IJAS Iranian Journal of Applied Animal Science

The Effect of Native Grass Substitution Using Jengkol (*Archidendron jiringa*) Peel and Leaves Powder on *in vitro* Rumen Fermentation

Research Article

N. Hidayah[1,2], R. Lubis[3], K.G. Wiryawan[4], S. Suharti[4*], W. Rita[1] and R. Zurina[1]

[1] Department of Animal Science, Faculty of Agriculture, Bengkulu Muhammadiyah University, Bengkulu, Indonesia
[2] Department of Animal Science, Faculty of Agriculture, Tidar University, Magelang, Indonesia
[3] Department of Biology, Faculty of Teacher Training and Education, Bengkulu Muhammadiyah University, Bengkulu, Indonesia
[4] Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Bogor, Indonesia

Received on: 2 Nov 2019
Revised on: 14 Jan 2020
Accepted on: 19 Jan 2020
Online Published on: Sep 2020

*Correspondence E-mail: sri_suharti@apps.ipb.ac.id

Online version is available on: www.ijas.ir

ABSTRACT

The effect of substituting native grass with jengkol (*Archidendron jiringa*) by-product on fermentation characteristics, rumen microbial profile, methane production, and hydrogen balance using *in vitro* method was investigated. Seven treatments (different composition of native grass, jengkol peel, jengkol leaves, and concentrate) with five replications in a block randomized design were used. Substitution of native grass with jengkol peel powder up to 22.5% decreased rumen pH and protozoa, increased N-NH_3, did not change feed digestibility (dry matter digestibility (DMD) and organic matter digestibility (OMD)), total and proportional volatile fatty acid (VFA) production, microbial protein synthesis, methane production, and hydrogen balance. The use of jengkol leaves powder up to 45% decreased rumen pH, increased N-NH_3, feed digestibility (DMD and DMO) and microbial protein synthesis, but did not affect total and proportional VFA production, protozoa population, hydrogen balance, and methane production. It is concluded that native grass can be substituted with jengkol peel powder up to 22.5% and leaves powder up to 45%.

KEY WORDS fermentation characteristic, jengkol leave, jengkol peel, methane, rumen microbes.

INTRODUCTION

Agricultural by-products are abundant in Indonesia. Many farmers have been using these by-products as the main source of livestock feed, especially for ruminants. Azevêdo *et al.* (2012) reported that ruminants are able to convert renewable natural resources, such as agricultural and agro-industrial by-products into high-quality feed for ruminants. Several reasons for using agricultural by-products as feed, among others include helping farmers to reduce feed costs, utilizing and optimizing by-products, and minimize the environmental impacts of this by-products. Kasapidou *et al.* (2015) reported that the utilization of agricultural wastes in farm animal nutrition has a significant effect on environmental, economic, and social factors. Van Dyk *et al.* (2013) stated that various by-products from agricultural and food processing, based on the nutrient content, it had potential as animal feeds. Many researches have been reported that the use of various by-products as an animal feed can improve rumen fermentation. The *in vitro* study by Jeon *et al.* (2016) showed that substitution of annual ryegrass straw with by-products of pickled radish improved ruminal fermentation that increased dry matter (DM) degradability, volatile fatty acids (VFAs) concentration, and total gas production. Research by Tona (2014) reported that 10% cassava peels combination with 60 % *Panicum maximum* + 30 % *Gliricidia sepium* made efficient rumen fermentation and optimize feed utilization.

Based on Hidayah *et al.* (2019), jengkol by-products, like peel and leaves have high potential to be used as ruminant feed. They are available in high quantity. The weight proportion of jengkol peel (59.99%) is higher than seed (40.01%), so if Indonesia produced 66,065 tons of jengkol (Badan Pusat Statistik, 2018), there would be 36,065 tons of peels available. In terms of nutritional value, jengkol peel and leaves contain 25.14-35.28% of crude fiber. This value is within the range of the recommended crude fiber value for ruminant, making jengkol peel and leaves potentials to be used as crude fiber sources.

Jengkol leaves also contain a good source of protein, with 15.17-19.26% of crude protein. Jengkol peel and leaves have a high content of total digestible nutrients (51.56-65.82%). This makes them as good partial source of energy for ruminants. Lastly, jengkol peel and leaves are also good potential source of saponin (8.26-35.13%) which can be used as an alternative natural feed additive to increase animal productivity.

However, there is not much information reported yet regarding the effect of jengkol peel and leaves to substitute native grass as an alternative of energy source for ruminants. So, this research was designed to find out how much powder of jengkol peel and leaves can be used to substitute native grass and its effect on fermentation characteristics, rumen microbial profile, methane production, and hydrogen balance of ruminants by using the method of Tilley and Terry (1963).

MATERIALS AND METHODS

The material in this research used jengkol peel and leaves powder. *In vitro* fermentation was conducted according to the method of Tilley and Terry (1963). Into each 100 mL fermentation tube, 500 mg substrate, 40 mL McDougall buffer, and 10 mL rumen fluid was added and the temperature was maintained at 39 ˚C. The substrate contained 60% forage (native grass, jengkol peel and leaves) and 40% concentrate mixture (rice bran, corn, tofu by-product, rucah fish meal and NaCl) with 10-11% CP and 65-68% total digestible nutrients (TDN) (Tables 1 and 2). The rumen fluid for this experiment was collected after 3 h morning feeding from the 3 rumens fistulated Ongole grade beef cattle with Ethical Approval from Animal Care and Use Committee (AUAC). Samples from aliquot were taken after 4 h incubation for pH, VFA, NH_3, protozoa, total bacterial analysis and after 48 h incubation for dry matter digestibility (DMD) and organic matter digestibility (OMD) analysis.

The rumen pH was measured with a HANA pH meter. Ammonia (N-NH_3) concentration was measured by micro-diffusion Conway method (General Laboratory Procedures, 1966).

Total VFA concentration and molar proportion of VFA were analyzed using gas chromatography (GC 8A, Shimadzu Crop., Kyoto, Japan, Capillary column type containing 10% SP-1200, 1% H_3PO_4 on 80/100 Cromosorb WAW and nitrogen as the gas carrier). Prior to analysis, the pH of rumen liquid from *in vitro* incubation was adjusted to 3-4 with H_2SO_4. The DMD and OMD were measured using Tilley and Terry (1963) method. Protozoa population was determined using Fuch Rosenthal Counting Chamber (4×4×0.2 mm) under a microscope (40×). The 0.5 mL liquid sample from 4 h incubation tubes were mixed with 2 mL methyl green formaldehyde saline solution. Microbial protein synthesis was measured using Makkar *et al.* (1982) method and then proceeds with Lowry's *et al.* (1951) method. The stages of protein synthesis measured: 1) production of complex reagents (solution A: 2% b/v Na_2CO_3 in 0.1 N NaOH) and solution B: 0.5% b/v $CuSO_4.5H_2O$ in K-Na-Tartrate 1%), 2) solution of NaOH 2N and folinciocalteu reagents. Methane production was estimated from molar proportions of VFA according to Moss *et al.* (2000) (CH4=0.45 C_2–0.275 C_3+0.40 C_4), meanwhile hydrogen balance was estimated from molar proportion of VFA according to Mitsumori *et al.* (2012) [2HP (Hydrogen production)= 2 × C_2 + C_3 + 4 × C_4 + 2 × Ci_5 + 2 x C_5], [2HUS (hydrogen utilization)= 2 × C_3 + 2 × C_4 + C_5] and 2H recovery in sohrt chain fatty acids [SCFA (%)= (2HUS/2HP) × 100].

The experiment was conducted in a randomized block design with seven treatments and five replications. The treatments tested were the ration: P1: concentrate (40%) + native grass (60%); P2: concentrate (40%) + native grass (52.5%) + jengkol peel powder (7.5%); P3: concentrate (40%) + native grass (45%) + jengkol peel powder (15%); P4: concentrate (40%) + native grass (37.5%) + jengkol peel powder (22.5%); P5: concentrate (40%) + native grass (45%) + jengkol leaves powder (15%); P6: concentrate (40%) + native grass (30%) + jengkol leaves powder (30%); P7: concentrate (40%) + native grass (15%) + jengkol leaves powder (45%). Data were tested using analysis of variance (ANOVA) and the differences among treatments means were examined by Duncan multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Fermentation characteristic

The substitution of native grass with jengkol peel powder up to 22.5% reduced ($P<0.05$) rumen pH, increased ($P<0.05$) N-NH_3, but did not affect DMD and OMD, total and proportional VFA production. Utilization of jengkol peel powder up to 45% decreased ($P<0.05$) rumen pH, increased ($P<0.05$) N-NH_3, DMD, and OMD but did not affect total and proportional VFA production (Table 3).

Table 1 Proximate analysis of native grass (NG), concentrate (C), jengkol peel (JP), and jengkol leaves (JL) (DM basis)

Materials	DM	Ash	CP	EE	CF	NFE	TDN*	Tannin	Saponin
					(%)				
NG	94.1	10.4	5.96	1.27	26.9	55.5	49.3	-	-
C	88.9	5.35	15.8	11.8	12.6	54.5	59.0	-	-
JP	89.6	3.48	7.90	0.65	33.1	54.9	51.6	1.43	35.1
JL	90.6	3.00	19.3	2.50	26.7	48.6	65.7	1.26	19.3

* 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 Nutritional composition of feed with subtitution of jengkol peel and leaves powder

Treatments	DM	Ash	EE	CP	CF	NFE	TDN	Tannin	Saponin
					(%)				
P1	92.0	8.38	5.48	9.89	21.2	55.1	66.1	-	-
P2	91.7	7.86	5.43	10.0	21.6	55.0	66.1	0.11	2.63
P3	91.3	7.34	5.38	10.2	22.1	55.0	66.1	0.21	5.27
P4	91.0	6.82	5.34	10.3	22.6	54.9	66.1	0.32	7.90
P5	91.5	7.27	5.66	11.9	21.1	54.1	67.1	0.19	2.90
P6	91.0	6.16	5.84	13.9	21.1	53.0	67.7	0.38	5.79
P7	90.4	5.05	6.03	15.9	21.1	52.0	68.5	0.57	8.69

DM: dry matter; CP: crude protein; EE: ether extracts; CF: crude fibre; NFE: nitrogen free extract and TDN: total digestible nutrients.
P1: concentrate (40%) + native grass (60%); P2: concentrate (40%) + native grass (52.5%) + jengkol peel powder (7.5%); P3: concentrate (40%) + native grass (45%) + jengkol peel powder (15%); P4: concentrate (40%) + native grass (37.5%) + jengkol peel powder (22.5%); P5: concentrate (40%) + native grass (45%) + jengkol leaves powder (15%); P6: concentrate (40%) + native grass (30%) + jengkol leaves powder (30%) and P7: concentrate (40%) + native grass (15%) + jengkol leaves powder (45%).

Table 3 Fermentation characteristic with subtitution jengkol peel and leaves powder

Parameters	Treatments							SEM	P-value
	P1	P2	P3	P4	P5	P6	P7		
pH	7.12^{b}	7.04^{ab}	7.00^{a}	7.00^{a}	6.96^{a}	6.96^{a}	6.94^{a}	0.04	0.01
N-NH_3 (mM)	5.59^{a}	7.00^{b}	6.80^{b}	7.34^{b}	6.99^{b}	6.93^{b}	7.37^{b}	0.15	0.00
VFA total (mM)	125.97	159.95	145.78	153.31	146.82	144.03	146.02	3.52	0.15
Proportion of VFA (%)									
Acetate	69.9	73.2	73.6	72.0	72.2	72.5	74.1	0.89	0.85
Propionate	16.1	13.9	14.6	15.1	13.6	13.5	13.3	0.41	0.32
Butyrate	10.9	9.3	8.9	9.45	11.3	10.9	9.37	0.25	0.63
Valerate	3.19	3.65	2.95	3.47	2.95	3.12	3.20	0.10	0.77
A:P	4.39	5.40	5.23	5.10	4.91	5.47	5.71	0.20	0.93
DMD (%)	55.9^{a}	59.0^{abc}	57.9^{abc}	56.9^{ab}	60.2^{bc}	59.3^{abc}	61.4^{c}	0.54	0.03
OMD (%)	61.0^{a}	64.1^{ab}	62.9^{ab}	61.2^{a}	65.2^{b}	63.9^{ab}	65.5^{b}	0.49	0.03

VFA: volatile fatty acid; A:P: acetate:propionate; DMD: dry matter digestibility and OMD: organic matter digestibility.
P1: concentrate (40%) + native grass (60%); P2: concentrate (40%) + native grass (52.5%) + jengkol peel powder (7.5%); P3: concentrate (40%) + native grass (45%) + jengkol peel powder (15%); P4: concentrate (40%) + native grass (37.5%) + jengkol peel powder (22.5%); P5: concentrate (40%) + native grass (45%) + jengkol leaves powder (15%); P6: concentrate (40%) + native grass (30%) + jengkol leaves powder (30%) and P7: concentrate (40%) + native grass (15%) + jengkol leaves powder (45%).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The highest pH value (7.12) on the control treatment is an indication of that the additions of jengkol peel and leaves powder increased fermentation process. This is probably related to saponin on jengkol peel leaves powder which acts as a defaunation agent for protozoa. Saponin can reduce protozoa population which caused rumen bacteria population increased. The response found in this study was decreasing and linear in protozoa population count (P<0.05) (Table 4).

Busquet *et al*. (2006) reported that addition of Yucca as saponin source at high level (0, 3, 30, 300, 3.000 mg/L) decreased (P<0.01) rumen pH value.

Patra and Yu (2014) stated that utilization of *Quillaja saponaria* as a saponin source at 0.6 g/L significantly decreased protozoa and increased *S. ruminantium, R. amylophilus, P. ruminicola, P. bryantii, C. aminophilum, C. sticklandii* (P<0.05) bacteria. Istiqomah *et al.* (2011) reported that the higher dose addition of saponin from *H. tiliaceus* on the basal diet (0, 5, 10, 15, 20%), the lower population protozoa present (P<0.05) whereas ruminal pH decreased just numerically. Substitution of jengkol peel and leave powder increased protein ration which increased N-NH_3 concentration. Nuswantara *et al.* (2001) reported that high protein feed content increased N-NH_3 concentrations

as a result of protein degradation. Jengkol peel and leaves presumably have high solubility protein, which is easily degraded by rumen microbes. Andrade-Montemayor *et al.* (2009) stated that soluble protein easily degraded by rumen microorganisms resulting in an increase in the NH_3 rumen concentration. The substitution of native grass with jengkol peel and leaves powder did not increase total and proportional VFA production. Similarly, Gunun *et al.* (2017) found that the total VFA concentration, acetate (C2), butyrate (C4) and C2:C3 were similar among treatments (P>0.05) with addition rambutan peel powder. Total VFA production in this research ranged from 125.97-159.95 mM which is standard for optimum levels of total VFA production in the rumen and able to provide energy for ruminant and protein microbial synthesis process. McDonald *et al.* (2002) stated that the optimum level of total VFA in the rumen range from 70-150 mM. The proportional VFA in this study was 69.86-74.09% acetate, 13.34-16.078% propionate, 9.33%-11.30% butyrate, 2.95%-3.65% valerate and A/P ratio at 4.39-5.71. The proportional VFA rumen depends on the type of feed and species of livestock. The utilization of 60% concentrate and 40% forage on cattle produced 61% acetate, 18% propionate, 13% butyrate and 8% others. Whereas on goats produced 52% acetate, 34% propionate, 12% butyrate and 3% others (McDonald *et al.* 2002). The substitution of native grass with jengkol leaves powder at 45% increased feed digestibility (DMD and OMD), but did not on jengkol peel powder substitution. These results could be due to this that nutrients of jengkol leaves were digested easier than peel in the rumen. Hidayah *et al.* (2019) reported that jengkol leaves contained acid detergent fiber (ADF) at 36.03-39.72% and TDN at 63.87-65.82% that is classified as high-quality forage feeds. The jengkol peel with ADF of 40.84-43.78% is classified as a low quality forage feed. The lignin content of jengkol peel (15.48-16.42%) is almost the same as lignin content of rice straw with 16.62% (Dewi, 2002), coffee hull with 17.5% and cocoa seed hull (Azevêdo *et al.* 2012). Lignin has complex components which are difficult to be degraded because lignin influences formation of cross-linkages between cellulose and hemicellulose.

Rumen microbial profile

The substitution of native grass with jengkol peel powder up to 22.5% decreased protozoa but did not increase microbial protein synthesis. This was the opposite with the utilization of jengkol leaves powder upto 45% which increased microbial protein synthesis but did not affect protozoa population (Table 4).

Saponin content of jengkol peel with defaunation action can lyse the protozoal cell. Wallace *et al.* (2002) stated that saponin might kill or damage protozoa by reacting with the cholesterol contained in the membrane of protozoa, which can lead to increase permeability of cell walls. This result showed that jengkol peel was more effective than jengkol leaves to reduce protozoa population. This may be due to the differences in the type of saponin between two matterials.

Patra *et al.* (2012) found that sapogenin of Yucca s. is less effective in inhibiting the growth of protozoa than the triterpenoid sapogenin of Quillaja s. The different type of saponin showing different magnitudes of effect on rumen fermentation and microbes (Patra and Saxena, 2009).

The substitution of native grass with jengkol peel powder at 22.5% was not able to increase microbial protein synthesis and its efficiency. While the substitution with jengkol leaves powder increased microbial protein synthesis and its efficiency. This result could be due to the increasing level of jengkol leaves powder substitution increased the protein content on feed ration (11.85-15.88%).

Research by Hidayah *et al.* (2019) reported that jengkol leaves potentially to be used as a source of protein for ruminants. Crude protein content of TMR was an important factor determining the amount of synthesis protein microbes per unit of fermented organic matter (Boguhn *et al.* 2006).

Protein of jengkol leaves presumably had a high solubility, which is easily degraded by rumen microorganisms that resulted more availability of nitrogen in the rumen. Clark *et al.* (1992) reported that energy and nitrogen as the most limiting factors affecting microbial protein synthesis. Saponin and tannin content on jengkol leaves powder might be able to increase microbial protein synthesis.

Hu *et al.* (2005) observed that by increasing the content of saponins, the *in vitro* rumen fermentation pattern of a mixture of meal of forage and corn was affected by increasing the microbial protein synthesis. Similar to the report by Puchala *et al.* (2005) that addition of condensed tannin-containing forage increased microbial protein synthesis.

Saponin and tannin have potential to modify favorably rumen fermentation which increased the efficiency in microbial synthesis and microbial yield outflow of microbial proteins (Rodríguez *et al.* 2007).

The value of microbial protein synthesis in this research ranged from 128.74-193.28 g/h with the efficiency of 51.05-80.32 g/kg BOMR, where the highest value was found on the substitution at 30% jengkol leaves powder.

Table 4 Profile of rumen microbial population with subtitution of jengkol peel and leaves powder

Parameters	Treatments							SEM	P-value
	P1	P2	P3	P4	P5	P6	P7		
Protozoa (Log CFU/mL)	5.62^{b}	5.34^{b}	5.30^{b}	4.74^{a}	5.45^{b}	5.18^{b}	5.43^{b}	0.07	0.08
Microbial protein syntesis (g/h)	147.92^{abc}	142.99^{ab}	153.14^{bc}	128.74^{a}	158.97^{bc}	193.28^{d}	168.63^{c}	4.50	< 0.00
Microbial protein syntesis efficiency (g/kg BOFR)	58.5^{ab}	59.6^{ab}	62.6^{bc}	51.1^{a}	67.5^{bc}	80.3^{d}	71.7^{cd}	2.02	< 0.00

P1: concentrate (40%) + native grass (60%); P2: concentrate (40%) + native grass (52.5%) + jengkol peel powder (7.5%); P3: concentrate (40%) + native grass (45%) + jengkol peel powder (15%); P4: concentrate (40%) + native grass (37.5%) + jengkol peel powder (22.5%); P5: concentrate (40%) + native grass (45%) + jengkol leaves powder (15%); P6: concentrate (40%) + native grass (30%) + jengkol leaves powder (30%) and P7: concentrate (40%) + native grass (15%) + jengkol leaves powder (45%).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SPM= 6.25 × N microbe and BOFR= 0.65 × organic matter digestibility (International Atomic Energy Agency, 1997).
SEM: standard error of the means.

Table 5 Methane production and hydrogen balance with subtitution of jengkol peel and leaves powder

Parameters	Treatments							SEM	P-value
	P1	P2	P3	P4	P5	P6	P7		
CH_4 (%)	18.5	15.5	17.3	18.9	16.9	18.8	18.6	0.59	0.56
CH_4/total VFA	0.15	0.10	0.11	0.13	0.11	0.13	0.14	0.01	0.13
CH_4/OMD (mL/100 mg)	0.31	0.24	0.28	0.31	0.26	0.29	0.28	0.01	0.32
Hydrogen production (mmol/L)	122.18	96.41	107.74	119.46	106.05	118.74	114.47	3.72	0.52
Hydrogen utilization (mmol/L)	33.3	22.3	25.6	29.5	25.4	28.9	25.9	1.39	0.53
H2 recovery (%)	26.8	23.2	23.7	24.9	24.1	23.8	22.6	0.75	0.50

VFA: volatile fatty acid and OMD: organic matter digestibility.
P1: concentrate (40%) + native grass (60%); P2: concentrate (40%) + native grass (52.5%) + jengkol peel powder (7.5%); P3: concentrate (40%) + native grass (45%) + jengkol peel powder (15%); P4: concentrate (40%) + native grass (37.5%) + jengkol peel powder (22.5%); P5: concentrate (40%) + native grass (45%) + jengkol leaves powder (15%); P6: concentrate (40%) + native grass (30%) + jengkol leaves powder (30%) and P7: concentrate (40%) + native grass (15%) + jengkol leaves powder (45%).
SEM: standard error of the means.

The efficiency value of the results in this study is higher than the efficiency value reported by Santoso *et al.* (2007) which added the saponin source from *Biophytum petersianum Klotzsch* until 26 mg of saponin/kg body weight just at 24.3-36.8 g/kg DOMR.

Methane production and hydrogen balance

The substitution of native grass with jengkol peel and leaves powder did not decrease methane (CH4) gas production and did not affect hydrogen balance (Table 5). This condition is presumably because saponin and tannin from jengkol peel and leaves did not give a significant effect on the decreased methanogenic population. Sirohi *et al.* (2001) stated that the formation of methane by ruminal methanogens is autotrophic and seems to occur mainly from CO_2 and H_2. The same result was reported by Patra *et al.* (2012) that the addition of quillaja or yucca at 0.2, 0.4, 0.6 g/L did not influence total gas or methane production.

Methane gas production in this research was 15.48-18.85% and lower than the result reported by Patra *et al.* (2012) at 24.00-27.40%. The different result reported by Poungchompu *et al.* (2009), that supplementation of soap berry fruit-mangosteen peel pellets until 4% DM of total diets with 12% DM of crude tannin and 15% DM of crude saponin was significantly decreased (P<0.01) CH_4 gas production by measurement and calculation from VFA production.

Jayanegara *et al.* (2009) reported that tannin effect on methane gas production was not consistent yet. This is depending on the plant of tannin source, the tannin structure, condensed or hydrolysis tannins that had many variations each other.

The type of compound or its concentration in the plant or extract used in the study may be several factors determining the efficacy of plant secondary compound to reduce methane production (Bodas *et al.* 2012).

Hydrogen production and utilization were similar with control treatment. Range of hydrogen production of the treatments was 96.41-122.18 mmol/L, hydrogen utilization from 22.34-33.31 mmol/L, and hydrogen recovery from 22.60-24.90%. Moss *et al.* (2000) stated that metabolic hydrogen is produced during degradation and fermentation of feed polymers (mainly carbohydrates, both structural and non-structural carbohydrates) under anaerobic condition in the rumen. H_2 production/utilization is the basis to classify them into three groups, bacteria which produce propionate, butyrate, ethanol and/or lactate; bacteria which produce acetate and H_2; and methanogenic microorganisms (Bodas *et al.* 2012). Moss *et al.* (2000) reported that the H_2 used during VFA synthesis or incorporated into microbial organic matter. Propionate formation pathway is a ruminal metabolic pathway that used H_2, and acetate formation produced H_2. Further, utilization of H_2 is possible since a number of rumen microbes such as H_2-utilizing bacteria and methanogens are able to consume H_2. Sliwi´nski *et al.* (2002) reported that the addition of saponin from *Yucca schidigera* extract (1, 20 and 100 mg sarsaponin/kg DM) and *Castanea sativa* wood extract containing hydrolyzable tannins (0.5 and 2.5 g tannins/kg) and pure sulphonate-free lignin (2.5g/kg) were the same as control treatment on hydrogen balance.

Hydrogen produced ranged from 0.107-0.134 mol/day, hydrogen utilized ranged from 0.077-0.091 mol/day, and hydrogen recovered ranged from 0.68-0.75 mol/day. The same result was reported by Jayanegara *et al.* (2015) who utilized hydrolyzable and condensed tannins extracted and purified from chestnut, sumach, mimosa, and quebracho on hay, the hydrogen balance was similar to control. Hydrogen production and utilization ranged from 3.51-4.47 mmol. The hydrogen recovery of the treatments ranged from 86.7-95.3%.

CONCLUSION

Substitution of native grass with jengkol peel powder up to 22.5% decreased rumen pH and protozoa, increased N-NH_3, did not change feed digestibility (DMD and OMD), total and proportional VFA production, microbial protein synthesis, methane production, and hydrogen balance. The use of jengkol leaves powder up to 45% decreased rumen pH, increased N-NH_3, feed digestibility (DMD and DMO) and microbial protein synthesis, but did not affect total and proportional VFA production, protozoa population, hydrogen balance, and methane production. Native grass can be substituted with jengkol peel powder up to 22.5% and leaves powder up to 45%.

ACKNOWLEDGEMENT

The authors acknowledge the financial support from the Ministry of Research, Technology and Higher Education of Indonesia through "PKPT" grant No. 2128/SP2H/LT/K2/KM/2018. Thank to D. Anggraeni, A.A.A.P.P. Gustari and M.R. Hibatullah for assistance in sampling and laboratory analyses. The authors also thank Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia to support for research facilities.

REFERENCES

Andrade-Montemayor H., Garca T.G. and Kawas J. (2009). Ruminal fermentation modification of protein and carbohydrate by means of roasted and estimation of microbial protein synthesis. *R. Bras. Zootec*. **38,** 277-291.

Azevêdo J.A.G., Filho V., Pina D.S., Detmann E., Pireira L.G.R., Valadares R.F.D., Fernandes H.J., Silva L.F.C. and Benedeti P.B. (2012). Nutritional diversity of agricultural and agro-industrial by-products for ruminant feeding. *Arq. Bras. Med. Vet. Zootec*. **64,** 1246-1255.

Badan Pusat Statistik. (2018). Produksi Tanaman Sayuran Jengkol (ton). Available at: https://www.bps.go.id/site/resultTab.

Bodas R., Prieto N., Garcia-Gonzalez R., Andres S., Giraldez F.J. and Lopez S. (2012). Manupulation of rumen fermentation and methane production with plant secondary metabolites. *Anim. Feed Sci. Technol*. **176,** 78-93.

Boguhn J., Kluth H. and Rodehutscord M. (2006). Effect of total mixed ration composition on fermentation and efficiency of ruminal microbial crude protein synthesis *in vitro*. *J. Dairy Sci*. **89,** 1580-1591.

Busquet M., Calsamiglia S., Ferret A. and Kamel C. (2006). Plant extracts affect *in vitro* rumen microbial fermentation. *J. Dairy Sci*. **89,** 761-771.

Clark M.J., Klusmeyer T.H. and Cameron M.R. (1992). Microbial-protein synthesis and flow of nitrogen fraction to the duodenum of dairy-cows. *J. Dairy Sci*. **75,** 2304-2323.

Dewi D. (2002). Hidrolisis limbah hasil pertanian secara enzimatik. *J. Akta Agrosia*. **5,** 67-71.

General Laboratory Procedures. (1966). Report of Dairy Science. University of Wisconsin, Madison, USA.

Gunun P., Gunun N., Cherdthong A., Wanapat M., Polyorach S., Sirilaophaisan S., Wachirapakorn C. and Kang S. (2017). *In vitro* rumen fermentation and methane production as affected by rambutan peel powder. *J. Appl. Anim. Res*. **11,** 1-6.

Hartadi H., Reksohadiprodjo S., Lebdosukojo S. and Tillman A.D. (1980). Tabel Komposisi Pakan untuk Indonesia. Gajah Mada University Press, Yogyakarta, Indonesia.

Hidayah N., Lubis R., Wiryawan K.G. and Suharti S. (2019). Phenotypic identification, nutrients content, bioactive compounds of two jengkol (*Archidendron jiringa*) varieties from Beng-

kulu, Indonesia and their potentials as ruminant feed. *Biodiversitas.* **20,** 1671-1680.

Hu W.L., Liu J.X., Ye J.A., Wu Y.M. and Guo Y.Q. (2005). Effect of tea saponin on rumen fermentation *in vitro*. *Anim. Feed Sci. Technol*. **120,** 333-341.

International Atomic Energy Agency. (1997). Estimation of Rumen Microbial Protein Production from Purine Derivatives in Urine. Vienna, Austria.

Istiqomah L., Herdian H., Febrisantosa A. and Putra D. (2011). Waru leaf (*Hibiscus tiliaceus*) as saponin source on *in vitro* ruminal fermentation characteristic. *J. Indonesian Trop. Anim. Agric*. **36,** 43-49.

Jayanegara A., Makkar H.P.S. and Becker K. (2015). Addition of purified tannin sources and polyethylene glycol treatment on methane emission and rumen fermentation *in vitro*. *Med Peternakan*. **38,** 57-63.

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

Jeon S., Sohn K.N. and Seo S. (2016). Evaluation of feed value of a by-product of pickled radish for ruminants: analyses of nutrient composition, storage stability, and *in vitro* ruminal fermentation. *J. Anim. Sci. Technol*. **58,** 34-43.

Kasapidou E., Sossidou E. and Mitlianga P. (2015). Fruit and vegetable coproducts as functional feed ingredients in farm animal nutrition for improved product quality. *Agriculture*. **5,** 1020-1034.

Lowry O.H., Rosenbrough N.J., Farr A.L. and Randall R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 265-270.

Makkar H.P.S., Sharma O.P., Dawra R.K. and Negi S.S. (1982). Simple determination of microbial protein in rumen liquor. *J. Dairy Sci*. **65,** 2170-2173.

McDonald P., Edward R.A., Greenhalgh J.F.D. and Morgan C.A. (2002). Animal Nutrition. Scientific and Tech John Willey and Sons. Inc., New York, USA.

Mitsumori M., Shinkai T., Takenaka A., Enishi O., Higuchi K., Kobayashi Y., Nonaka I., Asanuma N., Denman S.E. and McSweeney C.S. (2012). Responses in digestion rumen fermentation and microbial populations to inhibition of methane formation by a halogenated methane analogue. *British J. Nutr*. **108,** 482-491.

Moss A.R., Jouany J.P. and Newbold J. (2000). Methane production by ruminants: Its contribution to global warming. *Annal Zootechnol*. **49,** 231-253.

Nuswantara L.K., Soejono M. and Widyobroto B.P. (2001). Sintesis protein mikroba pada sapi peranakan ongole dan kerbau yang diberi pakan tunggal glirisidia, jerami jagung dan kaliandra. *Agrosains*. **14,** 165-174.

Patra A.K. and Saxena J. (2009). A review of the effect and mode of action of saponins on microbial population and fermentation in the rumen and ruminant production. *Nut. Research Rev*. 22, 204

Patra A.K. and Yu Z. (2014). Effects of vanillin, *quillaja* saponin, and essential oils on *in vitro* fermentation and protein-degrading microorganisms of the rumen. *Appl. Microbiol. Biotechnol*. **98,** 897-905.

Patra A.K., Stiverson J. and Yu Z. (2012). Effects of quillaja and yucca saponins on communities and select populations of rumen bacteria and archaea, and fermentation *in vitro*. *J. Appl. Microbiol*. **113,** 1329-1340.

Poungchompu O., Wanapat M., Wachirapakorn C., Wanapat S. and Cherthong A. (2009). Manipulation of ruminal fermentation and methane production by dietary saponins and tannins from mangosteen peel and soapberry fruit. *Arch. Anim. Nutr*. **63,** 389-400.

Puchala R., Min B.R., Goetsch A.L. and Sahlo T. (2005). The effect of a condensed tannin-containing forage on methane emission of goats. *J. Anim. Sci*. **83,** 182-188.

Rodríguez R., Sosa A. and Rodríguez Y. (2007). Microbial protein synthesis in rumen and its importance to ruminants. *Cuban J. Agric. Sci.* **41,** 287-294.

Santoso B., Kilmaskossub A. and Sambodo P. (2007). Effects of saponin from *Biophytum petersianum Klotzsch* on ruminal fermentation, microbial protein synthesis and nitrogen utilization in goats. *Anim. Feed Sci. Technol*. **137,** 58-68.

Sirohi S.K., Pandey N., Singh B. and Puniya A.K. (2001). Rumen methanogen: A review. *Indian J. Microbiol*. **50,** 253-262.

Sliwi´nski B.J., Soliva C.R., Machmüller A. and Kreuzer M. (2002). Efficacy of plant extracts rich in secondary constituents to modify rumen fermentation. *Anim. Feed Sci. Technol.* **101,** 101-114.

Steel R.G.D. and Torrie J.H. (1980). Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc., New York, USA.

Tilley J.M.A. and Terry R.A. (1963). A two-stage technique for the *in vitro* digestion of forage crops. *J. British Grassland Soc.* **18,** 104-111.

Tona G.O. (2014). Investigation of proximate composition and *in vitro* fermentation characteristics of *Panicum maximum, Gliricidia sepium* with cassava peels as feed for ruminants in Nigeria. *Int. J. Curr. Microbiol. Appl. Sci.* **3,** 188-197.

Van Dyk J.S., Gama R., Morrison D., Swart S. and Pletschke B.I. (2013). Food processing waste: Problems, current management and prospects for utilisation of the lignocellulose component through enzyme synergistic degradation. *Renew Sust. Energ. Rev*. **26,** 521-531.

Wallace R.J., McEwan N.R., McIntosh F.M., Teferedegne B. and New Bold C. (2002). Natural product as manipulators of rumen fermentation. *Asian-Australasian J. Anim. Feed Sci. Technol.* **15,** 1458-1468.