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Volatile Fatty Acids and Methane Profile of Dairy Cattle Ruminal Fluid was Gived Legumes in Ration Based on Synchronization Protein-Energy Index

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ABSTRACT

An experiment was aimed to assess the use of the legumes as a source of protein feedstuff and levels of protein-energy synchronization (PES) index in the diet of dairy cattles on volatile fatty acids (VFA) and methane (CH_4) profile. The research was applied in In vitro techniques used a completely randomized design (CRD), with factorially pattern (2x3), the first factor was the two species of legumes (Sesbania and Leucaena) and the second factor was the three level of the PES index (0.4, 0.5, and 0.6), there were 6 treatment combinations and each was 4 replicates. The results showed that interaction between legumes with PES index was not significantly affected ($P>0.05$) on all variable. Legumes was not significantly affected ($P>0.05$) on all variables and PES index was significantly affected ($P<0.05$) on propionate, A:P ratio, and methane. The study concluded that the use of turi and lamtoro leaves combined with the protein-energy synchronization index (PES) at medium level (0.5) could increasing the production of VFA, especially propionate, suppressing A:P ratio and decreasing methane production.

Key words: Legumes, Methane, Protein-energy synchronization index, Volatile fatty acids

Introduction

Dairy cattle ration consists of forages and concentrates, with a proportion of forage higher than concentrate. Common forages are from grass, such as elephant grass and king grass. The general characteristics of forages are containing high moisture and fiber content, but with low protein levels. Forage is an alternative fiber, namely leaves from the legumes group. Legumes is a proteinceus roughages which is a high protein fiber feed ingredient. Legumes protein levels reached 22% (Kushartono and Iriani, 2004), it can be used as a basis for growth that is only given in the dry season.

Fulfillment of protein requirements in ruminants is not only focused on the quantity in the ration, but must have degradability that is in accordance with the energy source used. Protein and energy must be available simultaneously (synchronously) for the development of optimum rumen microorganisms, thus kinetic degradation of proteins must be in accordance with carbohydrate degradation. The balance of degradation can be achieved through the preparation of rations based on protein and energy synchronization (PES) index. The ration based on the PES index is determined as a ration that has a level of harmoniously degradation of protein and carbohydrates, expressed quantitatively with an optimum index, namely 1 (Ginting, 2005).

The synchronization ratio of protein and carbohydrate degradation for local feed ingredients in the rumen is 20 g N / kg OM, meaning that to achieve the optimum PES index (1) it takes 20 g N in each kg of OM feed. This ratio is the standard used in determining the PES index in the rumen, thus the percent use of a feed ingredient in the ration must be adjusted to its index value to achieve a standard ratio or reach the optimum synchronization index (Hermon *et al.*, 2008). Legumes is a feed ingredient with a low synchronization index, because of the protein matrix bond with the tannin. Legumes commonly used as animal feed are *Leucaena glauca* and *Sesbania grandiflora*, each of which has an PES index of 0.31 and 0.34 (Syamsi *et al.*, 2015).

The ration with a good synchronization index will increase the efficiency of microbial protein synthesis (MPS) and will maximize feed utilization in the rumen fermentation process. The simultaneous use of protein and energy will increase volatile fatty acids (VFA) production and reduce methane production. The aim of the study was to examine the use of legumes as feed ingredients for protein sources and PES index levels in dairy cattle rations against the profile of VFA and methan through in vitro tests.

Materials and Methods

Experiment design

Experimental studies were conducted to measure VFA and pH of dairy rumen fluid in vitro (Tilley and Terry, 1963). The material used was the rumen fluid of the Friesian Holstein Grade Bull taken from the Mersi slaughterhouse, Purwokerto, immediately after the bull was slaughthered. The experimental design used was a completely randomized design (CRD) with a factorial pattern (2x3), factor A was legumes (turi and lamtoro), factor B was the PES index (0.4; 0.5; and 0.6). There were 6 combination of treatments and each was repeated 4 times. The combination of treatments that will be tested was compiled based on Syamsi *et al.* (2015) and can be illustrated in Table 1.

Measure protein-energy synchronization index (PES) of feed ingredients

PES index of feed ingredients based on Syamsi *et al.* (2015) which was determined by measuring protein and organic matter (OM) degradation of feed ingredients through in vitro modified by the Ørskov and Mcdonald (1979) method. The time interval used for concentrates was 2, 4, 6, 8, 12, 24 and 48 hours, while for forages was 2, 4, 6, 8, 12, 24, 48, and 72 hours. The degradation rate of protein and OM at each time interval was then analyzed in regression to obtain the degradation rate of gram N and kilogram OM per hour. After that, it was used to calculate the PES index of each ingredient which was then used as the basis for the preparation of feed treatment rations with the following equation.

$$\text{Synchronization Index} = \frac{20 - \sum_{1-24}^{n} \frac{\sqrt{\left(20 - \frac{N}{OM}\, per\ hour\right)^2}}{24}}{20}$$

Note : n : observation time, N / OM per hour: rate of protein degradation compared to the rate of degradation of organic matter every hour (Hermon *et al.*, 2008).

Analysis of variable

The total content of VFA of rumen fluid was analyzed according to the steam distillation procedure (General Laboratory Procedure, 1966). The partial VFA levels (acetate, propionate, butyrate, valerate, iso butyrate, iso valerate) rumen fluid was measured using the Goering and Van Soest (1970) method known as gas chromatography. The rumen liquid was taken using a 4 ml pipette then centrifuged at 10,000 rpm for 15 minutes. Supernatant was added as much as 2 ml into a small 5 ml plastic tube and added 30 mg 5-sulphosalicylic acid, then shaken and centrifuged at 3,000 rpm for 10 minutes at 4^0C then filtered with milpore until it obtained clear liquid. As much as 1 µl of clear liquid was injected into gas chromatography, previously injected with standard VFA solution (acetate 52.54% molar, propionate 13.42% molar, iso butyrate 5.40% molar, n-butyrate 10.89% molar, iso valerate 4.23% molar, n-valerate 4.61% molar). The concentration in the sample was calculated by using the formula, individual VFA = ((sample height / standard height) x standard concentration, the sum of all partial VFA expressed as the total VFA.

Methane (CH_4) gas levels were not measured directly through laboratory tests, but were calculated based on the proportion of partial VFA that has been obtained. CH_4 gas levels were calculated by the mM formula developed by Ørskov and Ryle (1990), namely CH4 = 0.5 a - 0.25 p + 0.5 b (a = acetate, p = propionate, and b = butyrate).

Analysis of data

Data were analyzed using variance analysis (ANOVA). If the interaction of legumes and PES index had a significant influence on the measured variables, only interactions were further tested with honestly significant difference (HSD) and polynomial orthogonal tests. If the

Table 1. The arrangement of rations treatment

Feedstuff	R1	R2	R3	R4	R5	R6
	.... %					
King grass	35	50	54	35	50	54
Daun turi (*Sesbania grandiflora*)	**10**	**10**	**10**	0	0	0
Daun lamtoro (*Leucaena l.)*	0	0	0	**10**	**10**	**10**
Cassava dregs	3	6	15	3	6	15
Rice bran	26	14	1	26	14	1
Pollard	8	5	3	8	5	3
Soybean curd dregs	14	9	3	14	9	3
Coconut meal	3	5	13	3	5	13
Mineral	1	1	1	1	1	1
Total	100	100	100	100	100	100
Syincronization index	**0,4**	**0,5**	**0,6**	**0,4**	**0,5**	**0,6**
	Nutrient					
Dry matter (%)	92.61	93.30	92.75	92.58	93.27	92.71
Ash (% BK (DM)	7.40	8.44	9.08	7.40	8.44	9.08
Crude protein (% BK (DM)	12.29	12.24	12.53	12.25	12.19	12.49
Crude fat (% BK (DM)	5.31	5.35	5.97	5.49	5.54	6.16
Crude fiber (% BK (DM)	21.21	23.18	23.13	21.17	23.14	23.08
Nitrogen free extract (% BK (DM)	53.79	50.79	49.29	53.69	50.69	49.19
TDN (%)	68.02	66.39	67.16	68.23	66.59	67.37

* The arrangement of rations and level of nutrient based on Syamsi *et al.* (2015).

interaction of legumes and PES index did not significantly influence the measured variables, then each factor (legumes and PES index) would be tested individually. If factor A (legumes) had a significant influence on the measured variable, then it would be further tested by the HSD test. If the PES index had a significant influence on the measured variable, then it would be tested further with the polynomial orthogonal test.

Result and Discussion

Volatile fatty acids (VFA) total

Volatile fatty acids (VFA) is the main energy source for ruminants. The product is mostly produced from the fermentation process of carbohydrates and a small portion is produced from the process of deamination of amino acids into ammonia. The high and low levels of VFA in the rumen fluid depend on the type of feed ingredients used and the performance of rumen microorganisms in fermenting the feed. The use of legumes and setting of the synchronization index can influence the level of VFA in the rumen fluid (Seo *et al.*, 2010; Anggraeny *et al.*, 2014). The influence of the treatment of legumes and the PES index in the ration on VFA is presented in Table 2.

The results of the variance analysis showed that the interaction between legumes and the PES index was not significantly affected (P> 0.05) on the total production of rumen fluid VFA. This can be caused due to the occurrence of equilibrium in the supply of energy and protein, thus microbial growth activities and feed fermentation run well on each treatment and also because the percentage of legumes used in each treatment was the same at 10%. Table 2 shows that the total VFA production is in the range of 81.10-85.05 mM rumen fluid. This is in accordance with Khampa *et al.* (2006) that the total VFA concentration in normal rumen fluid is in the range of 70-130 mM rumen fluid.

Putra (2006) explained that rations supplemented with defaunation agents had a tendency to produce VFA production in the normal range. The use of *Leucaena glauca* and *Sesbania grandiflora* indirectly acts as a defaunation agent. *Leucaena glauca* has defaunative ability because it contains tannins about 3.48-6% per g of dry matter (Pamungkas *et al.*, 2008; Soltan *et al.*, 2013), while *Sesbania* has an average tannin content of 3.4% per g of dry matter (Arfan *et al.*, 2016). *Sesbania* also contains another defaunative agent in the form of saponins with an average content about 16.38 mg / ml of *Sesbania* leaf extract (Rachmawati *et al*., 2006).

The role of defaunation agents is to suppress the growth of protozoa in the rumen fluid. Excessive amounts of protozoa will become a predator for bacteria. The mechanism of suppressing the amount of protozoa that occurs then causes the fiber or starch digesting bacteria to develop properly. This process is also supported by setting the PES index in each treatment, thus the availability of protein and energy is balanced and causes the production of VFA to be stable in each treatment.

Volatile fatty acids (VFA) parsial

Volatile fatty acids (VFA) consists of several components of fatty acids. Most VFA compositions in rumen fluids are acetic, propionic and butyric acids, whereas in small amounts they are formic acid, iso butyrate, valerate, iso valerate and iso kaproat. Each fatty acids is produced through a different process and is influenced by various factors. Factors that can influence are the types of feed supplementation such as legumes and synchronization of the availability of protein and energy in the rumen fluid. The influence of legumes and protein-energy synchronization index (PES) treatments in ration on partial VFA is presented in Table 2.

The results of the variance analysis showed that the interaction between legumes and PES index was not significantly affected (P> 0.05) on the levels of acetate and butyrate of rumen fluids. This happened because the use of legumes in rations combined with the PES index caused the fiber content of each treatment to be slightly different (Table 1). Suhartanto *et al.* (2014) stated that the level of acetate in rumen fluid is influenced by the fiber content in the ration. The average yield of acetate in each treatment was 42.39-47.42 mM rumen fluid or 60-63% of the total VFA. The results of this study are accordance with Chiba (2014), that dairy cattle feed with a forages base produces a minimum of 32 mM acetate of rumen fluid or reaches 65% of the total VFA to meet his body's needs. Forages and concentrate ratio in each treatment are different, but the fiber content of all treatments is in the range of above 20% dry matter (DM) ration.

Butyrate is a ketogenic fatty acids and it value is always lower than acetate and propionate. The average butyrate in each treatment was in range of 2-2.7 mM rumen fluid or 1-3% of the total VFA. These results were

Table 2. The average of volatile fatty acids (VFA), A:P ratio and methane each treatment

Variables	*Treatments*					
	R1	R2	R3	R4	R5	R6
VFA total (mM)	85,05±8,91	83,57±2,02	81,10±2,08	82,74±4,65	83,36±5,84	81,56±5,41
Asetat (mM)	47,42±5,70	45,99±0,95	44,91±1,15	46,28±4,27	44,12±4,39	42,39±5,99
Propionat (mM)	24,93±3,16	25,61±1,65	23,48±0,91	24,95±2,38	26,58±3,40	21,71±2,31
Butirat (mM)	2,06±1,13	2,09±1,19	2,70±0,29	2,75±0,40	2,66±0,22	2,46±0,35
A:P rasio (Ratio)	1,90 ± 0,06	1,81 ± 0,12	1,91 ± 0,03	1,81 ± 0,16	1,68 ± 0,23	1,95 ± 0,08
CH4 (mM)	25,10±0,55	24,04±1,38	25,23±0,25	24,28±1,50	22,84±2,49	25,48±0,66

R1 = Turi (*Sesbania*) + Indeks (Index) 0,4, R2 = Turi (*Sesbania*) + Indeks (Index) 0,5, R3 = Turi (*Sesbania*) + Indeks (Index) 0,6, R4 = Lamtoro (*leucaena*)+ indeks (Index) 0,4, R5 = Lamtoro (*leucaena*) + indeks (Index) 0,5, R6 = Lamtoro (*leucaena*) + indeks (Index) 0,6 different from Chiba (2014), that fiber-based feed will produce approximately 12% butyrate and concentrate-based feed will produce approximately 20% butyrate. Mitsumori and Sun (2008) stated that feed with high fiber sources will increase the activity of convertion pyruvate to acetyl coenzyme A (AC-CoA). The pathways for the formation of acetate and butyrate are both derived from AC-CoA, so the formation of acetate and butyrate will show the same graph even though butyrate production has a lower average than acetate. Permana *et al.* (2014) stated that acetate formation has a shorter pathway compared to butyrate. The formation of butyrate begins with the change of AC-CoA to aceto acetate, then β hydroxybutyrate to butyrate, while the formation of acetate is directly coming from AC-CoA.

Observation of propionate levels in rumen fluid showed that the type of legumes did not significantly affected ($P > 0.05$) on propionate levels in rumen fluid. Legumes is a source of fiber, while the level of propionate is more influenced by starch and other fermentable carbohydrates. The PES index significantly affected ($P < 0.05$) on propionate levels in rumen fluid. The polynomial orthogonal test showed that the PES index had a quadratic effect (Figure 1) on the propionate content of the rumen liquid with the equation Y = -26.181 + 220.83 X - 232.55 X2, the coefficient of determination (R2) = 0.30 and the peak point P (0,47, 26,24).

Figure 1 shows that the highest propionate average was achieved at the PES index of 0.47 (medium). Campbell and Reece (2005) stated that the proportion of propionic acid in rumen fluid increased with the increase in the proportion of feed concentrates or glucogenic compounds in the rumen. Budiman *et al.* (2006) added that non-structural carbohydrates such as starch or saccharide group were components of nitrogen free extract (NFE). This is accordance with Figure 1, that the lowest level of propionate is found in the ration with an index of 0.6, because it has the lowest NFE content (Table 1).

PES index of 0.5 produces average propionate content higher than the PES index of 0.4, even though it has a lower NFE level. This can be caused by the low rate of availability of protein and energy in the process of microbial protein synthesis in rations with a low index. Syamsi *et al.* (2015) proved that rations with a low synchronization index produce low microbial protein synthesis, including groups of microorganisms that initiate the formation of propionate either through the succinate or lactate pathways. This can be attributed to the production of methane in each treatment. Table 1 shows that methane in the ration with an index of 0.5 is lower than the index of 0.4. This means that archea metanogenesis uses more fermented H_2 to produce methane, because the bacterial growth rate is slow at a low synchronization index.

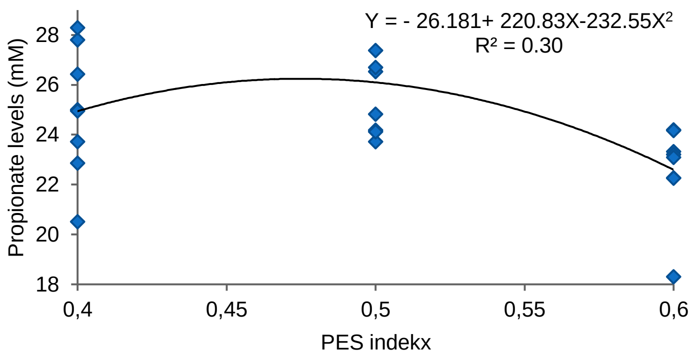

Figure 1. Effect of PES index on level of rumen fluid propionate.

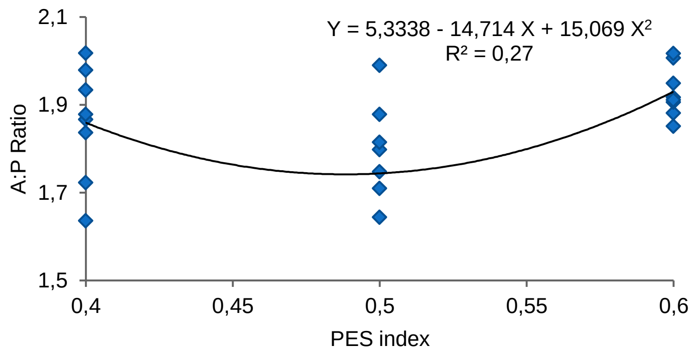

Figure 2. Effect of PES index on A:P ratio of rumen fluid.

A:P ratio

The influence of legumes treatment and setting of synchronization index in ration on A: P ratio is presented in Table 2. The results of analysis of variance showed that the type of legumes did not significantly affected (P> 0.05) on A:P ratio. Rodriguez *et al.* (2014) stated that protein from legumes influences more ammonia levels than VFA in rumen fluid. In contrast to legumes, the PES index was significantly affected (P <0.05) on A:P ratio. The polynomial orthogonal test shows that the influence is quadratic (Figure 2) with the equation Y = 5,3338 – 14,714 X + 15,069 X^2 , the coefficient of determination (R^2) = 0,27.

Figure 2 shows that the average value of A: P ratio decreases from the PES index of 0.4 to the PES index of 0.5 and continue to increase at the PES index of 0.6. The minimum point of the graph is reached at P (0.49, 1.74). Coe *et al.* (2011) stated that A:P ratio decreases with increasing propionate levels in the rumen fluid, and conversely the value of A:P will increase with decreasing propionate levels in rumen fluid. This is in accordance with the results of the study, it can be compared in Figure 1 and Figure 2. The proportion of molar propionate in rumen fluid increased from the PES index of 0.4 to the PES index of 0.5 then dropped back to the higher PES index of 0.6. The molar proportion or percent of propionate molar in the rumen has a negative relationship with A:P ratio. The A:P ratio of the study was in the range of 1.68-1.95 lower than the results of Gulmez and Turkmen (2007), which is around 3.31-4.21. Suwandyastuti and Rimbawanto (2015) stated that the A:P ratio is very important to be concerned, because it is related to the formation of methane. A high ratio of A:P will cause high production of methane and will cause energy inefficiency.

Methane (CH_4)

Influence of legumes treatment and setting of synchronization index in ration on levels of CH_4 is presented in Table 2. The results of the variance analysis showed that the type of legumes did not significantly affected (P>0.05) CH_4 levels in the rumen fluid. Sesbania and Leucaena actually have potential to reduce methane, because it contains defaunative antinutrients (Ridla, 2003). Antinutrient activity in suppressing the growth of protozoa in the rumen fluid is thought to be minimal, because the use of legumes is not too high in the rumen (10%). PES index treatment significantly affected (P<0.05) CH_4 levels in rumen fluid. The polynomial orthogonal test showed that the influence is quadratic (Figure 3) with the equation Y = 61,384 – 155,09 X + 158,41 X^2 and the coefficient of determination (R^2) = 0,29.

Tanuwiria *et al*. (2013) stated that CH_4 production is influenced by the production of acetate and butyrate. Campbell and Reece (2005) stated that the process of forming one mole of acetate will produce two moles of CO_2 and four moles of H_2, whereas in the formation process one mole of butyrate produces two moles of CO_2 and two moles of H_2. The process of forming one mole of CH_4 requires one mole of CO_2 and four moles of H_2. The formation of high acetate and butyrate will cause CH_4 production to be high. The results showed that the levels of acetate and butyrate not significantly different. Figure 3 shows that CH_4 levels was decreased from the PES index of 0.4 to the PES index of 0.5 , but continued to increase at the PES index of 0.6. The minimum point of the line equation is reached at P (0.49, 23.42). This shows that CH_4 is not absolutely influenced by the levels of acetate and butyrate. Suwandyastuti and Rimbawanto (2015) stated that nutritionists need to concern on A:P ratio compared to acetate and butyrate in relation to CH_4 production. Suwandyastuti (2013) stated that the high ratio of A:P can describe the condition of low energy efficiency, therefore the lower the A:P ratio, the more efficient use of energy. This is consistent with the results of the study, CH_4 graph (Figure 3) has a positive correlation with A:P ratio graph (Figure 2). The decrease in CH_4 from the PES index of 0.4 to the PES index of 0.5 is caused by a decrease in the A:P ratio and an increase in CH_4 from the PES index of 0.5 to the PES index of 0.6 due to an increase in the ratio A:P ratio.

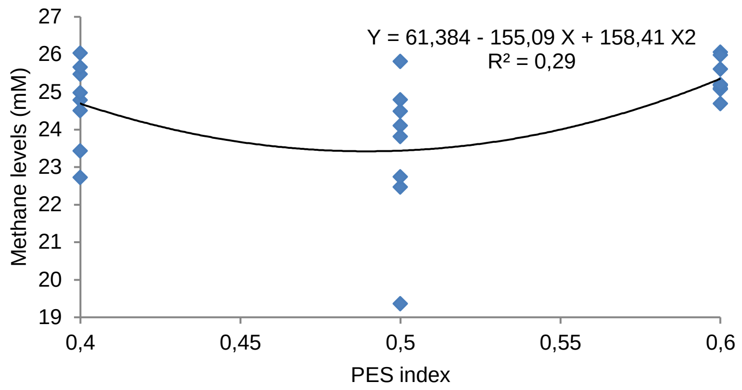

Figure 3. Effect of PES index on methane (CH_4) of rumen fluid.

Conclusions

The study concluded that the use of turi (*Sesbania grandiflora*) and lamtoro (*Leaucaena glauca*) leaves combined with the protein-energy synchronization index (PES) at the medium level (0.5) can increase VFA production especially propionate, suppress A:P ratio and reduce methane production.

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