In vitro rumen fermentation characteristics of ammoniated stover of Samurai 2 sorghum fertilized with different level of Urea

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In vitro rumen fermentation characteristics of ammoniated stover of Samurai 2 sorghum fertilized with different level of Urea

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Abstract. The limitation stock of forages in dry season cause serious problem for ruminant productivity. Various efforts have been made to obtain an alternative feed. Samurai 2 has a high productivity, immune to leaf rust disease and midrange rot and suitable for animal feed. However, sorghum stover is a poor-quality fodder, low in protein and digestibility. It is needing to be processing to increase nutritive value by providing a source of protein and or non-protein and certain minerals through ammoniation. The effect of ammoniation treatments on a sorghum stover on *in vitro* feed fermentation and gas production on ruminal fluid was the main objective of this study. P12 treatment gave highest gas production than other treatment. Total gas production 48 hours, gas production from soluble fraction (*a*) and insoluble fraction (b) also total fraction (*a*+*b*) of the sorghum stover was significantly affected by treatments ($P<0.05$). Volatile Fatty acid (VFA), *in vitro* degradability, NH_3, and biomass microbial rumen indicated no significant difference among treatments. In this study sorghum stover using basic fertilizer 90 kg/ha with ammoniation treatment has the best degradability on rumen fermentation characteristics *in vitro* of sorghum stover.

1. Introduction

The limitation stock of forages in dry season cause serious problem for ruminant productivity. Various efforts have been made to obtain an alternative feed, which is easy to obtain, cheap and contains nutrients to overcome scarcity of forages in dry season [1]. Sorghum is an upright growing plant that has a wide tolerance of soil fertility and waterlogging as well as drought and is relatively resistant to pest and diseases, high production, also requires less input [1]. Sorghum has a high nutritional content, 332 calories and 11.0 g protein / 100 g seeds in the seeds, and vegetative / forage 12.8% crude protein, so it can be cultivated intensively as a source of forage for ruminants mainly in dry season [2]. Pahat, Samurai 1 and Samurai 2 have produced by National Nuclear Energy Agency of Indonesia (BATAN) as sorghum mutant varieties to be developed in dry and ideal areas in the food industry, bioethanol and has a good palatability for animal [3]. Samurai 2 sorghum varieties is the result of breeding with radiation mutations derived from the Pahat varieties. It is has a high productivity, biomass of 47 ton/ha, immune to leaf rust disease and midrange rot [4]. This type of sorghum is suitable for animal feed [3,4]. *In vitro* research showed that leaves, stems, and bagasse Samurai 2 sorghum varieties can be used for animal feed [4], while *in vivo* testing using sweet sorghum bagasse can improve buffalo productivity [5].

Balanced fertilization can increase yields [6;7]. Nitrogen fertilizer promotes sucrose content, protein percentage, growth rate, production and quality of forage crops also can slow maturation of sorghum seeds (extending vegetative period) [8;9]. Nitrogen has an important role in growth of plant through cell division [10;11;12]. Addition dose of urea fertilization leads more N, will be available as raw material of protein formation so that the higher protein content of sorghum [9]. Added information by [9] explains that the end product of urea is in the form of the NH4 + and NO3 ions to be absorbed by the plant. K fertilizers also increase yield of sorghum responses from increasing levels of nitrogen fertilizer alone [13]. K is needful for efficient transformation of solar energy into chemical energy [14]. Biomass and carbohydrate of sweet sorghum can be stimulated with accurate application of N and K fertilizers which is considered as an important factor in food and industrial usage.

Sorghum stover is a poor-quality fodder, low in protein and digestibility. It is needing to be processing to increase nutritive value. Ruminant productivity can be improved by providing a source of protein and or non-protein and certain minerals. Nutritive value of rice straw could be increased through urea treatment [15], [16]. Alkali source like urea has been reported to be effective in improving fibrous feed quality [17], [18]. The urea also supplies N for rumen microbial growth besides improves fiber utilization. Volatile fatty acid (VFA), gas production and microbial biomass are the main product of feed of organic matter fermentation in ruminant [19]. The effect of ammoniation treatments on a sorghum stover on *in vitro* feed fermentation and gas production on ruminal fluid was the main objective of this study. However, the study of rumen fermentation characteristics *in vitro* of sorghum roughage is still limited in scientific publication. The comparative study of rumen fermentation characteristics *in vitro* of sorghum with different levels of fertilization and ammoniation process is needed, to determine a good product quality of sorghum ammoniation, and to evaluate the effect of different levels of fertilization and ammoniation treatment on rumen fermentation characteristics *in vitro*.

2. Materials and methods

2.1. Sample preparation

Samurai 2 sorghum varieties was planted in field laboratory of BPTBA LIPI using basic fertilizer urea. Sorghum plant was harvested at 150 days after harvesting the seeds. The sorghum straw used includes the leaves and stems. After this process leaves and stems were weighed and chopped manually around ± 5 cm. Ammoniation treatment using urea as much as 5% with simple open clip (SIMPOC) method refers to [20]. All treatment kept for one month at room temperature. Each treatment was conducted in three replicates. The treated sorghum analyzed for dry matter (DM) by following the procedure from [21].

2.2. Treatment and experimental design

This research was conducted based on Factorial Block Randomized Design (FBRD) with eight treatments and three replications based on [22]. Main factor is ammoniation (treated and untreated) and second factor is fertilizer used (0 kg/ha, 30 kg/ha, 60 kg/ha, 90 kg/ha). Treatments described as follows:

P3	:	0 kg/ha urea fertilizer SIMPOC
P3.o	:	0 kg/ha urea fertilizer SIMPOC.o
P6	:	30 kg/ha urea fertilizer SIMPOC
P6.o	:	30 kg/ha urea fertilizer SIMPOC.o
P9	:	60 kg/ha urea fertilizer SIMPOC
P9.o	:	60 kg/ha urea fertilizer SIMPOC.o
P12	:	90 kg/ha urea fertilizer SIMPOC
P12.o	:	90 kg/ha urea fertilizer SIMPOC.o

Information: “o” it is mean without ammoniation (untreated)

Nutrient value of Samurai 2 Sorghum stover after ammoniation process conducted based on paired t-test [22].

Table 1. Nutrient value of Samurai 2 Sorghum stover after ammoniation treatment.

Treatment	Dry Matter (%)	Ether Extract (%)	Crude Protein (%)	Crude Fiber (%)	Ash (%)	NFE (%)
Non-amoniation	90.74±0.33	0.74±0.38	5.45±5.62	35.59±52.61[a]	8.19±2.58	50.02±28.05[a]
Ammoniation	91.43±0.44	0.75±0.07	4.40±0.97	45.56±6.47[b]	6.57±2.39	42.72±1.56[b]

2.3. Fermentability and in vitro degradability assessment

The sample of sorghum roughage each treatment and rumen liquid were prepared before *in vitro* assessment. Two fistulated Ongole crossbreed cattle used as rumen liquor donor, those adapted by feeding consisted of forage (*P*. hybrid) and concentrated (80:20 in dry matter basis). Rumen fluid was taken using aspirator, and immediately transported in pre-warmed vacuum flask (39 ºC water temperature) then filtered.

In vitro gas production technique according to [23] used to evaluate *in vitro* fermentability. The exponential equation according to [24] 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, c is the rate of gas production, t is the time of incubation and e is Euler's constant (2.7183…). Fitting curve method using Neway Software [25] apply to the estimated value of *a*, *b* and *c*. Fermentation evaluation used 100 mL syringe glass (Fortuna model, Poulten and Graft Gmbh Germany). Three syringes are containing rumen-buffer without sample (blank) used in the experiment. All of the syringes consisted of blank and samples were randomly incubated for 48 hours in an incubator at 39 ºC [26].

Gas production cumulative recorded at 0, 2, 4, 8, 12, 24, 36 and 48 hours incubation. Gas was released after 48 h of incubation and the fluid contained in the syringe taken for analysis of VFA, and *in vitro* dry matter degradability. Substrate from each syringe measured according to [21] method to get dry matter (DM) value. Percentage of DM differences between initial and after incubation and then corrected with blank were calculated as *in vitro* degradability as followed the formulas previously described by [27]:

$$\text{IVDMD} = \frac{[\text{DMf}-(\text{DMr}-\text{DMb})]}{\text{DMf}}$$

$$\text{IVOMD} = \frac{[\text{OMf}-(\text{OMr}-\text{OMb})]}{\text{OMf}}$$

Where: IVDMD *in vitro* dry matter degradability. DMf: a dry matter of feed, DMb: a dry matter of blank, DMr: a dry matter of residue.
IVOMD in vitro organic matter degradability. OMf: an organic matter of feed, OMb: an organic matter of blank, OMr: an organic matter of residue.

Volatile fatty acid (VFA) product from fermentation measured according to [28]. Meta-phosphoric acid added to the sample and stored at -20 ºC before analysis. Gas chromatography (Shimadzu type 8A) was used to analyzed VFA content using GP10% SP-1200/1% H3PO4 column with 80/100 Chromosorb WAW (Supelco, Bellefonte, PA). NH3 measured using [29] method. Measurement microbial cell protein using principle gradually modified centrifugation from [30].

2.4. Data Analysis

Variables measured were *in vitro* degradability (IVDMD & IVOMD) fermentability (gas production kinetics, a, b and c), NH3 and biomass microbial rumen and individual volatile fatty acids (VFA). Analysis of covariance (ANCOVA) was used to evaluated data, and post hoc test of Duncan's Multiple Range Test was used to analyzed differences among mean treatments that performed by the CoSTAT statistical software [31].

3. Result and discussion

Sorghum with basic fertilizer urea (urea) 90 kg/ha with ammoniation treatment (P 12) was generated the highest gas production compared to the others (Figure 1).

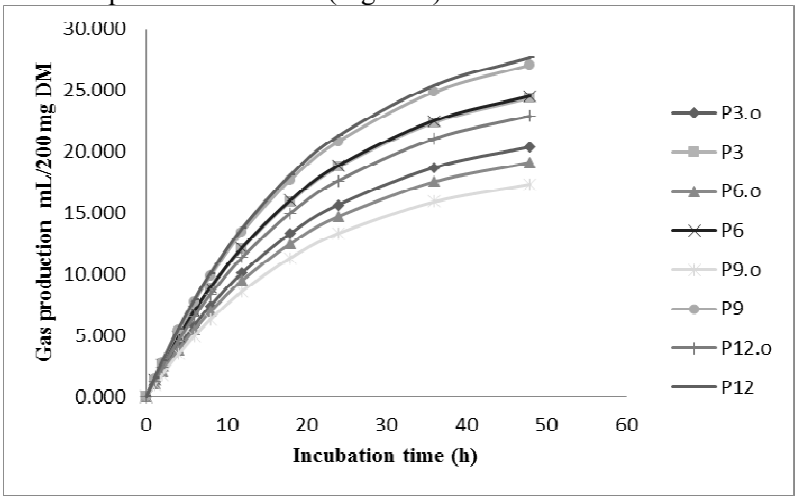

Figure 1. Cumulative gas production of sorghum ammoniation and without ammoniation treatment used different level of fertilizer incubated during 48 hours.

Figure 1. showed that P12 treatment (fertilizer 90 kg/ha with ammoniation treatment) gives the highest gas production than other treatment. Kinetic gas production parameters used as an indication of fermentability evaluation by *in vitro* gas production [28]. 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 [26]. The gas production kinetics continue to increase from hours 0 to 48 hours. The gas production curve continued to increase at 24 hours incubation time, while at 48 hours the incubation curve started to look linear although there was still no slowing. This condition may be due to the incubation time of 24 hours the presence of readily available carbohydrate sources (Readily Available Carbohydrate) in sufficient quantities to produce gas [20]. The highest cumulative gas production found at P12 treatment followed by P9, P6, P3, P12.o, P3.o, P6.o and P9.o. It can say that ammoniation treatments in sorghum stover with fertilizer 90kg/ha produce the best sorghum stover degradability than other treatment. Urea treatment with the highest fertilizer used showed to be effective in improving the nutritive value of sorghum stover. The reason is that urea supplement may act as nonprotein nitrogen source that rumen microbes could utilize it and then passed as microbial protein for the host animal [32].

Table 2. Gas production of sorghum stover with different level of fertilizer and ammoniation treatment

Variables	Level of Fertilizer (Urea)				Average
	0 kg/ha	30 kg/ha	60 kg/ha	90 kg/ha	
Gas production kinetic parameters					
a (mL)					
Ammoniation	-0.27 ± 1.17	-0.17 ± 1.29	-0.16 ± 1.23	0.20 ± 1.11	-0.10 ± 0.18[a]
Non-ammoniation	-0.82 ± 0.38	-0.91 ± 0.24	-0.87 ± 0.04	-0.78 ± 0.28	-0.85 ± 0.05[b]
Average	-0.55 ± 0.77	-0.54 ± 0.77	-0.52 ± 0.64	-0.29 ± 0.69	
b (mL)					
Ammoniation	30.73 ± 11.78	37.33 ± 14.64	40.06 ± 14.49	39.78 ± 13.22	36.98 ± 13.53[a]
Non-ammoniation	41.33 ± 20.56	30.79 ± 13.84	30.50 ± 16.16	33.26 ± 11.40	33.97 ± 15.49[b]
Average	36.03 ± 16.17	34.06 ± 14.24	35.28 ± 15.33	36.52 ± 12.31	
c (mL/h)					
Ammoniation	0.03 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
Non-ammoniation	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02
Average	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	
a+b (mL)					
Ammoniation	40.50 ± 19.39	36.42 ± 13.35	39.19 ± 13.26	39.00 ± 12.11	38.78 ± 14.53[a]
Non-ammoniation	30.46 ± 11.40	30.62 ± 13.60	30.44 ± 16.07	33.45 ± 11.68	31.24 ± 13.19[b]
Average	35.48 ± 15.40	33.52 ± 13.47	34.81 ± 14.66	36.23 ± 11.90	
Gas (48 h) (mL)					
Ammoniation	27.31 ± 4.17	27.12 ± 3.65	28.12 ± 1.44	29.62 ± 2.75	28.04 ± 3.00[a]
Non-ammoniation	22.96 ± 3.67	21.76 ± 3.76	19.43 ± 2.96	22.55 ± 0.52	21.68 ± 2.72[b]
Average	25.14 ± 3.92	24.44 ± 3.71	23.78 ± 2.20	26.08 ± 1.64	

Table 3. *In vitro* digestibility and ruminal fermentation profiles of sorghum stover with different level of fertilizer and ammoniation treatment

Variables	Level of Fertilizer (Urea)				Average
	0 kg/ha	30 kg/ha	60 kg/ha	90 kg/ha	
In vitro digestibility					
IVDMD (%)					
Ammoniation	42.33 ± 8.27	39.99 ± 11.14	42.48 ± 9.27	39.02 ± 10.56	40.96 ± 9.81
Non-ammoniation	37.45 ± 9.95	39.45 ± 9.59	35.78 ± 16.69	39.41 ± 3.11	38.02 ± 9.83
Average	39.89 ± 9.11	39.72 ± 10.37	39.13 ± 12.98	39.21 ± 6.84	
IVOMD (%)					
Ammoniation	87.71 ± 1.67	83.98 ± 0.76	89.34 ± 1.02	85.87 ± 1.87	86.72 ± 1.33
Non-ammoniation	88.96 ± 0.49	95.74 ± 10.82	85.05 ± 1.27	86.63 ± 1.97	89.09 ± 3.64
Average	88.33 ± 1.08	89.86 ± 5.79	87.19 ± 1.15	86.25 ± 1.92	
Ruminal Fermentation					
MCP					
Ammoniation	5.69 ± 1.33	4.08 ± 1.18	4.09 ± 0.73	3.88 ± 1.03	4.43 ± 1.07
Non-ammoniation	3.98 ± 1.57	3.61 ± 1.42	4.26 ± 0.81	4.00 ± 0.60	3.96 ± 1.10
Average	4.83 ± 1.45	3.84 ± 1.30	4.18 ± 0.77	3.94 ± 0.82	
Ammonia					
Ammoniation	128.66 ± 3.40	105.52 ± 4.50	107.99 ± 14.88	103.69 ± 7.61	111.47 ± 7.60
Non-ammoniation	92.56 ± 16.46	78.07 ± 19.56	99.49 ± 4.84	52.73 ± 74.58	80.71 ± 23.09
Average	110.61 ± 9.93	91.80 ± 12.03	103.74 ± 9.86	78.21 ± 41.10	

IVDMD = *in vitro* dry matter digestibility, IVOMD = *in vitro* organic matter digestibility, MCP = microbial cell protein

Table 4. Volatile fatty acids characterictics of sorghume stover with different level of fertilizer and ammoniation treatment

Variables	Level of Fertilizer (Urea)				Average
	0 kg/ha	30 kg/ha	60 kg/ha	90 kg/ha	
VFA total (mM)					
Ammoniation	168.46 ± 56.81	135.10 ± 58.18	316.96 ± 180.89	205.79 ± 84.21	206.58 ± 95.02
Non-ammoniation	208.29 ± 138.58	299.79 ± 293.62	163.28 ± 52.69	218.08 ± 105.71	222.36 ± 147.65
Average	188.37 ± 97.70	217.44 ± 175.90	240.12 ± 116.79	211.93 ± 94.96	
Acetate (%)					
Ammoniation	55.94 ± 1.29	56.91 ± 0.73	56.61 ± 4.14	57.57 ± 3.77	56.76 ± 0.74
Non-ammoniation	59.53 ± 4.96	55.43 ± 1.06	56.11 ± 1.74	58.04 ± 2.77	57.28 ± 0.71
Average	57.74 ± 3.86	56.17 ± 1.15	56.32 ± 2.70	57.80 ± 3.13	
Propionate (%)					
Ammoniation	28.53 ± 0.64	28.34 ± 15.26	27.73 ± 1.64	27.32 ± 1.47	27.98 ± 0.35
Non-ammoniation	26.51 ± 2.68	28.83 ± 83.46	28.54 ± 0.55	27.12 ± 1.17	27.75 ± 0.33
Average	27.52 ± 2.10	28.58 ± 49.36	28.19 ± 1.11	27.22 ± 1.26	
Butyrate (%)					
Ammoniation	15.54 ± 0.10	14.76 ± 0.38	15.66 ± 2.49	15.11 ± 2.60	15.27 ± 0.46
Non-ammoniation	13.97 ± 2.38	15.74 ± 1.35	15.35 ± 1.27	14.84 ± 1.65	14.98 ± 0.44
Average	14.75 ± 1.89	15.25 ± 1.06	15.48 ± 1.70	14.98 ± 2.06	
A/P ratio					
Ammoniation	1.96 ± 0.08	2.01 ± 0.11	2.05 ± 0.28	2.12 ± 0.25	2.04 0.18
Non-ammoniation	2.28 ± 0.44	1.92 ± 0.02	1.97 ± 0.10	2.15 ± 0.52	2.08 0.27
Average	2.12 ± 0.26	1.97 ± 0.06	2.01 ± 0.19	2.13 ± 0.38	

Ruminal fermentability characteristic productions were shown in Table 2. Total gas production 48 hours, gas production from soluble fraction (a) and insoluble fraction (b) also total fraction (a+b) of the sorghum stover was significantly affected by ammoniation treatments ($P<0.05$), but were not affected by the level of fertilizer treatment ($P>0.05$). The production of gas formed will increase with the length of the incubation time. The cumulative gas production increased during 48 hours incubation period [20]. The highest cumulative gas production found at P12 during 48 hours incubation. This result may be due to the higher digestibility of ammoniation treatment of sorghum stover with 90 kg/ha fertilizer. This study has a similar outcome with [33] that urea ammoniated sugarcane trash (USCT) more digestible than sugarcane trash (SCT) that similarly, concentrates are highly edible hence higher gas production recorded. The kinetics of gas production appear to be determined by two distinct phases; the first one insoluble but potentially fermentable fraction and second suitable to the degradation of the soluble fraction of the tested mixtures. 'a' is nutritionally represented by soluble carbohydrates of rapid ruminal degradation. The fraction 'b' is represented by cell wall components, of slow ruminal degradation, due to structural arrangements of these components with lignin [34]. Ruminal microbes need an adaptation time (*lag* phase) before degrading the soluble particle this condition reflected in negative results on gas production produced from the soluble fraction (*a*) this results in accordance with the previous study reported by [26;34; 35], that possibility there was negative "a" value arising from lag time of soluble fraction degradation activity by ruminal microbes and then to adhere to cellulosic fraction.

In vitro dry matter degradability (IVDMD) and *In vitro* organic matter degradability (IVOMD), Microbial cell protein (MCP) and ammonia (NH_3) shown in Table 3. IVDMD had a tendency ($P\sim0.11$) affected by ammoniation treatments, but IVOMD was not influenced by ammoniation treatment ($P>0.05$). Moreover, both of IVDMD and IVOMD were not influenced by the level of fertilizer treatment ($P>0.05$). Biomass microbial rumen was not influenced by ammoniation and level of fertilizer treatment ($P>0.05$). NH3 had a tendency ($P\sim0.11$) affected by ammoniation treatments, but not controlled by the level of fertilizer treatment ($P>0.05$). Value of IVDMD booth of treated and untreated ammoniation is 40.96, and 38.02 include in the low category of IVDMD. Grain-sorghum residues, like residues from small grain crops, had a low IVDMD (37.7%) [37]. Ammoniation treatment has tendency effect on IVDMD than without ammoniation treatment even though there are no significant. IVDMD value of ammoniated sorghum stover in this study was 7,708% units higher than untreated, this result lower than the result of [37] treated ammoniation 17-18% units higher than untreated. Urea treatment showed to be effective in improving the nutritive value of sorghum stover by reducing ADF content of sorghum stover [1;32]. Urea provided ammonia after being hydrolyzed to produce stepwise ammonium carbonate and ammonium carbamate [17]. Such treatment reduces physical strength of primarily fibrous feed, disrupts the silicified cuticular barrier and cleavages of some lignin-carbohydrate bonds [17], [38].

Higher IVDMD values compared than untreated appeared as an effect of increasing ammoniation-treated sorghum stover degradability. Apart from modifying structural carbohydrate of the sorghum stover, urea addition could contribute to feeding the microbes an additional nitrogen source for microbial protein synthesis purposes and subsequent utilization of the protein by the host animal; it will give better digestibility than other treatment [1;32]. IVDMD affected by degradation rate of particle and gas production. On the other value of *in vitro,* organic matter degradability (IVOMD) for treated and untreated ammoniation were 86.72 and 89.09%. Contrast results with IVDMD, untreated ammoniation has higher IVOMD than treated. It might be caused by existence rumen microbes that can not utilize organic matter on a regular basis maximum for growth, lack of time fermentation of soluble carbohydrates, lack of nitrogen [35; 39].

Microbial cell protein (MCP) was not influenced by ammoniation and level of fertilizer treatment ($P>0.05$), while NH_3 had a tendency ($P\sim0.11$) affected by ammoniation treatments, but not influenced by the level of fertilizer treatment ($P>0.05$). Ammonia value of all treatment ranged between 78-111 mg/L. Ammonia concentration that can support microorganisms in synthesizing microbial proteins is between 6 - 21 mM or 85 - 300 mg / l [40]. Ammonia is one of the fermentation products in the rumen derived from

proteins degradation that will used by rumen microbes for its growth [39]. The rumen microbe will be utilizing ammonia as nitrogen source used in protein synthesis. Increased synthesis of microbial proteins shows that there is the growth of rumen bacteria in utilizing a substrate. This result is proportional to the production of ammonia of each treatment. Synthesis of microbial proteins associated with ammonia concentration, where ammonia is a source of nitrogen which is used for protein synthesis. Value low ammonia indicates utilization of ammonia as the source of nitrogen for microbial growth rumen [39]. A lot of nutrient supply also provides more substrate for rumen microbes to grow and to yield more microbial protein from their biomass [41], [42], which also observed in this study.

A/P ratio, NGR, Total VFA, Acetate, Propionate, and Butyrate were not influenced by the level of fertilizer and ammoniation treatment ($P>0.05$) (Table 4). The resulting gas production shows the fermentation process of feed by rumen microbes, which hydrolyzes carbohydrates into monosaccharides and disaccharides which are further fermented into fatty acids (VFAs) such as acetic acid, propionate and butyrate and methane (CH_4) and CO_2 [40]. Total VFA varied between 135-316 mM per mL of rumen fluid. VFA range required for rumen microbial growth was 80-165.81 mM [43], added by [26] that total VFA production ranged from 202-268 mM, the VFA production was sufficient condition for optimal rumen microbial protein synthesis. Increased VFA concentration correlated with increasing microbial production [44]. An increase in the number of VFA shows quickly or not the feed is degraded by rumen microbes. The composition of VFA at rumen changes with the difference of physical form, the composition of feed, level and feeding frequency, also feed processing. Production of total VFA from non ammoniation higher 7,64% than ammoniation treatment ($P>0.05$). However, the contrast result in NH_3 also rumen microbial protein, ammoniation treatment give higher result than non ammoniation treatment (Table 2). This condition may be because low VFA production is not necessarily bad it could be that VFA will be converted into microbial protein. A/P ratio seemed constant in all treatments with value ± 2. In Table 2, ammoniation treatment using resulted in the ratio of acetate to propionate (A/P) lower than untreated. A/P ratio of ammoniation treatment was 2.04 while the A/P ratio untreated was 2.08. It has been recommended that there is an increased proportion of propionate in the rumen compared to acetate. Urea treatment plays a role in glucose metabolism affected the production of propionate that was glucogenic. This result in line with [45] 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. The proportion of C2: C3: C4 was constant about 57:28:15 for all treatments. Based on the ruminal fermentation stoichiometry, individual VFA proportion consisting of C2, C3, and C4 were 55-62%, 21-33% and 8-11% respectively [45,46]. Urea treatment could improve the nutritive value of cocoa pod; it will reduce fiber content and increase the protein content of the cocoa pod [20]. Production of VFA mostly depends on the fermentation of the carbohydrate feed, a fraction of the protein [47], [48].

4. Conclusion

In vitro degradability indicated nutrient utilization in the rumen which attributed by gas production kinetics parameters, NH3, rumen microbial protein and VFA. In this study sorghum stover using basic fertilizer 90 kg/ha with ammoniation treatment has the best degradability on rumen fermentation characteristics *in vitro* of sorghum stover

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