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Characteristic of different level of fermented concentrate in the rumen metabolism based on *in vitro*

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ABSTRAK

Kecernaan merupakan gambaran dari kemampuan bahan pakan yang dapat digunakan oleh ternak. Semakin tinggi suatu kecernaan, semakin banyak bahan pakan yang dapat dicerna di dalam saluran pencernaan. Penelitian ini bertujuan untuk mendapatkan proporsi penggunaan konsentrat terfermentasi pada fermentasi rumen secara in vitro. Pakan yang digunakan terdiri dari tujuh perlakuan sebagai berikut : P1 (20% *Pennisetum purpureum* : 80% konsentrat), P2 (30% *P.purpureum* : 70% konsentrat), P3 (40% *P.purpureum* : 60% konsentrat), P4 (50% *P.purpureum* : 50% konsentrat), P5 (20% konsentrat: 80% *P.purpureum*), P6 (30% konsentrat: 70% *P.purpureum*) dan P7 (40% konsentrat : 60% *P.purpureum*). Kecernaan bahan kering (KcBK) dan kecernaan bahan organik (KcBO) dilakukan menggunakan metode Theodorou. Produksi gas diukur pada saat sampel diinkubasi pada suhu 39°C di dalam penangas air pada jam ke- 2, 4, 6, 8,10, 12, 24, dan 48. Sebanyak 0,75 g formulasi pakan dicampurkan dengan 75 mL cairan buffer rumen. Cairan rumen diambil dari 3 sapi fistula sebelum pemberian pakan di pagi hari. Rancangan acak lengkap dengan tiga kali ulangan digunakan. Produksi dan kinetika gas diestimasi menggunakan model Orskov. Hasil penelitian menunjukkan bahwa pada perlakuan P2 dan P3 menunjukkan kecernaan tertinggi dibanding perlakuan lainnya dengan KcBK masing-masing sebesar 71,63% ; 71,06% dan KcBO masing-masing sebesar 76,42% ; 71.65%. Disimpulkan bahwa perlakuan P2 dan P3 dapat digunakan sebagai pakan untuk sapi potong.

Kata kunci : sapi; fermentasi rumen, konsentrat terfermentasi, kecernaan, in vitro.

ABSTRACT

Digestibility is a description of the ability of feed material that can be utilized by livestock. Higher digestibility of feed material means the total amount of feed content that can be digested in the digestive tract. This study aimed to obtain the proportion of the use of fermentation concentrate in rumen fermentation based on in vitro. The feed used consisted of 7 treatments as follows P1 (20% *Pennisetum purpureum*: 80% concentrate), P2 (30% *P.purpureum* : 70% concentrate), P3 (40% *P.purpureum* : 60% concentrate), P4 (50% *P.purpureum* : 50% concentrate), P5 (20% concentrate: 80% *P.purpureum*), P6 (30% concentrate: 70% *P.purpureum*) and P7 (40% concentrate: 60% *P.purpureum*). Dry matter digestibility (DMD) and organic matter digestibility (OMD) were done using Theodorou method. Gas production was measured when the samples were incubated at 39°C in the waterbath incubator at hours 2, 4, 6, 8, 10, 12, 24, and 48. Approximately 0.75 g of feed formulation was mixed with 75 mL of rumen fluid buffer. The rumen fluid is taken from 3 fistulated cattles prior to morning feeding. A complete randomized design with three replicates was used. Asymptotic gas production and

kinetics were estimated by the Orskov's model. The results of this study showed that P2 and P3 treatment showed the best digestibility among others treatment with 71.63% ; 71.06% of DMD and 76.42% ; 71.65% of OMD. In conclusion, P2 and P3 treatment can be used as feed for beef cattle.
Keywords: cattle, rumen fermentation, femented concentrate, digestibility, in vitro

INTRODUCTION

The improper storage technique of concentrate for a long time period and in humid conditions can trigger contamination of concentrate by microorganisms causing rancid odor (Canibe and Jensen, 2012). This may affect the nutritional quality of the concentrate as well as the palatability of feed. Therefore, to reduce the contamination of the concentrate by microorganisms, it can be processed through fermentation. The fermentation technique can inhibit the growth of microbial pathogens and also can improve the quality of nutrient concentrates (Lee and Mau., 2008; Cao *et al*., 2012). In addition, the process of fermentation increases the vitamins, enzymes, and growth factors of fermented products (Ng *et al*., 2011; Dei *et al.,* 2008).

The use of microbial inoculants to ferment concentrate have been commonly studied in livestock, especially in pigs and poultry. Nevertheless, there have been limited studies of the use of microbial inoculant to ferment concentrate in ruminant. In addition, there have been few studies about the use of lactic acid bacteria in fermented concentrate could increase the availability of phosphorus in cereal based-diets, able to reduce IP6 (Reale *et al*., 2004). The effects of fermentation on feed depend on the activity and type of microbial used (Niba *et al*., 2009).

Many researchers reported the beneficial of the use of microbial inoculant in fermented concentrate. As reported by Niba *et al* (2008) that the use of lactic acid bacteria can increase the concentration of lactic acid. Ahmed *et al* (2016) also reported that fermented feed contains large number of Lactobacilli with high concentrations of lactic acid and other volatile fatty acids and has a low pH. Moreover, Kobashi *et al* (2008) reported that the proportion of chlortetracycline-resistant *Escherichia coli* strains was significantly reduced in the gut of weaned piglets compared with dry feed. Fermented feed may provide more rich nutrition, better palatability, and increased digestibility so the needs of cattle protein could be met and could improve the performance of beef cattle (Niba *et al*., 2009; Humer *et al*., 2013). However, further research is needed to determine optimal proportion of fermented concentrate. The objective of this study was to characterize the effects of different level of fermented concentrate and elephant grass on the rumen fermentation in vitro.

MATERIALS AND METHODS

Sample preparation

Concentrate was made from the following formulation : 22% of rice bran, 5% of maize flour, 5% of coffee pulp, 10% of corn gluten feed, 6% of cassava flour waste, 9% of coconut meal, 18% of palm kernel cake, 18% of pollard brand, 6% of soy bean meal, and 1% of mineral mix. The formulation concentrate was fermented by adding 2% of *L. plantarum (*2.5×10^6 cfu/mL) and kept for 24 hours incubation. *Lactobacillus plantarum* TSD-10 was from the laboratory of Applied Microbiology, Research Center for Biotechnology. *Pennisetum purpureum* were freshly collected from Agrostology Laboratory Field, Research Center for Biotechnology, LIPI. These materials were oven-dried at 60^0C for 24 h, milled using a grinder to pass a 1 mm sieve.

Chemical and pH analysis

Samples of *P.purpureum* and fermented concentrate were analyzed for dry matter (DM) and ash according to AOAC (2005). Crude protein (CP), crude fiber (CF), ether extract (EE) were determined followed the manufacture procedure of FOSS. The NH_3-N concentration was determined by a Conway micro-diffusion method. Volatile Fatty Acids (VFA) were determined in centrifuged samples (1mL) by Gas Chromatography (GC) as described by Goering and Van Soest (1970). pH measurement of mix buffer rumen fluids were measured using pH meter (Jenway Model 3505, UK). Prior to the measurements, pH meter was calibrated by using pH 7 buffer solution and it was prepared carefully as the manufactures instructions. All chemical composition analyses were conducted in triplicate.

Table 1. Chemical Composition (g/kg DM)

Item[a]	(%) DM	(%) CP	(%) CF	(%) EE	pH
Pennisetum purpureum	97.40	9.04	29.72	1.53	
Concentrate	89.37	13.70	21.14	1.85	6.24
Fermented Concentrate	89.96	14.82	18.13	1.70	4.47

DM= dry matter; CP= crude protein; CF= crude fiber; EE= ether extract ; [a]Data are the means of three replicate analysis.

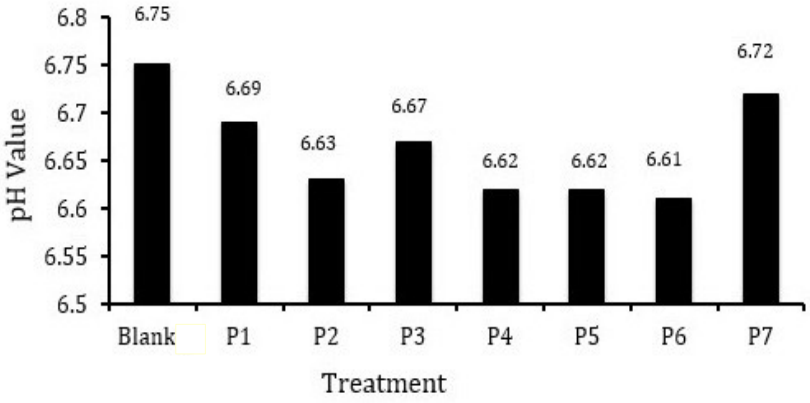

Figure 1. pH value of fermented concentrate. P1 = 20% *Pennisetum purpureum*: 80% concentrate; P2 = 30% *P.purpureum*: 70% concentrate ; P3 = 40% *P.purpureum* : 60% concentrate ; P4 = 50% *P.purpureum*: 50% concentrate ; P5 = 20% concentrate: 80% *P.purpureum*; P6 = 30% concentrate: 70% *P.purpureum* ; P7 = 40% concentrate: 60% *P.purpureum*

***In vitro* Incubation and Gas Production**

Samples of *P.purpureum* and fermented concentrate were arranged and mixed according to the following experimental treatments (dry matter basis). These treatments were subjected to *in vitro* analysis according to Theodorou *et al.* (1994):

P1 : 20% *P. purpureum* : 80% concentrate feeds
P2 : 30% *P. purpureum* : 70% concentrate feeds
P3 : 40% *P. purpureum* : 60% concentrate feeds
P4 : 50% *P. purpureum* : 50% concentrate feeds
P5 : 80% *P. purpureum* : 20% concentrate feeds
P6 : 70% *P. purpureum* : 30% concentrate feeds
P7 : 60% *P. purpureum* : 40% concentrate feeds

Rumen contents were obtained from three fistulated cattles, then was mixed and strained through four layers of cheesecloth into an erlenmeyer flask with headspace. The use of the cattle in this experiment was approved No. 9879/WK/HK/XI/2015 by the Ethic Clearance Committee of Indonesian Institute of Sciences. Approximately 0.75 g of substrates were put inside the serum bottle glass and filled with 73 mL mixture of 24.33 mL rumen fluid and 48.67 mL of Mc'Dougall buffer ($NaHCO_3$ 0.98 g, $Na_2HPO_4.7H_2O$ 0.7 g, KCl 0.057 g, NaCl 0.0472 g, $MgSO_4.7H_2O$ 0.012 g, CaCl 0.004 g, and H_2O up to 100 mL). Anaerobic condition in the bottles was achieved by filling CO_2 gas until saturated. Serum bottles were sealed with butyl rubber stoppers and aluminum crimp seals shortly before starting the incubation. The bottles were placed in a water bath at 39ºC for 48 h. After 2, 4, 6, 8, 10, 12, 24 and 48 h of incubation, gas production from each serum bottle was vented and recorded by using disposable syringe.

In vitro gas production was analysed in seven different treatments of P1, P2, P3. P4, P5, P6, and P7 in three replicates. In addition, three blank (no sample; rumen fluid + buffer mixture) were used to calculate the total gas production. Gas production kinetics were estimated by following Ørskov method (Ørskov and McDonald, 1979).

Table 2. Gas Production Kinetics during 48 Hours of Incubation

Time (Hr)	Treatment (mL)							
	Blank	P1	P2	P3	P4	P5	P6	P7
2	0 ± 0	8.5±1.5^{ab}	5.2±2.3^{a}	6.2±3.6^{a}	8.8±2.85^{ab}	20.0 ± 6.6^{c}	20.50±0.5^{c}	13.8 ± 5.4^{bc}
4	0 ± 0	18.7±2.8^{ab}	12.3±3.55^{a}	14.0±7.2^{a}	19.5±5.0^{ab}	37.3 ±10.3^{c}	38.2± 0.8^{c}	28.3 ±9.8^{bc}
6	1.2±1.0	35.5±4.3^{ab}	22.3±3.9^{a}	25.3±12.0^{a}	34.6 ±7.3^{ab}	60.0 ±13.3^{c}	62.0±03.9^{c}	45.8 ±14.6^{bc}
8	2.7±4.5	53.5±3.8^{ab}	34.0±3.9^{a}	37.7±14.7^{a}	50.7±7.9^{ab}	78.5±14.5^{c}	79.8±4.0c	61.7 ± 16.4^{bc}
10	7.3±6.8	79.3±3.8^{c}	51.7±3.6^{a}	55.3±17.5^{ab}	74.2±865^{bc}	101.3±13.8^{d}	101.7±3.7^{d}	8.02 ±17.8^{cd}
12	14.3±7.4	95.3±2.8^{b}	65.7±3.3^{a}	70.0±17.7^{a}	90.8±8.8^{b}	117.7±14.4^{c}	118 ± 3.9c	97.8 ±18.8^{bc}
24	27.5±6.1	137.8±3.3^{b}	97.5±3.6^{a}	110.5 ±23.4^{a}	143.2±15.5^{b}	173.2±13.3^{c}	175.0±5.7^{c}	154.8±19.69^{bc}
48	37.5±6.1	165.0 ±3.8^{bc}	119.5±3.6^{a}	141.5±23.4^{ab}	175.7±15.9^{bc}	212.8±17.9^{e}	216.3±7.4^{e}	194.5±21.3^{de}

P1 = 20% *Pennisetum purpureum*: 80% concentrate; P2 = 30% *P.purpureum*: 70% concentrate; P3 = 40% *P.purpureum* : 60% concentrate; P4 = 50% *P.purpureum*: 50% concentrate; P5 = 20% concentrate: 80% *P.purpureum*; P6 = 30% concentrate: 70% *P.purpureum*; P7 = 40% concentrate: 60% *P.purpureum*; a-e Different superscripts within the same column are significantly different at P<0.05.

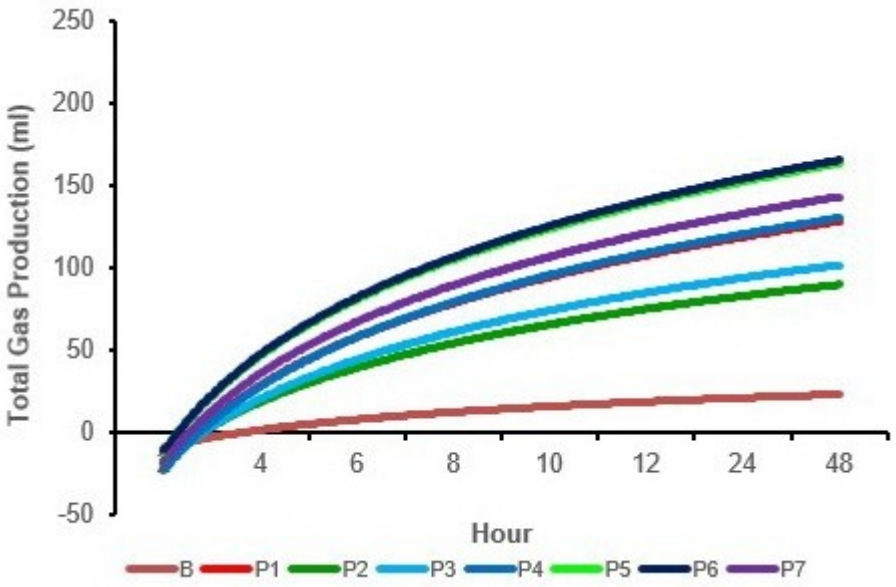

Figure 2. Total Gas Production during 48 Hours of Incubation. P1 = 20% *Pennisetum purpureum*: 80% concentrate; P2 = 30% *P.purpureum*: 70% concentrate; P3 = 40% *P.purpureum* : 60% concentrate; P4 = 50% *P.purpureum*: 50% concentrate; P5 = 20% concentrate: 80% *P.purpureum*; P6 = 30% concentrate: 70% *P.purpureum*; P7 = 40% concentrate: 60% *P.purpureum*

Microbiology analysis

Total lactic acid bacteria and rumen bacteria were counted as colony forming units (CFU) per mL. The lactic acid bacteria were grown on MRS agar medium and counted by using total plate count (Cappucino, 2001), while the rumen bacteria on 98-5 agar medium were grown anaerobically by following the method of roll tube (Hungate, 1969). Incubation was done at 38^{0}C for 3-4 days. Aliquots of fresh filtrate (1 mL) were added to 1 mL of protozoal staining solution (MFS solution, Ogimoto and Imai, 1987). Ciliate

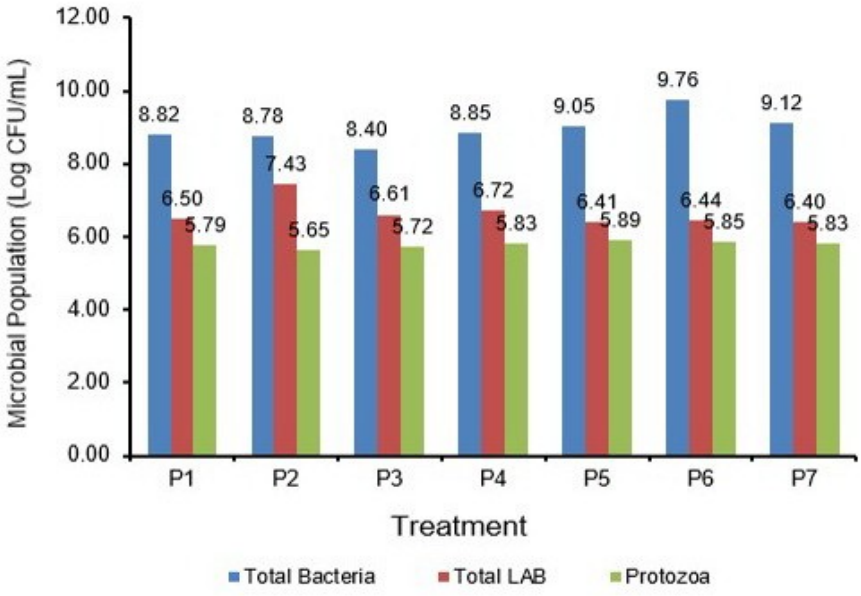

Figure 3. The Comparison among Total Bacteria, Total Lactic Acid Bacteria and Protozoa in Each Treatment . P1 = 20% *Pennisetum purpureum*: 80% concentrate; P2 = 30% *P.purpureum*: 70% concentrate; P3 = 40% *P.purpureum* : 60% concentrate; P4 = 50% *P.purpureum*: 50% concentrate; P5 = 20% concentrate: 80% *P.purpureum*; P6 = 30% concentrate: 70% *P.purpureum*; P7 = 40% concentrate: 60% *P.purpureum*

protozoa were counted as described by Veira *et al*. (1983) using a Fuchs-Rosenthal counting chamber. Duplicate preparations of each sample were counted, and if either value differed from the average by more than 5%, the counts were repeated. After being incubated for 24 hours, protozoa can be observed by using microscope at 200x magnification

Statistical analysis

The experiment design was a block randomized. The statistical analysis of data was performed using SPSS 16 for Windows. The difference among groups was determined by oneway ANOVA analysis. The Duncan's Multiple Range Test, one of the multiple comparison tests, was used when the difference among groups was found to be significant. Significance was defined at $P<0.05$. Mocrobial diversity data were analyzed descriptively.

RESULTS AND DISCUSSIONS

Chemical and pH analysis

Chemical analysis of feed material is very important to evaluate the nutrient content of feed. Proximate analysis is one of parameter to determine the quality of feed material. The nutrition composition of feed material that used in this study is shown in Table 1.

The pH rumen indicate rumen conditions that are appropriate or inappropriate for the growth of rumen microbes. This study showed that the use of different levels of fermented concentrate did not affect the pH of rumen (Figure 1). The same pH values as control showed that the use of different levels of fermented concentrate does not interfere rumen fermentation activity. According to Dehority (2004), the rumen's normal pH is about 5.5-7.0. The pH value actually represents the amount of acid produced by microbial activity in the rumen (Elghandour *et al*., 2015; Hutton *et al*., 2012). The lowest pH value is generally achieved about 2-6 hours after meals, in accordance with the maximum acid production. Changes in pH are affected by time after meal, lactic acid supply, feed properties, and feeding frequency of animal. (Dehority, 2004; Seo *et al*. 2010).

***In vitro* Incubation and Gas Production**

Total gas production for 48 hours incubation is presented in Table 2. The gas production comes from direct substrate fermentation (CO_2 and CH_4)

Table 3. Production of VFA and NH_3 during 48 h Incubation

Variable	Treatment						
	P1	P2	P3	P4	P5	P6	P7
C_2	44.51^{a}	41.38^{a}	42.94^{a}	43.86^{a}	39.48^{a}	43.65^{a}	47.43^{b}
C_3	30.17^{b}	31.40^{b}	31.89^{b}	27.84	21.57	23.99	24.46
C_4	13.45^{a}	14.71^{a}	13.73^{a}	16.28^{ab}	19.62^{ab}	16.64^{ab}	12.73^{a}
Iso-C_4	5.26^{a}	7.08^{a}	5.56^{a}	5.87^{a}	13.11^{ab}	9.33^{a}	7.95^{a}
C_5	2.5^{a}	1.27^{a}	1.59^{a}	1.82^{a}	1.53^{a}	2.16^{a}	3.26^{ab}
Iso-C_5	4.11^{a}	4.16^{a}	4.29^{a}	4.33^{a}	4.69^{a}	4.23^{a}	4.17^{a}
C_2/C_3	1.47^{a}	1.31^{a}	1.35^{a}	1.58^{a}	1.83^{a}	1.82^{a}	1.94^{a}
NH_3	6.09^{bc}	6.59^{c}	6.39^{c}	6.94^{c}	4.98^{b}	5.00^{b}	4.80^{b}

P1 = 20% *Pennisetum purpureum*: 80% concentrate; P2 = 30% *P.purpureum*: 70% concentrate; P3 = 40% *P.purpureum* : 60% concentrate; P4 = 50% *P.purpureum*: 50% concentrate; P5 = 20% concentrate: 80% *P.purpureum*; P6 = 30% concentrate: 70% *P.purpureum*; P7 = 40% concentrate: 60% *P.purpureum*; a-eDifferent superscripts within the same column are significantly different at $P<0.05$.

and indirect gas production (CO_2) reactions from organic acids with bicarbonate buffers (Goel *et al*., 2008; McDonald *et al*. 2010; Hook *et al*., 2010). In addition, according to McDonald *et al*. (2010) gas composition in the rumen is consist of 40% carbon dioxide (CO_2), 30-40% methane (CH_4), 5% hydrogen, and a small portion of oxygen and nitrogen. Based on 24 hours incubation presented in Table 2, it was showed that the average of gas production was over 75% of the total gas during 48 hours incubation, this result is coherent with Jayanegara *et al*. (2009), which stated that more than 75% of the gas is generated after 24 hours of incubation period. Differences in gas production between treatments can be caused by rumen fluids, incubation conditions, and also substrates. Table 2 showed that the rate of gas production of each treatment decreased every hour in accordance with the reduced amount of substrate that can be fermented during the incubation period. Therefore, the rate of gas production will demonstrate the time required for rumen microorganisms to digest the entire substrate.

Figure 2 showed that the gas was still being produced until 48 hours of incubation time. This means that the tested substrate remained until the 48 hours of incubation. Consequently, the zero point of gas production could not be known. It takes up to 96 hours to obtain an accurate kinetic coefficient (Jayanegara *et al*., 2009) because incubation for 96 hours the substrate will be exhausted. If the substrate has been fermented out, then the zero point of the gas production rate will be known and the shape of the graph will be constant by forming horizontally as in the blank (Figure 2).

Microbiology Analysis

In this study, it was showed in the Figure 3 that the treatment of P1, P2, P3, and P4 had higher amounts of lactic acid bacteria than other treatments. In contrast, the total number of bacteria on treatment P1, P2, P3, and P4 was decreased. We assumed that *L.plantarum* TSD 10 has an antimicrobial ability that can inhibit the presence of pathogenic bacteria in the rumen thus affecting the reduction of total bacteria in the rumen. Fermentation with with *L. plantarum* might prevent harmful microbe in the digestive tract and increase the immune enhancing activity of fermented products (Rizzello *et al*., 2013; Ashraf and Shah., 2014; Fu *et al.*, 2014; Li *et al.*, 2012).

In addition, concentrate which is fermented by lactic acid bacteria not only as a preservation, but also as probiotic , therefore animals can obtain both nutrients and the probiotics from fermented feed (Sánchez *et al*., 2015; Hu *et al*., 2015). Ellis *et al.* (2016) found that the viable cells of lactic

Table 4. Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) *In vitro*

Treatment	DMD (%)	OMD (%)
P1	65.80 ± 1.32^{cd}	66.48 ± 1.53^{b}
P2	71.63 ± 2.73^{d}	76.42 ± 2.39^{c}
P3	71.06 ± 3.69^{d}	71.65 ± 3.93^{c}
P4	60.36 ± 2.94^{bc}	60.76 ± 4.52^{b}
P5	50.89 ± 3.49^{a}	59.28 ± 1.75^{b}
P6	55.07 ± 5.81^{ab}	45.49 ± 5.75^{a}
P7	50.03 ± 1.59^{a}	46.93 ± 4.54^{a}

P1 = 20% *Pennisetum purpureum*: 80% concentrate; P2 = 30% *P.purpureum*: 70% concentrate; P3 = 40% *P.purpureum* : 60% concentrate; P4 = 50% *P.purpureum*: 50% concentrate; P5 = 20% concentrate: 80% *P.purpureum*; P6 = 30% concentrate: 70% *P.purpureum*; P7 = 40% concentrate: 60% *P.purpureum*; a-eDifferent superscripts within the same column are significantly different at P<0.05.

acid bacteria in silage can play a role as probiotic in the rumen during fermentation process. Sari *et al.* (2017) also reported that lactic acid bacteria has an important role for producing lactic acid, which results in quicker drop of pH. This finding suggested that *L. plantarum* population in fermented concentrate was sufficiently established during fermentation process and able to utilize the high concentration of NH_3-N. The NH_3-N in the fermented concentrate was used as a source of N for microbial protein synthesis and for microbial growth (Calsamiglia *et al*., 2010; Belanche *et al*., 2012b).

The population of ciliated protozoa is more dominant in the rumen, while the population of flagellated protozoa is minor (Dehority, 2004). In this study the protozoa population presented in Figure 3 in the treatment of P4, P5, P6, and P7 were higher among other treatments. This is because the presence of protozoa naturally develops in the rumen to assist digestion of nutrients from forage crops containing high crude fiber (Noziere *et al*., 2011). However, the impact of protozoa on the rumen digestion depends on their concentration and the generic composition of their population (Belanche *et al*., 2012a).

NH_3 and VFA Production

Ammonia is an important component for the synthesis of amino acids and microbial cell proteins. Protein from feed will be degraded by proteolytic bacteria with the help of protease enzyme into peptide then hydrolyzed to amino acid, and amino acid will go through deamination process to become ammonia. McDonald *et al*. (2010) states that ammonia is produced in conjunction with several small peptides and free amino acids, then it is utilized by rumen organisms for microbial protein synthesis. In addition, some of the amino acids will be further digested into organic acids, ammonia (NH_3), and carbon dioxide (CO_2).

The results of the average concentration of NH_3 are presented in Table 3. This study showed that proportion of fermented concentrate can increase the production of NH_3 in the treatment of P2, P3 and P4, while in the treatment of P5, P6, and P7 the amount of NH_3 decreases. This is due to the tendency of increasing bacterial population in the treatment of P5, P6, and P7 (Figure 3) will reduce the value of NH_3 concentration due to NH_3 is an important source of N for rumen microbes for the microbial synthesis process.

Production of VFA after incubation for 48 hours is presented in Table 3. The use of feed which is dominated by fermented concentrate such as P1, P2, and P3 had significant effect (P<0.05) on propionic acid formation. In contrast, the use of feed which is dominated by forage on treatment P7 had significant effect (P <0.05) on acetate production.

C_2/C_3 ratio shows a pattern of microbial fermentation in the rumen that has the potential to form acetic acid or propionate. The lowest C_2/C_3 ratio is produced by P2 as shown in Table 3, an be explained that in this treatment has a higher fermentable organic content than others with 76.42% of IVOMD (Table. 4). The low of C_2/C_3 ratio is due to the high amount of fermentable organic matter enabling the formation of higher propionic acid than acetic acid. As reported by Moran (2005) that the fermentative digestion of carbohydrates especially starch will produce lactic acid, this lactic acid will be converted to propionic acid by microbial lactate utilization, such as *Propionibacteria sp, Veillonella alkalescens and Peptostreptococcus elsdeini.* The low of C_2/C_3 ratio will stimulate fattening and tend to body fat formation. In the treatment of P5, P6, and P7 showed higher C_2/C_3 ratio values than other treatments, this indicates that the microbial

fermentation pattern leads to the formation of acetic acid because the carbohydrates contained in the feed are still dominated by fibrous carbohydrates and microbes that grow well are cellulolytic and hemicelulolytics microbes.

Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD)

The results of DMD and OMD during 48 hours incubation for all treatments are presented in Table 4. Dry matter digestibility value is one of indication for feed quality, the higher digestibility value, the more nutrition will be absorbed by the body.

Based on DMD value it was found that P2 and P3 resulted the highest percentage of DMD with 71.63 and 71.06 respectively. It also has a similarity in OMD pattern, that P2 and P3 resulted the highest percentage of OMD with 76.42 dan 71.65 respectively. This study found that digestibility is affected by the presence of protozoa. The lower protozoa population, the higher digestibility value will be generated. This is because protozoa will consume fibrolytic bacteria so that the population will be less and will affect the process of feed degradation in the rumen. This is evidenced by the number of protozoa in the treatment of P2 and P3 is less than other treatments (Figure 3). In Table 4. It is showed that the increase of DMD value will increase the OMD value. This is in accordance with as reported by Muhtarudin and Liman (2006), that the dry matter digestibility will be in line with the organic matter digestibility value. Digestibility can also be affected by components in the feed. As reported by De Boever *et al.* (2005) that the structural components of plant feed material such as cellulose, lignin, cell wall, NDF and ADF will reduce the nutrient digestibility of feed, whereas soluble carbohydrates (starch) and crude protein can increase the nutrient digestibility.

CONCLUSION

Based on this study it could be concluded that fermented concentrate with the range proportion from 60 -70% (P2 and P3) can be used for feed, particularly in beef cattle. In addition, characteristic of fermented concentrate can improve the digestibility of feed in the rumen metabolism.

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