang, Bengkulu Province, and sun-dried for 4-5 days. The dry leaves were then powdered using a hammer mill with 80-100 mesh size.

PREPARATION OF LOCAL MICROORGANISMS (NURHAITA ET AL., 2016).

The formula for preparing the local microorganism (MOL) was 2 kg of goat's rumen fluid, 15 L of coconut water, and 4 kg of molasses. Furthermore, these ingredients were put into a jerry can, closed tightly, and mixed evenly. Then a

smaller jerry can was prepared with water and closed tightly. The caps of both jerry cans were perforated and connected using a small tube. Finally, the small jerry can that contained water functioned to accommodate the gas formed during the MOL production process (10 days incubation).

FERMENTED OIL PALM FRONDS (NURHAITA ET AL., 2019).

Oil palm fronds were chopped and sun-dried until the water content was at $\pm 60\%$. Then 10 ml MOL/kg FOPF, 1% urea, and 5% molasses were added to the chopped OPF. Subsequently, the materials were mixed properly, put in a plastic bag, compacted, tied, and fermented for 7 days. The fermented oil palm fronds were then evaluated for their physical quality and powdered using a hammer mill with 80-100 mesh size.

IN VITRO FERMENTATION (TILLEY & TERRY, 1963)

The 100 ml fermentation tube was filled with 10 ml of rumen fluid, 40 ml buffer solution (McDougall), and 500 mg substrate. The temperature was maintained at 39 °C. Subsequently, the mixture was incubated for 4 hours to collect samples of pH (using a HANA pH meter) NH_3 (with micro diffusion Conway), partial VFA (with gas chromatography), protozoa (with Fuch Rosenthal Counting Chamber (4×4×0.2 mm) under a microscope (40×)), total and cellulolytic bacteria, according to the method of Ogimoto & Imai (1981) were obtained. The incubation for 48 hours to ascertain the dry matter digestibility (DMD) and organic matter digestibility (OMD). Methane production was estimated from partial VFA according to Moss et al. (2000) ($\text{CH}_4=0.45 \text{ C}_2-0.275 \text{ C}_3+0.40 \text{ C}_4$).

STATISTICAL ANALYSES

Randomized block design with four treatments was used in this experiment. Treatments were supplementation of tea leaves powder at 0, 2, 4, and 6% and four groups as replications. Data were analyzed using ANOVA and the differences among the means of the treatments were examined using DMRT (Steel & Torrie, 1980).

RESULTS

NUTRIENT PROFILES OF THE FERMENTED OIL PALM FRONDS, TEA LEAVES, AND SUBSTRATE

The nutrient profiles of the OPF, FOPF, tea leaves powder, and substrate are shown in Table 1. Fermentation using MOL increased the nutrient content of OPF, as the ash content, EE, CP, and TDN increased by 50.71, 56.17, 54.71, and 19.07%, respectively. Meanwhile, DM, CF, tannin, and saponin decreased by 8.66, 37.36, 38.25, and 28.90%, respectively. The Tea leaves powder had its CP at 16.68%, CF at 12.96%, TDN at 57.70%, tannin at 7.20%, and saponin at 23.62%. Furthermore, the supplementation

Table 1: Nutrient profile of fermented oil palm fronds, tea leaves powder and substrate *in vitro* (%DM)

Materials	DM	Ash	EE	CP	CF	NFE	TDN*	Tannin	Saponin
Oil palm fronds (OPF)	92.57	7.25	1.67	5.86	32.15	53.07	51.86	1.49	13.84
Fermented oil palm fronds (FOPF)	84.55	14.71	3.81	12.94	20.14	48.40	64.07	0.92	9.84
Tea leaves powder (TLP)	91.97	8.77	3.89	16.68	12.96	57.70	78.44	7.20	23.62
FOPF+ 2% TLP	86.38	14.88	3.89	13.27	20.40	49.56	65.64	1.14	10.96
FOPF + 4% TLP	88.22	15.06	3.96	13.61	20.66	50.71	67.21	1.28	11.63
FOPF + 6% TLP	90.06	15.23	4.04	13.94	20.92	51.86	68.78	1.43	12.10

* TDN calculated according to Hartadi *et al.* (1980).

DM: dry matter; CP: crude protein; EE: ether extracts; CF: crude fibre; NFE: nitrogen free extract and TDN: total digestible nutrients.

Table 2: Fermentation characteristic with supplementation tea leaves powder on fermented oil palm fronds

Parameters	Treatments				SEM	p
	P1	P2	P3	P4		
pH	6.73 ^b	6.65 ^a	6.67 ^{ab}	6.70 ^{ab}	0.01	0.20
NH ₃ (mM)	8.99 ^b	7.99 ^{ab}	7.41 ^{ab}	7.01 ^a	0.08	0.05
VFA Total (mM)	97.26 ^c	93.63 ^c	84.23 ^b	67.18 ^a	1.12	0.00
Proportion of VFA (%)						
Acetate	61.66	61.86	67.01	66.73	0.75	0.11
Propionate	19.94 ^c	19.08 ^{bc}	15.91 ^a	16.56 ^{ab}	0.42	0.02
Butyrate	18.39	19.05	17.08	16.71	0.40	0.42
A:P	3.13 ^a	3.28 ^{ab}	4.24 ^c	4.09 ^{bc}	0.11	0.04
DMD (%)	47.38 ^b	46.13 ^{ab}	43.92 ^a	44.91 ^{ab}	0.36	0.05
OMD (%)	46.47 ^b	45.66 ^b	42.71 ^a	43.67 ^{ab}	0.36	0.04

Notes:

P1: FOPF, P2: FOPF+ 2% TLP, P3: FOPF+ 4% TLP, P4: FOPF+ 6% TLP

Standard error of mean (SEM).

Table 3: Rumen microbes profile and methane production with supplementation tea leaves powder on fermented oil palm fronds

Parameters	Treatments				SEM	p
	P1	P2	P3	P4		
Total bacteria (10 ⁹)	3.46	3.60	3.89	4.11	0.04	0.96
Cellulolytic bacteria (10 ⁷)	8.73	6.87	8.33	8.80	0.20	0.29
Protozoa (10 ⁵)	3.37 ^c	3.10 ^{cb}	2.87 ^{ab}	2.57 ^a	0.03	0.01
Methane (%)	7.82	8.14	7.89	7.57	0.18	0.81

Notes:

P1: FOPF, P2: FOPF+ 2% TLP, P3: FOPF+ 4% TLP, P4: FOPF+ 6% TLP

Standard error of mean (SEM).

of tea leaves powder at 2-6% on the FOPF caused the CP to be at 13.27-13.94% and TDN at 65.64-68.78%.

FERMENTATION CHARACTERISTICS *IN VITRO*

Data of fermentation characteristics *in vitro* are shown in Table 2. The Rumen pH value ranged from 6.73-6.65, with the lowest value being due to the supplementation of 2% tea leaves powder. Furthermore, the supplementation of this powder on FOPF decreased NH₃ concentration (7.01-8.99 mM) and total VFA (67.18-97.26 mM), and

their lowest value was in the treatment with 6% supplementation (NH₃ at 7.01 mM; VFA total at 67.18 mM). The proportion of acetate (61.66-66.73%) and butyrate (16.71-19.05%) in the rumen was not affected by the dietary treatments. However, supplementation of the powder at 4% caused the lowest proportion of propionate (15.91%) and the highest A/P ratio (4.24). A similar result was obtained for the DMD (43.92%) and OMD (42.71%), where the lowest values were in the treatment with supplementation at 4%. Finally, the ranges of DMD and OMD were

43.92-47.38% and 42.71-46.47%, respectively.

RUMEN MICROBIAL PROFILE AND METHANE PRODUCTION *IN VITRO*

Data for rumen microbial profile and methane production *in vitro* were presented in Table 3. It was observed that tea leaves powder supplementation up to 6% on FOPF did not affect the total bacteria ($3.46\text{-}4.11 \times 10^9$), cellulolytic bacteria ($6.87\text{-}8.80 \times 10^7$), and methane production (7.57-7.82%). However, the supplementation decreased the protozoa population, with the lowest value being caused by the supplementation at 6% (2.57×10^5).

DISCUSSIONS

NUTRIENT PROFILE OF THE FERMENTED OIL PALM FRONDS, TEA LEAVES POWDER, AND SUBSTRATE

The MOL which consisted of microbes contributed to the increase in the CP of FOPF because these microscopic organisms mostly consist of protein. This was supported by Crueger and Crueger (1984) which stated that different kinds of microbes affect protein content, including bacteria, as they contain 70 to 78% protein (Crueger and Crueger, 1984). Based on the results of microbiology identification in the Indonesian Institute of Sciences (2019), the MOL in this study consisted of 5 species of *Bacillus* bacteria, namely *Bacillus* sp, *B. aureus*, *B. altitudinis*, *B. cereus*, and *B. megaterium*. Oboh (2006) stated that microbial proliferation or multiplication could increase protein content. Also, microorganisms may increase CP due to their ability to synthesize amino acids (Jokotagha & Amoo, 2012). A similar result was reported by Yao et al. (2018), which showed that the CP in the fermented by-product from the beverage industry with *Candida utilis* and *Bacillus subtilis* supplementation was higher than in the unfermented product.

The cell membrane of MOL bacteria contains inorganic compounds which contributed to an increase in ash content (Ahaotu et al., 2013). Moreover, Jokotagha & Amoo (2012) reported that some factors contributed to an increase in fat during the fermentation process such as an increase in microbial population that consists of lipid component, extracellular enzyme (lipase) from the activities of lipolytic microorganisms, and microbial oil substance in the fermentation medium. Fermentation technology could reduce CF content because the bacteria from MOL could degrade fibers. Obueh et al. (2014) reported that enzymes of hydrolysis and oxidation could convert the recalcitrant compounds from waste into utilizable compounds. Furthermore, it was discovered by Ojewumi et al. (2018) that CF content could be reduced through both aerobic and anaerobic fermentation. This was supported by Adeleke et al. (2017) which showed that the fiber content from

fermented cassava peels decreased by 33.77 and 23.46%. Bacteria from MOL also degraded tannin and saponin in OPF. The same result was reported by Ojewumi et al. (2018), that after fermentation with *Saccharomyces cerevisiae*, tannin and saponin from cassava waste reduced by 44 and 27.78% respectively.

Nutrient in tea leaves powder is almost similar to shrub legume of *Desmodium velutinum* that has CP at 16.00%, ash at 6.59%, and tannin at 7.70% (Heinritz et al., 2012). Moreover, supplementation of this powder at 2-6% on FOPF caused the CP to be at 13.27-13.94% and TDN at 65.64-68.78%, which conforms with the beef requirement for fattening in Indonesia (BSN, 2016).

FERMENTATION CHARACTERISTICS *IN VITRO*

Rumen pH value in this research was normal as Dehority (2005) reported that the normal rumen pH value is between 5.4-7.8. The lowest pH which occurred with supplementation at 2% might be due to the higher total VFA production than in the 4-6% supplementation groups. Roca-Fernandez et al. (2020) reported that 50% orchardgrass + 50% birdsfoot trefoil had the lowest pH and highest total VFA production. The increasing supplementation of tea leaves powder on FOPF, the NH_3 concentration decreased progressively. This is probably due to the increasing tannin content on the substrate as more tea leaves powder was added. Similar research was reported by Mohammadabadi & Chaji (2012), that the addition of 30 g/kg DM oak leaves and fruit, and pistachio hull and leaves that contained tannin in sunflower meal decreased ruminal NH_3 concentration compared to the control.

Tannin from tea leaves helps to protect dietary protein from rumen microbes degradation. This condition has a beneficial effect with respect to ruminant production because the absorption of amino acids in the small intestine could be increased. Waghorn (2008) reported that animal which grazed forage with high condensed tannin (CT) had a higher flow of metabolizable protein and essential amino acids in their small intestine compared to those that grazed forage with low CT.

There was a decrease in total VFA, DMD, and OMD with increasing levels of supplementation of tea leaves powder, probably due to the increasing formation of tannin with macromolecule complexes, which hinders microbial enzymes (McSweeney et al., 2001), therefore, reducing fermentative activities and depressing intestinal digestion (Makkar, 2003). Alipour & Rouzbehan (2010) stated that the addition of tannins might be due to a reduction of microbes in feed particles. Also, Gameda & Hassen (2014) reported that tannin from a different species of tropical browse plants decreased total VFA and IVOMD. Jaya-

negara et al. (2019) stated that with higher levels of the compound on *Indigofera* and *Moringa* silages, there was a greater reduction in total VFA concentration, DMD, and OMD rumen *in vitro*.

The increasing levels of supplementation of tea leaves powder, there was a continuous decrease in the proportion of propionate and an increase in A:P ratio, meanwhile, there was no effect on the proportion of acetate and butyrate. A similar result was obtained by Patra et al. (2010), that the addition of 0.50 mL extracts of clove (plant extract rich in tannins) increased A:P ratio and decreased the production of propionate by direct inhibition of *Selenomonas ruminantium* population. Also, Cieslak et al. (2016) found that the addition of tannin extract from *Sanguisorba officinalis* caused a significant decrease in propionate (linear $P < 0.01$). Castro-Montoya et al. (2011) in the research with dietary Quebracho, mimosa, and chestnut tannins demonstrated the opposite effect, that there was a significant linear decrease in the proportion of acetate ($P = 0.01$) and A:P ratio ($P < 0.001$) but an increase of propionate proportion descriptively.

RUMEN MICROBIAL PROFILE AND METHANE PRODUCTION *IN VITRO*

Supplementation of tea leaves powder did not affect total and cellulolytic bacteria population and methane production. However, the protozoa population decreased with increasing levels of supplementation. This condition occurred because the leaves contained saponin which has a defaunation effect that enables the lysis of protozoa cells. The study by Ramírez-Restrepo et al. (2016) is in support of this finding as it showed that tea leaves are one of the plant leaves with the potential to be used as an antiprotozoal agent. Saponin reacts with the cholesterol from the protozoa membrane, leading to an increase in the permeability of the cell walls, thus killing the protozoa (Wallace et al., 2002). Hidayah et al. (2020) also confirmed these findings, that the increasing substitution of native grass with jengkol (*A. jiringa*) peel powder that contained saponin, decreased the protozoa population. Finally, according to Cieslak et al. (2014), the use of *S. officinalis* root, which is a source of saponin, could reduce the population of protozoa (Cieslak et al., 2014).

Methane production was estimated using VFA profiles, as this method leads to accurate results with a very low RMSPE and a high coefficient of measurement (Jayane-gara et al., 2015). The results showed that the supplementation of tea leaves powder on FOPF until 6% did not decrease the methane gas production. This is supported by Ramírez-Restrepo et al. (2016) which reported that the addition of tea seed (*Camellia sinensis*) saponin (TSS) did not affect methane gas emissions. Besides, this condition

might be due to the level of tannin and saponin in the leaves, which was not sufficient to decrease the production of the gas.

Based on the research of Krueger et al. (2010), low tannins (hydrolysable tannin) have little or no effect on methane production, and on the proportion of acetate, propionate, or butyrate. However, Hassanat and Benchaar (2013), found a contradicting result that the methane gas production decreased compared to the control with the addition of up to 40% of acacia, chestnut or valonea (consist of tannins ≥ 50 g/kg), or quebracho (consist of tannins ≥ 100 g/kg). The varying impacts of tannin on methane production might be due to its chemical structure and concentration in the plant from which it was obtained (Ramírez-Restrepo et al., 2016).

CONCLUSION

Supplementation of tea leaves powder at 2% on fermented oil palm fronds was did not influence fermentation characteristics, rumen microbial profile, and methane production *in vitro*. The tea can be used in ration to optimize the utilization of oil palm fronds.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support from the Ministry of Research, Technology, and Higher Education of Indonesia through “Penelitian Terapan” grant No. 491/LPPM-UMB/2019. Furthermore, the authors are grateful to the students in the research team and Dian Anggraeni for their assistance in the sampling process and during the laboratory analyses.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Nurhaita formulated and supervised the experimental design, and obtained and analyzed the data. Meanwhile, Nur Hidayah checked the data analysis and drafted and revised the manuscript. Finally, all authors read and approved the final version of the manuscript.

REFERENCES

- Abdul Khalil HPS, Siti Alwani M, Mohd Omar AK (2006). Chemical composition, anatomy, lignin distribution, and cell wall structure of Malaysian plant waste fibres. *Bioresources*. 1: 220–232.
- Adeleke BS, Akinyele BJ, Olaniyi OO, Jeff-Agboola YA (2017).

- Effect of fermentation on chemical composition of cassava peels. *Asian J. Plant Sci.* 7: 31-38.
- Ahaotu I, Ogueke CC, Owuamanam CI, Ahaotu NN, Nwosu JN. Fermentation of under watered cassava pulp by linamarase producing microorganisms: Effect of nutritional composition and residual cyanide. *AJFN.* 3: 1-8. <https://doi.org/10.5251/ajfn.2013.3.1.1.8>
 - Alipour D, Rouzbehan Y (2010). Effects of several levels of extracted tannin from grape pomace on intestinal digestibility of soybean meal. *Livest. Sci.* 128: 87-91. <https://doi.org/10.1016/j.livsci.2009.11.003>
 - Astuti T, Rofiq MN, Nurhaita (2017). Evaluasi kandungan bahan kering, bahan organik dan protein kasar pelepah sawit fermentasi dengan penambahan sumber karbohidrat. *J. Peternak.* 14: 42-47.
 - Azevêdo JAG, Filho V, Pina DS, Detmann E, Pereira LGR, Valadares RFD, Fernandes HJ, Silva LFC, Benedeti PB (2012). Nutritional diversity of agricultural and agroindustrial by-products for ruminant feeding. *Arq. Bras. Med. Vet. Zootec.* 64: 1246-1255. <https://doi.org/10.1590/S0102-09352012000500024>
 - Badan Standarisasi Nasional (2016). Standar Nasional Indonesia (SNI) pakan ternak. Direktorat Jenderal Peternakan dan Kesehatan Hewan, Kementerian Pertanian. Jakarta, Indonesia.
 - Castro-Montoya JM, Makkar HPS, Becker K (2011). Chemical composition of rumen microbial fraction and fermentation parameters as affected by tannins and saponins using an *in vitro* rumen fermentation system. *Can. J. Anim. Sci.* 91: 433-448. <https://doi.org/10.4141/CJAS2010-028>
 - Chaves AV, Thompson LC, Iwaasa AD, Scott SL, Olson ME, Benchaar C, Veira DM, McAllister TA (2006). Effect of pasture type (alfalfa vs. grass) on methane and carbon dioxide production by yearling beef heifers. *Can. J. Anim. Sci.* 86: 409-418. <https://doi.org/10.4141/A05-081>
 - Cieslak A, Zmora P, Matkowski A, Nawrot-Hadzik I, Pers-Kamczyc E, El-Sherbiny M, Bryszak M, Szumacher-Strabel M (2016). Tannins from *Sanguisorba officinalis* affect *in vitro* rumen methane production and fermentation. *J. Anim. Plant. Sci.* 26: 54-62.
 - Cieslak A, Zmora P, Stochmal A, Pecio L, Oleszek W, Pers-Kamczyc E, Szczechowiak J, Nowak A, Szumacher-Strabel M (2014). Rumen antimethanogenic effect of *Saponaria officinalis* L. phytochemicals *in vitro*. *J. Agric. Sci.* 152: 981-993. <https://doi.org/10.1017/S0021859614000239>
 - Crueger W, Crueger A (1984). *Biotechnology, a textbook of industrial microbiology. science technology.* Sinaver Associates Inc. Madison, USA
 - Dehority BA (2005). Effect of pH on viability of *Entodinium caudatum*, *Entodinium exiguum*, *Epidinium caudatum*, and *Ophryoscolex purkynjei* *in vitro*. *J. Eukaryot. Microbiol.* 52: 39-342. <http://dx.doi.org/10.1111/j.1550-7408.2005.00041.x>
 - Directorate General of Estate Crops (2019). Tree crop estate statistic of Indonesia 2018-2020. Directorate General of Estate Crops, Jakarta, Indonesia.
 - Ebrahimi M, Mohamed AR, Goh YM, Shokryzadan P, Sazili AQ, Jahromi MF (2015). Feeding oil palm (*Elaeis guineensis*, Jacq.) fronds alters rumen protozoal population and ruminal fermentation pattern in goats. *Ital. J. Anim. Sci.* 14: 403-409 <https://doi.org/10.4081/ijas.2015.3877>
 - Gameda BS, Hassen A (2014). Effect of tannin and species variation on *in vitro* digestibility, gas, and methane production of tropical browse plants. *Asian-Australas. J. Anim. Sci.* 28: 188-199. <http://dx.doi.org/10.5713/ajas.14.0325>
 - Hartadi H, Reksodiprodjo S, Lebdosukojo S, Tillman AD (1980). *Tabel komposisi pakan untuk Indonesia.* Gajah Mada University Press, Yogyakarta, Indonesia.
 - Hassanat F, Benchaar C (2013). Assessment of the effect of condensed (acacia and quebracho) and hydrolysable (chestnut and valonea) tannins on rumen fermentation and methane production *in vitro*. *J. Sci. Food Agric.* 93: 332-339. <https://doi.org/10.1002/jsfa.5763>
 - Heinritz SN, Hoedtke S, Martens SD, Peters M, Zeyner A (2012). Evaluation of ten tropic(al legume forages for their potential as pig feed supplement. *Livest. Res. Rural Dev.* 24(1). <http://www.lrrd.org/lrrd24/1/hein24007.htm>
 - Hidayah N, Lubis R, Wiryawan KG, Suharti S, Rita W, Zurina R (2020). The effect of native grass substitution using jengkol (*Archidendron jiringa*) peel and leaves powder on *in vitro* rumen fermentation. *Iran. J. Appl. Anim. Sci.* 10: 421-427.
 - Hu WL, Wu YM, Liu JX, Guo YQ, Ye JA (2005). Tea saponins affect *in vitro* fermentation and methanogenesis in faunated and defaunated rumen fluid. *J. Zhejiang Univ. Sci. B.* 6: 787-792. <https://doi.org/10.1631/jzus.2005.B0787>
 - Jayanegara A, Yaman A, Khotijah L (2019). Reduction of proteolysis of high protein silage from *Moringa* and *Indigofera* leaves by addition of tannin extract. *Vet. World.* 12: 211-217. <https://doi.org/10.14202/vetworld.2019.211-217>
 - Jayanegara A, Makkar HPS, Becker K (2015). Addition of purified tannin sources and polyethylene glycol treatment on methane emission and rumen fermentation *in vitro*. *Med. Pet.* 38:57-63. <https://doi.org/10.5398/medpet.2015.38.1.57>
 - Jokotagha OA, Amoo IA (2012). Effect of fermentation on the nutritive value of *Aspergillus niger* and *Aspergillus fumigatus* fermented hura crepitans seed flour. *Greener. J. Phys. Nat. Sci.* 2: 85-88. <https://doi.org/10.5281/zenodo.3440912>
 - Khiaosa-ard R, Zebeli Q (2013). Meta-analysis of the effects of essential oils and their bioactive compounds on rumen fermentation characteristics and feed efficiency in ruminants. *J. Anim. Sci.* 91: 1819-1830. <https://doi.org/10.2527/jas.2012-5691>
 - Klevenhusen F, Muro-Reyes A, Khiaosa-ard R, Metzler-Zebeli BU, Zebeli Q (2012). A meta-analysis of effects of chemical composition of incubated diet and bioactive compounds on *in vitro* ruminal fermentation. *Anim. Feed Sci. Technol.* 176: 61-69. <https://doi.org/10.1016/j.anifeedsci.2012.07.008>
 - Krueger WK, Gutierrez-Bañuelos H, Carstens GE, Min BR, Pinchak WE, Gomez RR, Anderson RC, Krueger NA, Forbes TDA (2010). Effects of dietary tannin source on performance, feed efficiency, ruminal fermentation, and carcass and non-carcass traits in steers fed a high-grain diet. *Anim. Feed Sci. Technol.* 159:1-9. <https://doi.org/10.1016/j.anifeedsci.2010.05.003>
 - Makkar HPS (2003). Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Rumin. Res.* 49: 241-256. [https://doi.org/10.1016/S0921-4488\(03\)00142-1](https://doi.org/10.1016/S0921-4488(03)00142-1)
 - Mao HL, Wang JK, Zhou YY, Liu JK (2010). Effects of addition of tea saponins and soybean oil on methane production: fermentation and microbial population in the rumen of growing lambs. *Livest. Sci.* 129, 56-62. <https://doi.org/10.1016/j.livsci.2009.12.011>
 - McSweeney CS, Palmer B, McNeill DM, Krause DO

- (2001). Microbial interactions with tannins: nutritional consequences for ruminants. *Anim. Feed Sci. Technol.* 91: 83-93. [https://doi.org/10.1016/S0377-8401\(01\)00232-2](https://doi.org/10.1016/S0377-8401(01)00232-2)
- Mohammadabadi T, Chaji M (2012). The Influence of the plant tannins on *in vitro* ruminal degradation and improving nutritive value of sunflower meal in ruminants. *Pak. Vet. J.* 32: 225-228.
 - Moss AR, Jouany JP, Newbold J (2000). Methane production by ruminants: its contribution to global warming. *Ann. Zootech.* 49: 231-253. <https://hal.archives-ouvertes.fr/hal-00889894/>
 - Ng FY, Yew FK, Basiron Y, Sundram K (2011). A renewable future driven with Malaysian palm oil-based green technology. *JOPE.* 2: 1-7. <https://doi:10.5366/jope.2011.01>
 - Nurhaita, Ruswendi, Rita W, Robiyanto (2014). Pemanfaatan pelepah sawit sebagai sumber hijauan dalam ransum sapi potong. *J. Pastura.* 4(1): 38-41.
 - Nurhaita, Neli D, Suliasih (2016). *Fermentation with local microorganism to improve pod cacao quality as ruminants feed.* In: Proceeding ISEPROLOCAL, Fakultas Pertanian Universitas Bengkulu, Bengkulu, Indonesia, 23-24 October 2015, 218-221.
 - Nurhaita, Neli D, Hidayah N (2019). Fermentasi pelepah sawit dengan mol dan pengaruhnya terhadap pencernaan dan karakteristik cairan rumen secara *in-vitro*. In: Prosiding Seminar Nasional FAPERTA UMMY, Solok, Padang, West Sumatra, Indonesia, 4-5 September 2019, 57-65.
 - Nurhayu A, Ishak ABL, Ella A (2014). Pelepah dan daun sawit sebagai pakan substitusi hijauan pada pakan ternak sapi potong di kabupaten luwu timur sulawesi selatan. Balai Pengkajian Teknologi Pertanian Sulawesi Selatan, Makasar, Indonesia.
 - Oboh G (2006). Nutrient and antinutrient composition of condiments produced from some fermented underutilized legumes. *J. Food Biochem.* 30: 579-588. <https://doi.org/10.1111/j.1745-4514.2006.00083.x>
 - Obueh HO, Ikenebomeh MJ (2014). Bioethanol production and compositional changes during fermentation of cassava processing wastes from a local cassava mill. *Int. J. Curr. Res. Biosci. Plant. Biol.* 1: 43-51.
 - Ogimoto K, Imai S (1981). *Atlas of rumen microbiology.* Japan Scientific Societies, Tokyo, Japan.
 - Ojewumi ME, Omoleye AJ, Nyngifa AS (2018). Biological and chemical changes during the aerobic and anaerobic fermentation of African locust bean. *Int. J. Chem. Stud.* 2: 25-30.
 - Patra AK, Kamra DN, Agarwal N (2010). Effects of extracts of spices on rumen methanogenesis, enzyme activities and fermentation of feeds *in vitro*. *J. Sci. Food Agric.* 90: 511-520. <https://doi:10.1002/jsfa.3849>
 - Poh PE, Wu TY, Lam WH, Poon WC, Lim CS (2020). Oil palm plantation wastes. In: waste management in the palm oil industry, green energy and technology. Springer, Cham. Berlin, Germany.
 - Ramírez-Restrepo CA, Tan C, O'Neill CJ, López-Villalobos C, Padmanabha J, Wange J, McSweeney CS (2016). Methane production, fermentation characteristics, and microbial profiles in the rumen of tropical cattle fed tea seed saponin supplementation. *Anim. Feed Sci. Technol.* 216: 58-67. <http://dx.doi.org/10.1016/j.anifeedsci.2016.03.005>
 - Roca-Fernández AI, Leanne Dillard S, Soder KJ (2020). Ruminal fermentation and enteric methane production of legumes containing condensed tannins fed in continuous culture. *J. Dairy Sci.* 103:1-11. <https://doi.org/10.3168/jds.2019-17627>
 - Shahbandeh M (2020). Leading producer of palm oil worldwide from 2019/2020. [https://www.statista.com/statistics/856231/palm-oil-top-global-producers/\(November 28, 2020\)](https://www.statista.com/statistics/856231/palm-oil-top-global-producers/(November%2028,%202020))
 - Steel RG, Torrie JH (1991). Prinsip dan prosedur statistik, suatu pendekatan biometrik edisi 2. PT. Gramedia Pustaka Utama, Jakarta, Indonesia.
 - Tilley JMA, Terry RA (1963). A two-stage technique for the *in vitro* digestion of forage crops. *Grass. Forage Sci.* 18:104-111. <https://doi.org/10.1111/j.1365-2494.1963.tb00335.x>
 - Waghorn G (2008). Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production progress and challenges. *Anim. Feed Sci. and Technol.* 147: 116-139. <https://doi:10.1016/j.anifeedsci.2007.09.013>
 - Wajizah S, Samadi, Usman Y, Marian E (2015). Evaluasi nilai nutrisi dan pencernaan *in vitro* pelepah kelapa sawit (oil palm fronds) yang difermentasi menggunakan *Aspergillus niger* dengan penambahan sumber karbohidrat yang berbeda. *J. Agripet.* 15: 13-19. <https://doi.org/10.17969/agripet.v15i1.2286>
 - Wallace R.J., McEwan N.R., McIntosh F.M., Teferedegne B. and New Bold C. (2002). Natural product as manipulators of rumen fermentation. *Asian-australas. J. Anim. Sci.* 15: 1458-1468.
 - Wizna, Abbas H, Rizal Y, Dharma A, Kompiang IP (2009). Improving the quality of tapioca by-products (onggok) as poultry feed through fermentation by *Bacillus amyloliquefaciens*. *Pak. J. Nutr.* 8: 1636-1640.
 - Yao KY, Zhang TZ, Wang HF, Liu JX (2018). Upgrading of by-product from beverage industry through solid-state fermentation with *Candida utilis* and *Bacillus subtilis*. *Lett. Appl. Microbiol.* 67: 557-563. <https://doi.org/10.1111/lam.13078>
 - Zhou YY, Mao HL, Jiang F, Wang JK, Liu JX, McSweeney CS (2011). Inhibition of rumen methanogenesis by tea saponins with reference to fermentation pattern and microbial communities in Hu sheep. *Anim. Feed Sci. Technol.* 166: 93-100. <https://doi.org/10.1016/j.anifeedsci.2011.04.007>