Volatile Fatty Acids and Ammonia Levels in Local Sheep's Rumen Fluid Fed with Fermented Rice Straw

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Abstract- This study aims to determine the effects of providing suspension of *Acetobacter liquefaciens* bacterial isolate and *Geotrichum sp.* fungi isolate, as well as a mix between *Acetobacter liquefaciens* and *Geotrichum sp.* isolates extracted from the faeces of girrafes feeding on rice straw on pH value and Volatile Fatty Acids (VFA) and ammonia levels in Local sheep's rumen fluid. This experiment utilized twenty Local sheep randomly divided into four treatments where each treatment consisted of five repetitions, thus Completely Randomized Design method was applied (four treatments by five repetitions). The four treatments were T0: 60% (rice straw fermented without isolates+ 3% of urea + 3% of molasses) + 40% of concentrates, T1: 60% (rice straw fremented with *Acetobacter liquefaciens* bacteria + 3% urea + 3% molasses) + 40% of concentrates, T2: 60% (rice straw fermented with *Geotrichum sp.* fungi isolates + 3% of urea + 3% of molasses) + 40% concentrates, and T3: 60% (fermented rice straw with *Acetobacter liquefaciens* and *Geotrichum sp.* isolates + 3% of urea + 3% of molasses) + 40% of concentrates. The isolates of *Acetobacter liquefaciens* bacteria and *Geotrichum sp.* fungi were extracted from giraffes' faeces kept in Safari Park, Pandaan, East Java. At the end of this three months study, the rumen fluid was collected using a vacuum pump. The variables were then measured including pH value and the levels of VFA and ammonia. The result of the research shows that local sheep fed with rice straw fermented with suspension of *Acetobacter liquefaciens* bacteria, *Geotrichum sp.* fungi, as well as a mixture of *Acetobacter liquefaciens* and *Geotrichum sp.* experienced an increase on their pH level as well as the VFA and ammonia levels in their rumen fluid. The provision of the suspension of cellulolytic bacteria isolate mixture of *Acetobacter liquefaciens* and *Geotrichum sp.* fungi in the fermentation of rice straw yields the highest levels of acetic, propionic, butyric acids and total of Volatile Fatty Acids in Local sheep's rumen fluid.

Keyword- rice straw, cellulolytic bacteria, fungi, VFA, ammonia, rumen fluid.

I. INTRODUCTION

The provision of forage plays an important role in the production of ruminants. The need for forage for ruminants is increasingly rising along with the increase in livestock population. In addition, during the dry season, forage becomes scarce to obtain and even if it does exist, it is often of poor quality. As a result, agricultural waste such as sugarcane tops, rice straw and corn straw are often used as alternative fodders by cattle breeders.

Agricultural waste such as rice straw is very abundant at harvest time although, as fodder, rice straw has numerous drawbacks due to the low levels of protein and high coarse fiber. According to the FAO-Indonesia[1] , rice straw contains 28.8% crude fiber and 3.9% crude protein, making it least expected to meet cattles' basic needs. Cattle feed ingredients containing crude protein less than 7% may cause obstruction on rumen microbial activity due to the lack of nitrogen, causing less maximum carbohydrate utilization by rumen microbes, according to Crowder and Chedda[2].

Cellulolytic microbes are capable of producing 1.4 β-glucanase endo enzyme, 1.4 β- glucanase and β-glucosidase exo enzymes that can decompose raw fibers into soluble carbohydrates. Changes in the types of microbes in rumen may affect the feed's digestibility consumed by cattles and thus may affect feed intake, weight gain and milk production according to Beauchemin *et. al*. [3]. Meanwhile, a synergy between fungi and bacteria may be explained as a process in the digestion system where fungi can physically detach the surface

layer of cell walls and lignin (e.g. those on trunks or branches), making it penetrable by bacteria for further cell wall degradation according to Wang and Mc Allister [4]. Krehbiel, *et. al*. [5] that the provision of these microbes in ruminants may lower rumen acidosis, increase propionate concentration, the number of protozoa and immune response Omed, *et. al.* [6] that faeces is suspected to contain microbes derived from rumen, therefore allowing it to be an alternative source of microbial provider.

Fermentation in rumen is the result of physical and microbiological activities that transform the feed components into useful products (VFA, microbial protein, vitamin B); useless products (CH4, CO2) or harmful pruducts (ammonia, nitrate) for host animals, according to Owen and Goetsh [7]. After a widely-spread fermentation process by microbes, the fermentation products, especially VFA and microbial protein, may be consumed by cattles, according to Bannink and Tamminga [8] . Microbial fermentation end products, especially in the forms of VFA including acetic, propionic and butyric acids are the main energy sources for ruminants; while gases such as carbon dioxide and methane are the energy residue, periodically excreted; as well as ammonia which is useful for microbial protein production. More than 80% of the energy needs of ruminant animals can be provided by VFA produced in the rumen, depending on the types of feed consumed, according to Moran [9] .

This study aims to determine the effect of providing isolate suspension of *Acetobacter liquefaciens* bacteria and *Geotrichum sp.* fungi as well as a mix between *Acetobacter liquefaciens* and *Geotrichum sp*. isolates extracted from giraffes' faeces feeding on rice straw to the levels of Volatile Fatty Acids (VFA) and ammonia in Local sheep's rumen fluid.

II. MATERIALS AND METHODS

Experimental materials were twenty heads of male Local sheep (about 12 months age), rice straw fermented with *Acetobacter liquefaciens* bacteria, rice straw fermented with *Geotrichum sp* fungi isolate, urea, molasses, and concentrates.

This research was conducted by permission of animal ethics committee Faculty of Veterinary Medicine, Airlangga University.

The experiment utilized twenty Local sheep randomly divided into 4 treatments where each treatment consisted of 5 repetitions, thus Completely Randomized Design experiment pattern structure was used (4x5 repetitions). The four treatments were T0: 60% (rice straw fermented without isolates + 3% of urea + 3% of molasses) + 40% of concentrates; T1: 60% (rice straw fermented with *Acetobacter liquefaciens* bacteria + 3% of urea + 3% of molasses) + 40% of concentrates; T2: 60% (rice straw fermented with *Geotrichum sp* fungi isolate + 3% of urea + 3% of molasses) + 40% of concentrates; and T3: 60% (rice straw fermented with *Acetobacter liquefaciens* and *Geotrichum sp.* isolates + 3% of urea + 3% of molasses) + 40% of concentrates. The isolates of *Acetobacter liquefaciens* bacteria and *Geotrichum sp.* fungi were extracted from the faeces of girrafes kept at Safari Park, Pandaan, East Java. Each sheep was given drinking water *ad libitum.* At the end of the three months study, the rumen fluid was collected using a vacuum pump. The VFA levels were observed by using *chromatography gas* and the measurement of ammonia levels of rumen fluid was observed using *Conway method* , according to General Laboratory Procedures [24]

The data obtained in this study were then analyzed using Analysis of Variants (ANOVA) statistical method while the average differences between treatments were tested using Duncan Multiple Range Test method, according to stell and Torrie [23].

III. RESULTS AND DISCUSSION

The average values and standard deviation of pH level, ammonia and concentration in rumen fluid of sheep treated T0, T1, T2, and T3 can be observed in Table I.

TABLE I. The Average Values and Standard Deviation of pH Value, Ammonia Concentration, Acetic, Propionic, and Butyric Acid Values and the total VFA in Local Sheep's Rumen Fluid with the Treatments of Cellulolytic Bacteria and Fungi

Variables	Treatments			
	T0	T1	T2	T3
pH Value	6.80 ^{a}± 0.14	6.81^{a}±0.01	6.92^{b}±0.05	6.940^{b}±0.065
Ammonia Concentration N (mg/100ml)	11.55^{a}±1.021	13.61^{ab}±1.27	14.99^{b}±2.72	15.53^{b}±2.44
Acetic Acid (mmol/100cc)	77.44^{a}± 2.11	123.84^{b}±2.35	110.64^{b}± 2.35	171.78^{c}±37.87
Propionic Acid (mmol/100cc)	67.68 ^{a}±3.61	85.91^{ab}± 17.28	95.25 ^{bc}± 9.66	114.42^{c}±24.39
Butyric Acid (mmol/100cc)	41.26^{a}±4.62	43.42^{a}±1.03	53.71^{b}±3.32	57.36^{b}±14.32
Total of VFA (mmol/100cc)	186.38^{a}±3.86	253.17^{b}±14.47	259.60^{b}±21.06	343.50^{c}±71.51
Ratio of Acetate: Propionate: Butyrate	2.08:1.82:1.12	2.13:1.83 :1.02	2.45:1.69 :0.86	2.49 : 1.67 :0.83

Note: different superscripts in the same row indicate significant differences (P <0.05).

pH Value of Rumen Fluid

The acidity degree of sheep's rumen fluid (pH) treated T1, T2 and T3 has shown significantly different results. Sheep treated T2 and T3 have higher pH value in their rumen fluid than T0 and T1. The degree of acidity of rumen fluid is influenced by the type of feed consumed and the time of measurement. Feed materials containing non-structural carbohydrates will rapidly decrease the pH of rumen fluid. Low pH values after provision of concentrates is due to the fact that it is more easily fermented so that the Volatile Fatty Acid production per unit is higher than those of forage, according to Orskov and Ryle [10].

However, in this study, there has been an increase in pH value due to the fact that the feed given was in the form of rice straw and concentrates with a high content of crude fiber, resulting in low non-structural carbohydrate. This is in line with Utomo [11] that in cattles fed with rice straw basal, the pH value of rumen fluid reaches the highest at two hours after feeding, then gradually decreases 3 hours after feeding.

The result of pH value measurement in rumen fluid shows normal condition where the pH value ranges from 6.65 to 7.0. Concentrate feed will produce ruminal pH value of about 5.5 to 6.5 while fibrous feed will produce higher pH value of 6.2 to 7.0, according to Owen and Zinn[7]. According to Suharti et al. [12], constant normal rumen pH value is between 6.8 to 7.0 in a temperature of 38^0 C-40^0 C. The degree of acidity of rumen fluid is the main variable of rumen fermentation because the pH value change can alter the type of fermentation Orskov and Ryle [10].

The Ammonia Concentration in Rumen Fluid

The result of the study shows that the rumen fluid ammonia concentration of the sheep treated Ti, T2, and T3 show ammonia levels of 13,612; 14,988 and 15,529mg/100ml of rumen fluid respectively, which are significantly different from T0, reaching 11,545 mg/100ml. Arora [14] that ammonia in rumen fluid is derived from the degradation of feed protein and NPN (Non-Protein Nitrogen) by proteolytic microbes.

The concentration of NH_3 in the hours after feeding increased to a certain level of concentration and then gradually decreased. The increase of NH_3 in rumen fluid was due to the accumulation of NH_3 resulting from feed protein degradation. The decline occurred because some of the NH_3 was utilized by rumen microbes for body protein synthesis and partly for the synthesis of amino acid, nucleic acid and the transportation of amino acid into cells. According to Hidayah [15], the levels of NH_3 in the rumen fluid of sheep fed with hay increased and reached the peak three hours after feeding, reaching 11.38mg/100ml of rumen fluid and then gradually decreasing. Fermentation cassava flour noodle waste using *Aspergillus niger* level 1%, 2% and 3% had no significant effect on VFA and had a significant effect on ammonia by *In Vitro* Indriani *et.al.* [16]

In this study, rumen fluid was collected two hours after feeding, so that the levels of NH_3 was still high. High level of NH_3 in the treatment shows that the provision of suspension of *Acetobacter liquefaciens* bacterial isolate, *Geotrichum sp.* fungi isolate and the mixture bacteria *Acetobacter liquefaciens* bacteria

and *Geotrichum sp.* fungi have caused an increase on the degradation of protein and NPN of the feed. In this study, *Acetobacter liquefaciens* bacteria are capable of altering the color on Broth urea medium from red to pink, meaning that it is able to produce urease enzyme. This is in accordance with Owen and Zinn[7] statement that ammonia in rumen fluid is the result of NPN and feed protein degradation, microbial protein degradation and urea hydrolysis entering the rumen along with saliva and are diffused through the rumen wall.

The rumen fluid ammonia levels remain within normal range which is in accordance with Owen and Zinn [7] stating that for microbial growth, ammonia is required in the level of 0.35 to 29mg/100ml of the rumen fluid while according to McDonald *et.al.* [17], the ammonia concentration of rumen fluid under normal conditions ranges from 8.5 to 30mg/100ml of rumen fluid.

Volatile Fatty Acids in Rumen Fluid

The result of the study shows that the total Volatile Fatty Acids in rumen fluid is the highest in the sheep group treated T3 while no significant difference occurred in group treated T1 and T2

The synergy between *Acetobacter liquefaciens* bacteria and *Geotrichum sp.* fungi yields fermentation products of the best Volatile Fatty Acids based on the digestibility levels of Crude Fiber (CF), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF). If the digestibility levels of CF, NDF and ADF are high, the conversion of energy resources into VFA products in the rumen will increase respectively so that the energy formed in the metabolism in the body will produce acetic, propionatic and butyric acids in an adequate amount for the sheep to grow.

The rumen fluid was collected two hours after feeding so that the VFA levels were still relatively high. According to Perry [18] **,** Volatile Fatty Acids are the end product of carbohydrate or protein fermentation and are primary products in the fermentation process of monosaccharides in the rumen making it the main energy sources for ruminants. Furthermore, according to Utomo[11] , in basal hay feeding substituted with 15% of fine bran, the increase in VFA levels in rumen fluid occurs two hours after the supplementation. After two hours, the VFA concentration begins to decrease although at the levels of 25% and 35% of fine bran supplementation, VFA concentrations remain high until four hours afterwards. Supplementation of the protected flaxseed oil increased total VFA and proportion of propionic acid concentration as a potential energy source for the cattle. This result indicated that supplementation of protected fat did not disturb microbial activity in feed fermentation, according to Suharti *et. al.*[12]

The highest acetate acid level is in the sheep group provided T3, followed by the group provided T1 which does not have significant differences from the group provided T2. However the result shows a significant difference from T0. Chen et.al. [25] reported that the increasing of concentrate level from 20% to 50 % in the ration decresed the molar proportion ruminal acetic acid from 68.45% to 66.22%.

The highest propionic acid level occurs in the group provided T3 which is not significantly different from the group provided T2. Serment *et. al.* [26] reported that the increasing of concentrate level from 35 up to 705 in the ration increased the ruminal propionic acid from 18.0 to 24.1mol/100 mol during 10 weeks of investigation.

The highest butyric acid level occurs in T0 and is not significantly different from those provided T2 while the lowest level of butyric acid occurs in the group treated T3 which is not significantly different from the group provided T1. Widiyanto *et. al* [27] reported that the feeding field grass in pellet form for male Java Thin Thin sheep, increased the ruminal propionic acid molar proportion and decreased ruminal ratio of acetic acid : propionic acid.

The ratio of VFA acids from the highest to the lowest is acetatic acid, propionatic acid, and butyric acid respectively. According to Hume [19] , feeding basal feed in the form of hay produces the highest proportion of acetic acid, although the supplementation of granule concentrates reduces the proportion of acetic acid as the higher the level of concentrate provision, the higher the proportion of propionic acid. The high proportion of acetic acid in this study occurred because the feed given was in the form of rice straw and concentrates with high coarse fiber content.

Tillman *et. al* [21] found the main result of carbohydrate hydrolysis in the rumen is glucose that will be fermented into VFA as the main energy source for ruminants. McDonald *et.al.* [17] that VFA are mostly composed of acetatic acid (C_2), followed by propionic acid (C_3) and butyric acid (C_4). Acetic acid is used in forming body fat and milk fat, while propionic acid is the source of glucose in blood. Czerkawski [22] that acetic and butyric acids are synthesic precursors of milk fat and body fat whereas propionic acid is the precursor of body fat. Therefore, in the effort of cattle fattening, the latter fermentation orientation is expected. The total and composition of VFA of *CRM*-treated rumen liquor were significantly different ($P<0.05$) compared to that of rumen liquor of Control (ie. the total VFA: 85.3 vs 73.5 mM and the percentage of acetic acid: 67.8 vs 60.3%). It is concluded that *CRM* treatment resulted in positive effects on growth of ruminant fed high fibrous forages such as rice straw and could lower enteric methane production, according to Thalib *et.al*. [20].

IV. CONCLUSION

The provision of the suspension of cellulolytic bacteria isolate mixture of *Acetobacter liquefaciens* and *Geotrichum sp.* Fungi (T3) in the fermentation of rice straw yields the highest levels of acetic, propionic, butyric acids and total of Volatile Fatty Acids in Local sheep's rumen fluid.

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COMPETITING INTERESTS

The Authors declare that they have no conflict and competiting interest

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