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Rumen Fermentation Characteristic and *In Vitro* Digestibility of King Grass Silage Supplemented with Shredded Coconuts Pulp

(KARAKTERISTIK FERMENTASI RUMEN DAN KECERNAAN IN VITRO SILASE RUMPUT RAJA YANG DISUPLEMENTASI AMPAS KELAPA PARUT)

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ABSTRACT

Shredded coconuts pulps are the potential wastes of coconuts product that can be used as a feed alternative because it contains enough nutrients. In the other side fat content of shredded coconut pulp are high enough. High-fat will bother the fermentation process in the rumen. The fermentation process can improve the quality of the digestibility and nutrient content of shredded coconut pulp. The purpose of this study was to evaluate the effect of silage formulation of shredded coconut pulp on *in vitro* rumen fermentation characteristics. There are five treatments with three replication: P1: 16% rice bran of total silage composition, P2: 16% shredded coconut pulp of total silage composition, P3: 8% using rice bran and shredded coconut pulp of total silage composition, P4: 8% rice bran, 5.6% shredded coconut pulp and 2.4% "*gaplek*" flour of total silage composition and P5: 8% shredded coconut pulp, 5.6% rice bran and 2.4% "*gaplek*" flour of total silage composition. P2 treatment gave the highest gas production than other treatment. Percentage of acetate, propionate, and butyrate, acetate to propionate ratio (A:P), non glucogenic ratio (NGR), ammonia-N (NH_3) production of shredded coconut pulp was significantly affected by treatments ($P<0.05$). Total gas production 48 hours, gas production from soluble fraction (a), insoluble fraction (b), total fraction ($a+b$), total VFA and microbial rumen protein of silage gave no significant difference among treatments ($P>0.05$). While in total gas production 48 hours not significant, when it breakdown each hour show significant results, which means there is an increase in feed degradability. In this study silage formulation using, 8% rice bran, 5.4% of shredded coconut pulp and 2.6% "*gaplek*" flour, 83% king grass, 0.5% mineral mix and 0.5% molasses treatment has the best degradability on *in vitro* rumen fermentation characteristics.

Keyword: silage, shredded coconut pulp, rumen fermentation, in vitro.

ABSTRAK

Ampas kelapa parut merupakan limbah dari kelapa (proses pembuatan santan) yang dapat digunakan sebagai pakan alternatif karena masih mengandung nutrisi yang cukup. Namun dilain pihak ampas kelapa parut juga mengandung kandungan lemak yang cukup tinggi. Kandungan lemak yang tinggi akan mengganggu proses fermentasi di rumen. Proses fermentasi dapat meningkatkan kualitas kecernaan dan kandungan nutrisi dari ampas kelapa parut. Tujuan dari penelitian ini adalah untuk mengevaluasi

pengaruh formulasi silase ampas kelapa parut terhadap karakteristik fermentasi rumen secara *in vitro*. Ada lima perlakuan dan tiga ulangan yaitu P1: 16% dedak padi dalam total komposisi silase, P2: 16% ampas kelapa parut dalam total komposisi silase, P3: 8% dedak padi dan ampas kelapa parut dalam total komposisi silase, P4: 8% dedak padi, 5,6% ampas kelapa parut dan 2,4% tepung "gaplek" dalam total komposisi silase and P5: 8% ampas kelapa parut, 5,6% dedak padi dan 2,4% tepung "gaplek" dalam total komposisi silase. Perlakuan P2 memberikan produksi gas tertinggi dibandingkan perlakuan lainnya. Persentase asetat, propionat, dan butirat, rasio asetat terhadap propionat (A:P), rasio non glukogenik (NGR), produksi amonia-N (NH3) silase ampas kelapa parut dipengaruhi secara signifikan oleh perlakuan (P <0,05). Total produksi gas 48 jam, produksi gas dari fraksi terlarut (a), fraksi tidak larut (b), fraksi total (a + b), total VFA dan protein mikroba rumen silase tidak memberikan perbedaan yang signifikan antara perlakuan (P> 0,05). Sementara total produksi gas 48 jam tidak signifikan, namun ketika dipisahkan setiap jam menunjukkan hasil yang signifikan, yang berarti ada peningkatan degradabilitas pakan. Dalam penelitian ini formulasi silase menggunakan, 8% dedak padi, 5,4% ampas kelapa parut dan 2,6% tepung "gaplek", 83% rumput raja, 0.5% mineral mix and 0.5% molasses memiliki degradabilitas terbaik pada karakteristik fermentasi rumen in vitro.

Kata kunci: silase, ampas kelapa parut, fermentasi rumen, in vitro

INTRODUCTION

Shredded coconuts pulps are one of the potential wastes of coconuts product especially in food industries that can be used as feed alternative. In the Gunungkidul region, Yogyakarta province there are some food industries especially "*Jenang Ketan*" used coconut as a basic ingredient. In one batch production process (30 kg) of "*Jenang Ketan*," it required 80-100 pieces of coconuts or as much as 50-62 kg of coconuts and from this process will produce shredded coconuts pulp as much as 30% from weight of coconut or 17-19 kg/process. The pulp obtained from the remaining unutilized manufacture of coconut milk. Usually, it is only waste, whereas shredded coconut pulp can be used as an alternative feed because it contains sufficient nutrients. Shredded coconut pulp can be used as alternative feed because it's provided enough nutrition such as crude protein 5.78%, ether extract 38.24% and crude fiber 15.07% (Putri, 2010). Miskiyah *et al.* (2006) reported that nutrient content of shredded coconut pulp is crude protein 11.35%, ether extract 23.36% and crude fiber 14.97%. The crude fiber of shredded coconut pulp easy to digest, and it is a benefit to uses an alternative feed for ruminant (Derrick, 2005).

In the other side fat content of shredded coconut pulp are high enough. High-fat content in the feed will bother the fermentation process in the rumen (Kurniawan *et al*., 2016). Johnson and McClure, (1973) reported that usually ruminants not tolerance with a high level of fat (more than 6-8%) in dietary it will decrease feed consumption of animal. High-fat content in dietary feed will influence the metabolism of rumen microbe and post-ruminal digestive system (Onetti et al., 2001). High fat in shredded coconut pulp can increase rancidity process. Rancidity caused the smell and taste bad, thus reducing the quality and economic value of shredded coconut pulp itself. The fermentation process can improve the quality of the digestibility and nutrient content of shredded coconut pulp. Fermentation produces a product with flavor, texture, and palatability preferred by the animal. Fermentation is done through a process called ensilage, where feed or forage preserved by the action of lactic acid fermentation in anaerobic conditions (McDonald *et al.,* 2002). Gas production, volatile fatty acid (VFA), and microbial biomass are the main of feed organic matter fermentation product in ruminant (Alexander *et al.,* 2008). The aim of this study was to evaluate the effect of silage formulation of supplemented shredded coconut pulp on *in vitro* feed fermentation and gas production on ruminal fluid.

RESEARCH METHODS

Formulation and Preparation of Shredded Coconut Pulp Silage

The material of silage is king grass, shredded coconut pulp, rice bran, "*gaplek* flour," molasses and mineral mix. Five silage formulations used in this study, these were done to get the best result depending on silage quality and characteristic fermentation *in vitro*. The profile was presented in Table 1.

After 14 days ensilage process, sample of each formulation as substrate was grounded to pass a 1 mm screen and dried in an oven at 55°C

Table 1. Formulation of shredded coconut pulp silage

Material	Percentage (%)				
	Formula 1: P1 (100% RB; 0% SCP)	Formula 2: P2 (100% SCP; 0% RB)	Formula 3: P3 (50% SCP; 50% RB)	Formula 4: P4 (50% RB;35% SCP;15% GF)	Formula 5: P5 (50% SCP;35% RB;15% GF)
King grass	83	83	83	83	83
Shredded coconut pulp	0	16	8	5.6	8
Rice bran	16	0	8	8	5.6
"Gaplek" flour	0	0	0	2.4	2.4
Molasses	0.5	0.5	0.5	0.5	0.5
Mineral mix	0.5	0.5	0.5	0.5	0.5

Note: RB: rice bran, SCP: shredded coconut pulp; GF: "*gaplek*" flour

for 72 h. Substrate sample from each formulation was analyzed for nutrient content (ash, crude fiber, crude protein, ether extract, and nitrogen-free extract) using the Association of Official Analytical Chemists (AOAC) method (1995).

Treatments and Experimental Design

This study used Completely Randomized Design as an experimental design with five treatments with three replications described as follows:

P1 : Silage contains 100% rice bran (16% at total formula) and 0% shredded coconut pulp, 83% king grass, 0.5% mineral mix and 0.5% molasses in formulation.

P2 : Silage contains 100% shredded coconut pulp (16% at total formula) and 0% rice bran, 83% king grass, 0.5% mineral mix and 0.5% molasses in formulation.

P3 : Silage contains 50% rice bran (8% at total formula) and 50% shredded coconut pulp (8% at whole formula), 83% king grass, 0.5% mineral mix and 0.5% molasses in the formulation.

P4 : Silage contains 50% rice bran (8% at total formula), 35% shredded coconut pulp (5.6% at total formula) and 15% "*gaplek*" flour (2.4% at total formula), 83% king grass, 0.5% mineral mix and 0.5% molasses in the formulation.

P5 : Silage contains 50% shredded coconut pulp (8% at total formula), 35% rice bran (5.6% at whole formula) and 15% "*gaplek*" flour (2.4% at total formula), 83% king grass, 0.5% mineral mix and 0.5% molasses in the formulation.

Fermentability Assessment

The sample of silage content shredded coconut pulp each treatment and rumen liquid were prepared before *in vitro* assessment. Rumen liquor obtained from two fistulised Ongole crossbreed cattle those adapted by feeding consisted of concentrated and forage (*Pennisetum hybrid*) (20:80 in dry matter basis). Aspirator used to take rumen fluid, and quickly transported in pre-warmed vacuum flask (39ºC water temperature) then filtered.

Menke and Steingass (1988) *in vitro* fermentability method was used to evaluate *in vitro* gas production. Fermentation conducted in a glass size of 100 mL syringe. A total of 200 mg of silage substrate was put into the syringe and incubated in an incubator at 39ºC for one night. Then, along with CO_2 flow, a mixture of rumen fluid was added by 30 mL medium mixture with a ratio of 1:2 into the syringe. All the measurements repeated three times. Incubation continued for 48 h at 39°C. Measurements of gas production performed at 0, 3, 6, 9, 12, 24, 48 h. Incubation was stop and gas was released after 48 h.

The gas production observation was fitting by the exponential equation according to Ørskov and McDonald (1979) to calculate gas production kinetics**.** Ørskov and McDonald (1979) methods of exponential equation was used to calculate gas production kinetics. The exponential function is $P = a+b\ (1-e^{-ct})$ with describing P is cumulative total gas production, a is shared gas production from soluble fraction, b is the gas production from insoluble fraction, e is Euler's constant (2.7183…), c is the rate of gas production, and t is the time of incubation. The Neway Excel

Software according to Chen (1997) was used to the fitting data calculation and to revealed the estimated value of *a*, *b* and *c*. Fermentation evaluation used 100 mL syringe glass (Fortuna model. Poulten and Graf Gmbh. Germany). Three syringes as blank are containing rumen-buffer without sample used in the experiment. All the syringes consisted of samples and blank were randomly incubated at 39°C for 48 hours in an incubator (Sofyan *et al.,* 2017).

Gas production cumulative recorded at 0, 3, 6, 9, 12, 24, and 48 -hours incubation. After 48 h of incubation gas was release and the fluid contained in the syringe taken for analysis of ammonia-N (NH_3) production, microbial cell protein and VFA. Ammonia-N (NH_3) production measured using Chaney and Marbach (1964) method. Lowry *et al*. (1951) modified by Makkar *et al*. (1982) method used for measurement of microbial cell protein of rumen fluid. Sun *et al*. (2013) method used for measurement of volatile fatty acid (VFA) product from fermentation. Meta-phosphoric acid added to the sample then stored at -20°C before analysis. Gas chromatography (Shimadzu type 8A) was used to analysed VFA content using GP10% SP-1200/ 1% H3PO4 column with 80/100 Chromosorb WAW (Supelco, Bellefonte, PA).

Data Analysis

Variables measured were nutrient content, pH acidity of silage, rumen fermentability i.e. gas production kinetics, estimated gas production from soluble fraction symbols as "a", gas contribution from insoluble fraction symbols as "b", rate of the gas production symbols as "c", gas production from potentials degradable material symbols as "a+b", ammonia-N (NH_3) production, total and individual volatile fatty acids (VFA) production and biomass microbial rumen protein. Fix factors respond were evaluated by using analysis of variance (ANOVA) with completely randomized design and Post Hoc Least Significant Differences (LSD) Test was used to analysed differences among mean treatments (Gomez and Gomez, 1984). The CoSTAT statistical software used to perform all the statistical calculation (Cohort, 2008).

RESULT AND DISCUSSION

The results of the feed nutrient content before and after ensilage for all treatment are present in Table 2. The use of feed ingredients in various formulations causes varied results. Analysis of the chemical composition of feed based on a dry matter (DM).

There were significant results (P<0.05) for the content of ash, crude protein (CP), the extract ether (EE), crude fiber (CF) and nitrogen-free extract (NFE) in feed before and after ensilage. The highest ash content after ensilage treatment founded on P3 treatment after ensiling, which is 18.88%. The ash content after ensilage in each treatment increased compared with before ensilage treatment. Increase in ash content of silages possibly associated with organic matter loss (Table 3) due to the processes of fermentation and oxidation (Ribeiro *et al*., 2017). Increases in ash content of the deteriorated silage could be used as representation of percentage unit increases in DM loss (Borreani *et al*., 2018).

Significant result (P<0.05) founded in crude protein content (Table 2). The highest crude protein content was found in the treatment of P4 after silage process. The P4 treatment consist of 50% rice bran (8% at total formula), 35% shredded coconut pulp (5.6% at total formula) and 15% *"gaplek"* flour (2.4% at total formula) in the formulation. This is presumably due to both rice bran, shredded coconut pulp or "*gaplek*" flour contained high soluble fraction such as water-soluble carbohydrate and crude protein (Putri, 2010; Perera *et al*., 2018). Silage added by various soluble fraction possibly supported the fermentation process during ensilage. Fermentation process can preserve while increasing the nutrient content of the feed. This is in accordance with Anggraeni *et al*. (2015) revealed that the silage treatment increases and maintains the quality of the feed ingredients.

Ether extract (EE) content of silage was significant difference (P <0.05) between treatments, which was ranged 3.98-5.47% DM. Otherwise the lowest percentage of EE values was at P3 treatment after ensiling of 3.98% DM. After ensiling, the highest extract ether was found at the silage treated by P5. High content of EE in P5 silage contributed from the raw materials, i.e. rice bran and shredded coconut pulp contained EE were 11.4% and 38.24% respectively (Putri, 2010), EE content in silage might associated with TDN content.

There were significant results (P<0.05) for the content of crude fiber in feed before and after silage process (Table 2). Increase in crude fiber happen for all treatment after silage process. This is maybe caused of the processing of feed

Table 2. Nutrient content of silage at the before- and after-ensilage

Nutrient (DM%)	Before Ensiling					After Ensiling				
	P1	P2	P3	P4	P5	P1	P2	P3	P4	P5
Ash	14.49 ±0.43^{bc}	8.59 ± 0.61^{e}	11.30 ± 0.22^{de}	12.19 ±0.28^{bcd}	12.06 ± 0.15^{cd}	14.62 ± 2.94^{bc}	11.60 ± 2.12^{d}	18.88 ± 2.55^{a}	14.83 ± 0.65^{b}	12.57 ± 1.37^{bcd}
CP	7.93 ±1.67^{ab}	7.48 ± 1.52^{ab}	7.78 ± 0.22^{ab}	6.17 ± 0.46^{b}	8.40 ± 0.79^{ab}	9.18 ± 2.06^{a}	9.43 ± 1.38^{a}	9.65 ± 1.89^{a}	10.26 ± 2.39^{a}	9.44 ± 1.21^{a}
EE	4.17 ± 0.00^{c}	4.99 ±0.00^{ab}	4.58 ± 0.00^{bc}	4.13 ± 0.00^{c}	4.25 ± 0.00^{c}	5.13 ± 0.03^{ab}	5.04 ± 0.40^{ab}	3.98 ± 0.28^{c}	4.29 ± 0.17^{c}	5.47 ± 1.19^{a}
CF	24.77 ± 4.54^{d}	27.95 ±3.80^{cd}	33.37 ±3.52^{abc}	28.58 ± 3.33^{bcd}	35.54 ± 4.68^{ab}	35.07 ± 4.67^{abc}	32.13 ±5.83^{abc}	39.12 ± 5.15^{a}	32.50 ± 2.71^{abc}	33.09 ± 2.76^{abc}
NFE	52.62 ±6.55^{a}	53.95 ± 3.59^{a}	47,21 ± 8.46^{abc}	50.86 ± 6.10^{ab}	37.79 ± 5.29^{cd}	40.62 ± 6.47^{bcd}	38.68 ± 2.01^{cd}	29.38 ± 6.73^{d}	37.05 ± 6.38^{cd}	38.75 ± 2.93^{cd}

Note: DM: Dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, NFE: nitrogen free extract. The different superscript in same row means signiûcant differences (P <0.05).

Table 3**.** Difference of weight during ensilage process

Silage treatment	Before ensilage (g)	After ensilage (g)	Difference of weight during ensilage process (%)
P1	2000 ± 0.00	1985.33 ± 4.47	0.73 ± 0.22
P2	2000 ± 0.00	1989.87 ± 1.22	0.51 ± 0.06
P3	2000 ± 0.00	1926.40 ± 117.24	0.30 ± 0.35
P4	2000 ± 0.00	1990.07 ± 2.91	0.50 ± 0.15
P5	1200 ± 0.00	1196.00 ± 3.74	0.33 ± 0.31

Note: P1: 16% rice bran of total silage composition, P2: 16% shredded coconut pulp of total silage composition, P3: 8% using rice bran and shredded coconut pulp of total silage composition, P4: 8% rice bran, 5.6% shredded coconut pulp and 2.4% "*gaplek*" flour of total silage composition and P5: 8% shredded coconut pulp, 5.6% rice bran and 2.4% "*gaplek*" flour of total silage

through chemically, physically and biologically will give result in changes of the nutrient composition of a feed material, such as an increase or decrease in certain ingredients on 100% DM. In this study this condition was caused by a fermentation process that can improve the nutritional quality of the ingredients due to the chemical changes of organic compounds in both aerobic and aerobic conditions (Hapsari *et al*., 2014). Increase in fiber fraction proportion i.e. NDF may be due to fermentation of structural carbohydrates or water-soluble constituent losses or used by lactic acid bacteria to produce lactate together with effluents produced during fermentation or by gas losses (Valeriano *et al*., 2009).

Based on Table 2 the results of the variance analysis on Nitrogen-free extract (NFE) gave a significant difference (P <0.05). There was different content happen in this case increase in the content of CP, EE, CF, and ash, but there was a decrease in the NFE content after the ensilage while the highest NFE content founded in P1 before silage treatment. The NFE will be used first time as source of energy for microbe during ensilage process, it will give result in decrease on NFE content and increase percentage of other nutrient such as CP, CF, EE. This result accordance with increasing CF, CP and other nutrients during ensilage process that leads in changes of the nutrient composition of a feed material, such as an increase or decrease in certain ingredients on 100% DM (Hapsari *et al*., 2014). Kurniawan *et al*. (2016) explained that steaming resulted changes the composition of nutrient coconut pulp such as decreasing the content of EE coconut pulp which can change the composition of chemical compounds in steamed coconut pulp as a result of changes in the chemical composition of coconut pulp. In

this study this condition caused by a fermentation process that can rectify the nutritional quality of the components due to the chemical changes of organic compounds in both aerobic and aerobic conditions (Hapsari *et al*., 2014).

Table 4 show there is no significant effect of the formulation of shredded coconut pulp both of before and after the silage process on pH acidity of silage. The average value of the pH acidity of all formulation of shredded coconut pulp after ensilage was in the range 4.10-4.70. This range pH agreement with another study who reported that high-quality and a good silage should have a pH ranging from 3.80 to 4.70 (Mafakher *et al*., 2010; Falola *et al*., 2013; Ulger *et al*.,2018). A decrease in pH value happens in all treatment compared with before silage process. Decrease in pH due to the fermentation process by anaerobic bacteria. *Lactic acid* bacteria use carbohydrates dissolved in water (water-soluble carbohydrate/WSC) and produce lactic acid bacteria which plays a role in decreasing pH of silage which is indicated due to an increase in the LAB population in the feed (Santoso *et al*., 2009; Mugiawati *et al*., 2013; Ribeiro *et al.*, 2017).

The lowest pH value in silage treatment showed in P2. It probably caused by the substrate used which is shredded coconut pulp contributes carbohydrates as an energy source for anaerobic microorganisms during fermentation. These results are corresponding with the research of Kurniawan *et al.* (2016) that the low pH acidity of fermented coconut pulp probably caused by a large amount of fatty acid content in fermented coconut pulp which is the result of degradation coconut pulp fat.

Figure 1 showed that P2 treatment (16% SCP in formula treatment) gives the highest cumulative gas production 48 h than other treatment. An indication of fermentability evaluation by *in vitro* gas production reflected on kinetic gas production parameters (Sun *et al.,* 2013). Gas production is the result of fermentation caused by the amount of microbial activity occurring in the rumen, as well as showing the amount of digested organic material (Sofyan *et al.,* 2017). The gas production kinetics continue to increase from hours 0 to 48 hours. This condition may be due to the incubation time of 24 hours the presence of readily available carbohydrate sources (Readily Available Carbohydrate) in enough quantities to produce gas (Anggraeni *et al*., 2017). The highest cumulative gas production 48 h found at P2 treatment followed by P1, P4, P3, and P5. It can be said that use of 16% SCP in silage formula treatments produces the best degradability than other treatment. It probably caused by the substrate used which is shredded coconut pulp contributes carbohydrates as an energy source for anaerobic microorganisms during fermentation.

Gas Production and Kinetics

Based on Table 5, there is no significant result in a, b, c, a + b, and total gas production 48 hour among all treatment. The formed of gas kinetics continue to increase from hours to 0 to 48 hours. The gas production at 48 hours showed no significant result. This may be caused by the ration of each treatment not disrupting the rumen microbial activity in degrading feed. According to Derrick (2005) the crude fiber content in coconut pulp that is easily digested can be its advantage, especially in stimulating the rumen. While in total gas production is 48 hours not significant, when we break down the

Table 4. The acidity value (pH) of silage

Silage treatment	Before ensilage	After ensilage	pH Acidity decrease before and after ensilage (%)
P1	5.75 ± 0.69	4.19 ± 0.13	23.91 ± 9.23
P2	5.81 ± 0.38	4.10 ± 0.16	28.71 ± 5.37
P3	5.83 ± 0.56	4.31 ± 0.14	23.50 ± 10.82
P4	4.94 ± 0.09	4.29 ± 0.23	12.95 ± 6.11
P5	5.02 ± 0.60	4.20 ± 0.14	16.31 ± 3.61

Note: P1: 16% rice bran of total silage composition, P2: 16% shredded coconut pulp of total silage composition, P3: 8% using rice bran and shredded coconut pulp of total silage composition, P4: 8% rice bran, 5.6% shredded coconut pulp and 2.4% "*gaplek*" flour of total silage composition and P5: 8% shredded coconut pulp, 5.6% rice bran and 2.4% "*gaplek*" flour of total silage.

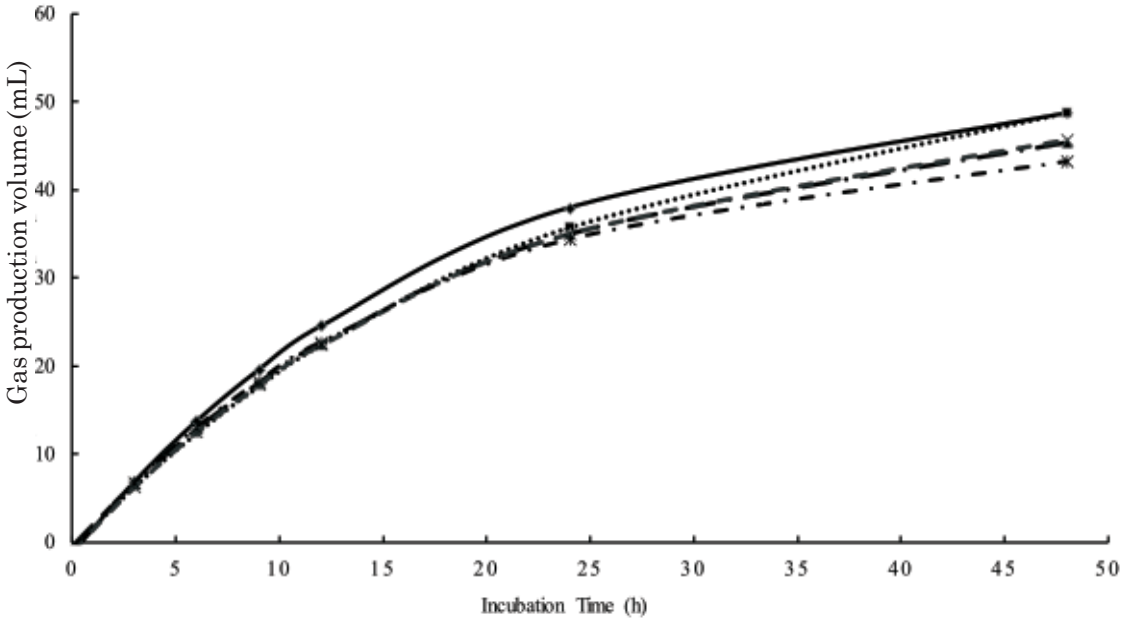

Figure 1. Fitted cumulative gas production of silage content shredded coconut pulp treatment incubated during 48 hours P1 (♦), P2 (■), P3 (▲), P4 (×), P5 (✲).

Table 5. *In vitro* fermentability parameters of silage content shredded coconut pulp treatment

Variables	Formulation of shredded coconut pulp				
	P1	P2	P3	P4	P5
Gas Production kinetic					
a (mL)	-1.16 ± 0.33	-0.71 ± 0.54	-0.46 ± 0.64	-0.98 ± 0.50	-0.61 ± 0.17
b (mL)	53.98 ± 4.37	59.77 ± 12.67	50.27 ± 2.73	51.56 ± 6.97	46.84 ± 1.14
c (mL/h)	0.05 ± 0.01	0.04 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
a+b (mL)	52.82 ± 4.65	59.06 ± 12.95	49.82 ± 2.39	50.57 ± 6.66	46.24 ± 1.27
Gas 48 hours (mL)	48.60 ± 5.22	48.70 ± 3.54	45.20 ± 1.75	45.70 ± 3.80	43.30 ± 0.66
VFA					
Total VFA mM	95.85 ± 2.92	112.84 ± 26.60	121.68 ± 10.69	94.02 ± 5.15	107.24 ± 4.07
Acetate (%)	78.50 ± 2.32^{ab}	81.98 ± 2.33^{b}	77.59 ± 0.01^{a}	74.72 ± 0.05^{a}	76.40 ± 1.12^{a}
Propionate (%)	16.92 ± 0.63^{b}	13.81 ± 1.27^{a}	16.47 ± 0.04^{b}	17.81 ± 0.28^{b}	16.91 ± 1.17^{b}
Butyrate (%)	4.58 ± 1.69^{ab}	4.21 ± 1.06^{a}	5.94 ± 0.04^{abc}	7.47 ± 0.33^{c}	6.69 ± 0.06^{bc}
A/P ratio	4.65 ± 0.31^{a}	5.97 ± 0.72^{b}	4.71 ± 0.01^{a}	4.20 ± 0.06^{a}	4.53 ± 0.38^{a}
NGR	9.56 ± 0.53^{a}	12.24 ± 1.38^{b}	9.78 ± 0.03^{a}	8.81 ± 0.15^{a}	9.46 ± 0.79^{a}
NH_3 (mg/100mL)	0.70 ± 0.38^{a}	10.21 ± 0.68^{b}	21.23 ± 1.26^{c}	39.79 ± 0.59^{d}	58.96 ± 1.75^{e}
Microbial Rumen Protein (mg/mL)	0.17 ± 0.03	0.14 ± 0.02	0.14 ± 0.02	0.14 ± 0.01	0.11 ± 0.06

Note: *a, b, c*, Constans from P = a+b ($1-e^{-ct}$) with P : Gas Production, *a* : gas contribution from soluble fraction, *b* : gas production from insoluble fraction, *c*: rate of gas production at *t* time, VFA : Volatile Fatty Acid, A/P : Acetic to Propionate Ratio. NGR : Non Glucogenic Ratio, NH_3 : Ammonia-N production. The different superscript in same row means signiûcant differences (P <0.05).

observation each hour it shows a significant result. It means there is an increase in feed for 48 hours' time incubation. The production of gas shows feed fermentation process by rumen microbes, that is hydrolyzing carbohydrates into monosaccharides and disaccharides which are fermented into volatile fatty acids (VFA) including acetic acid, propionate, butyrate and methane gas (CH_4) and CO_2 (McDonald *et al*., 2002).

Fermentation is relatively intensive (lag phase), during the first 24 hours of incubation, after which it reaches a stationary phase. The kinetics of gas production appear to be determined by two different phases, the first suitable for degradation of the soluble fraction and second being insoluble but potentially fermented. Negative results on the soluble fraction (*a*) gas production (Table 5) shows that rumen microbes in the lag phase it means that they still need adaptation time due to the delay in microbial colonization of the substrate during the initial stages of incubation before degrading the soluble particle (Chumpawadee *et al*., 2005). Some authors (Chumpawadee *et al*., 2005; Arhab *et al.,* 2010; Sofyan *et al*., 2015,) also reported negative values from the soluble fraction (*a*) for diverse substrates when using mathematical models to fit gas production kinetics. Lag time of soluble fraction degradation activity by ruminal microbes and then adhere to a cellulosic fraction, this reason possibility cause negative "a" value. Ideal fermentation of the dissolved fraction could be described from the absolute value of a (| a |) (Chumpawadee *et al*., 2005)

Gas volume in asymptote (b) describes insoluble fraction fermentation. The b value ranked from highest to lowest were formula P2, P1, P4, P3, and P5 (Table 5). The b value has the advantage for the prediction of feed intake because it accounts for 88% of the variance intake (Chumpawadee *et al*., 2005).

Although there was no significant result, the P2 treatment gave the highest maximum gas production (a + b) and 48 hours of gas production (Table 5). Ruminal microorganisms will easily attach the soluble fraction and lead to higher gas production (Table 5). Maximum gas production (a + b) at 24 hours incubation has produced more than 73% of the maximum gas production in almost all treatments of shredded coconut pulp formula. This result shows that the rate of *in vitro* gas production decreases with increasing incubation time, this is because there are decreasing in a number of fermented substrates (Hungate, 1966; Jayanegara and Sofyan, 2008, Jayanegara *et al*., 2009). Generally, high carbohydrate fraction (particularly NDF) be the reason higher maximum gas production of tropical feed production of non-forage high fibrous (Chumpawadee *et al*., 2005).

The rate of gas production (c) has ranked from the fastest was P5, and the lowest was P2 around (0.04-0.06) (Table 5), possibly influenced by the soluble carbohydrate fractions readily available to ruminal microbes. The carbohydrate fraction could affect the kinetics of gas production (Deaville and Givens, 2001). The product of carbohydrates fermentation when a feedstuff incubated with buffered rumen fluid *in vitro* are short-chain fatty acids (acetate, propionate, and butyrate), gases, and microbial cells. Gas production from carbohydrate fermentation is relatively big as compared to protein fermentation (Van Soest, 1994, Anggraeni *et al*., 2017).

Volatile Fatty Acid (VFA)

Productions of total volatile fatty acid showed no significant difference (P>0.05) for all treatments, while the percentage of acetate (C2), propionate (C3), butyrate (C4), A/P ratio and NGR showed a significant difference (P<0.05) for all treatments (Table 5). Total VFA varied between 94-121 mM per mL of rumen fluid in this study. This VFA production was enough condition for optimal rumen microbial protein synthesis, because VFA range required for rumen microbial growth was 80-160 mM (Van Soest, 1994), added by Istiqomah *et al*. (2011) that total VFA production ranged from 106.67-165.81 mM. Increased microbial production correlated with increasing VFA concentration (Istiqomah *et al*., 2011). Quickness of feed degraded by rumen microbes could be show on increasing in the number of VFA. The increase in total VFA production caused by the absence of VFA release through absorption in the *in vitro* system and VFA only used by rumen microbes (Woolcock, 1991). The difference of physical form, the composition of feed, level and feeding frequency, and feed processing can alter the composition of VFA in rumen. Furthermore, high VFA production is the energy adequacy for ruminant (Anggraeni *et al*., 2017).

Fermentation of feed with higher results of acetic acid (C2) and butyric acid (C4) produce greater ratio (C2 + C4) / C3 (NGR). This condition reflected on P2 formula, while another

formula shows an increased proportion of C3 especially in P4 formula, so that the value of the ratio of (C2 + C4) / C3 (NGR) is lower. Variation of VFA production (C2, C3, C4) causes differences in CH_4 production in the rumen (Gworgwor *et al.,* 2006). The C2 and C4 play a role in the formation of CH_4 when there is competition with the formation of H ions using propionate (C3) in the rumen. Increased ratio NGR, in this study case, happen in P2 formula will produce high availability C2 and C4 so that the higher the production of CH_4. C2 and C4 are the source of energy for oxidation. The C2 is a non-glucogenic compound, and almost all tissues of the body can oxidize it. Oxidation process produces high heat increment; it will cause lower feed efficiency. The C3 is a sugar compound precursor or primary glucogenic. The C3 of the reticulo-rumen is absorbed into the blood, through the portal vein to the liver to be converted into glucose through gluconeogenesis (Marhaeniyanto and Susanti, 2014). Rumen fermentation system that leads to an increase in glucogenic propionate also resulted in the value of non-glucogenic ratio (NGR) tends to decrease (Istiqomah *et al*., 2011). Change of NGR was closely related to C2, C3 and C4 in which C2 was dominantly affected due to 60% of VFA (Sofyan *et al*., 2015).

The A/P ratio of P2 higher than other treatment, while P4 formula produces lowest A/P ratio from all treatment. It has been recommended that there is a decreased proportion of acetate in the rumen compared to propionate happened in P4 formula. This result in line with (Hidayah *et al*., 2014) that supplementation of microencapsulated flaxseed oil resulted in the lowest proportion of acetate and the ratio of A:P, and the highest proportion of propionate. This result was presumably because microencapsulated flaxseed oil supplementation can stimulate the growth of bacteria propionate producers in the rumen system so that the ruminal propionate production increased. Another study (Chalupa *et al*., 1984) explained that in the free acid form, oleic acid increased production of propionate, reduced production of acetate hence, lowered A/P by 54% with-out a significant reduction of total volatile fatty acids. Formation of calcium salts of long chain fatty acid (LCFA) is an efficacious method of protecting ruminal microorganisms from adverse effects of fat, fatty acids induce smaller changes when in the form of triglycerides. The relationships between melting point of LCFA and production of volatile fatty acids indicate that hard fats (i.e., those with high melting points) are more inert in the rumen, and thus, less likely to coat either bacterial cells or feed particles. The proportion of C4:C3:C2 was constant about 6:16:78 for all treatments. Individual VFA proportion consisting of C2, C3, and C4 were 60-70%, 20-30% and 10-15% respectively, based on the ruminal fermentation stoichiometry (Bo *et al.,* 2013, Hidayah *et al.*, 2014, Anggraeni *et al*., 2017). The C2 will lead oxidation process, produces high heat increment and it will cause lower feed efficiency (Marhaeniyanto and Susanti, 2014). Production of VFA mostly depends on the fermentation of the carbohydrate feed, a fraction of the protein (Van Hourtert, 1993; Herdian *et al*.,2011). A lot of nutrient supply also provides more substrate for growing rumen microbes and yielding more microbial protein from their biomass (Clarck *et al*., 1992; Bach *et al.,* 2005). When the microbes are active and abundant, it is expected that there will be an increase in the VFA concentration as the final product of microbial fermentation in the rumen (Kamra, 2005).

Ammonia-N (NH_3)

Ammonia-N (NH_3) concentration of treatment was presented in Table 5. The results of the variance analysis showed that the ammonia concentration gave a significant difference ($P<0.01$) for each treatment. The NH3 value of P5 higher than P4, while protein content on P5 is lower than P4 it's presumably because in P4 formulation used higher rice bran than P5. Rice bran content higher protein than shredded coconut pulp, but on the other hand it's also content of anti nutrition that is phytic acid (Perera *et al.,* 2018). This phitatic acid has the ability to chelating with a number of minerals and proteins (Hernaman *et al* 2007., Selle *et al.,* 2000). The presence of binding protein with phytates causes the rumen proteolytic MO activity to decrease so that through phytase addition the amount of rumen MO increases (Yanke *et al.,* 1998). This is the potential cause of the decreasing proteolytic activity of the rumen MO to produce NH_3 in the P4 ration. Factors that effect on NH_3 include protein content in feed consumed, time length of feed in the rumen, and rumen pH. Ammonia (NH_3) is made from the activity of rumen microorganisms to produce proteolytic enzymes that play a role in degrading proteins in rations. The NH_3 content in rumen is a reflection of the degradation activity of feed

proteins and endogenous proteins by rumen microbes through the N balance mechanism of the animal body (Bondi, 1987). Ammonia concentrations produced in this study ranged from 0.70-58.96 mg/100 mL. The normal NH_3 concentration in rumen according to Bondi (1987) is between 2 and 50 mg per 100 ml. The NH_3 levels for optimal microbial growth ranges from a 5-8 mg/100 ml of rumen fluid (Marhaeniyanto and Susanti, 2014). Value low ammonia indicates utilization of ammonia as the source of nitrogen for microbial growth rumen (Imanda *et al,* 2016). There is evidence that changes in NH_3 content are strongly influenced by changes in carbohydrate content of feed. Öztürk (2008) reported that an increase in supplementation of topinambur (*Helianthus tuberosus L*) plants as much as 0.2-1.0 g /day has a source of inulin (polysaccharide: fructose â (2,1)) to substrates containing a mixture of straw and concentrates (5 : 4 g) can reduce NH_3 levels of sheep rumen fluid by *in vitro* method. This condition happens because of increased microbial growth as a result of an increase in carbohydrates which requires more NH_3 availability (Herdian *et al.,* 2011).

Microbial Rumen Protein

The results showed that treatments did not have a significant effect on microbial rumen protein. Similar results were found in Sakti *et al.* (2013) supplementation of *Cyclea barbata* did not significantly influence (P> 0.05) on rumen protein biomass (0.0047-0.0133 mg/mL). The non-significant results were probably due to non-significant result of total VFA in all treatments. Number of bacteria depends on the concentration of NH_3 and the availability of soluble carbohydrates, in this case concentration of VFA, higher VFA concentration will generate optimum growth of bacteria and produce greater number of bacteria (Woolcock, 1991). Production of microbial rumen protein indicates the number of rumen microbes (bacteria, protozoa, fungi) that play a role in degrading feed in the rumen (Marhaeniyanto and Susanti, 2014).

CONCLUSION

In vitro degradability expressed nutrient utilization in the rumen which related by gas production kinetics parameters, ammonia-N (NH_3), microbial rumen protein and VFA. In this study silage contains 50% rice bran, 35% shredded coconut pulp and 15% "*gaplek*" flour, 83% king grass, 0.5% mineral mix and 0.5% molasses in the formulation has the best degradability on *in vitro* rumen fermentation characteristics based on VFA composition and acetate : propionate ratio.

SUGGESTION

Further research should be conducted to evaluate the degradability and quality of silage supplemented with shredded coconut pulp through *in vivo* assessment. Based on VFA composition and acetate: propionate ratio we suggest that the silage is more suitable for beef cattle production.

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