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Identification and characterization of acidosis on *in vitro* rumen fermentation with feeds based on grass, rice bran, concentrate, and tofu pulp

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ABSTRACT

The present study evaluated and characterized various types of biomass fermented in the rumen culture. A series of batch experiments were conducted to assess any potential organic acid build-up in the rumen culture fermenting different substrates of biomass. Results showed that pH depression occurred within 2 h of incubation in the rumen culture fermenting rice bran and/or concentrate in which the pH dropped from the neutral level (pH 6.9 ± 0.15) to the acidic level (pH 5.6 ± 0.15). However, at the same period of incubation, the fermentation of grass and tofu pulp in the rumen culture did not have a dramatic drop of pH in which the pH was somewhat stable between 7.0 and 6.85. Organic acids, such as acetic acid and lactic acid, were accumulated in the digesters of rumen culture fermenting rice bran and/or concentrate, suggesting that the culture was acidified which led to ruminal acidosis.

1. INTRODUCTION

Rumen fermentation is an important process that could give a significant impact on the life of ruminants. This is because rumen fermentation could provide energy sources to the ruminant through the conversion of ingested feeds [1]. The feeds consisting of plant fibers and polysaccharides are degraded by rumen microbes to produce volatile fatty acids, proteins, vitamins, and/or other nutrients required for the growth and maintenance of the host [2]. The types of substrates digested normally would affect the metabolite generated and microbial community in the rumen [3]. The end-products and/or metabolites produced during the fermentation of substrates would significantly modulate the microbial population in the rumen [3,4]. The diversities in rumen microbiota may be related to cattle production and health characteristics including milk production and milk composition, feed efficiency, methane generation, and ruminal acidosis [5-8].

Some studies revealed that the feeds that contain high biodegradable materials including grain and/or carbohydrates-based feed could provide a large amount of energy for ruminant growth and production [9]. However, feeding a lot of highly biodegradable substrates would harm the ruminants. This is because the conversion of biodegradable materials through the fermentation process in the rumen would generate organic acids as the main end-products. An organic acid build-up in the rumen may be escalated when an excessive amount of biodegradable materials is fed to the ruminants [10]. Some studies reported that an accumulation of organic acids in the rumen would generate acidic conditions to the rumen and may lead to a ruminal acidosis [11-13]. Ruminal acidosis is typically related to an increase in hydrogen ion or proton concentration (H^+), leading to a drop of ruminal pH [14].

Ruminal acidosis is a metabolic disturbance caused by organic acids accumulated in the rumen that may potentially affect the health of ruminants [15,16]. The organic acids build-up during the rumen fermentation would significantly decrease the pH level in the rumen culture. Low pH could potentially induce the growth of lactic acid-producing bacteria and thereby would generate a large amount of lactic acid as the main metabolite [17]. The lactic acid build-up in the rumen may escalate and/or worsen the acidosis occurring in the rumen since lactic acid accumulation typically could generate a significant decrease in the rumen pH to <5.5 [18,19]. Acute ruminal acidosis is a metabolic disorder that occurs when blood pH and bicarbonate level are depressed due to the overproduction of lactic acid in the rumen [10]. In some cases, a severe lactic acidosis would cause mortality of the ruminants [20].

Since rumen fermentation is an essential process of the ruminant's digestive system that requires more attention, the present study aims to evaluate *in vitro* rumen fermentation of various types of biomass substrates. The potential of acidosis that may occur in the rumen fermentation system would also be evaluated through a series of batch experiments.

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2. MATERIALS AND METHODS

2.1. Rumen Fluid Collection

Rumen fluid was collected at a commercial slaughterhouse from a commercially raised cow that had previously been fed under controlled condition in which the feeds typically contain grasses and small amount of concentrates. The slaughterhouse is situated in Peunayong, Banda Aceh City, Aceh Province, Indonesia. The fresh collected rumen fluid was then immediately warmed under the temperature of 37 ± 0.5°C before the start of the experiments.

2.2. Substrates Preparation

The present studies used various types of substrates that were typically utilized as a feed for the ruminants including grass, tofu pulp, concentrate, rice bran, and corn bran. The type of grass used for this current experiment was elephant grass (*Pennisetum purpureum*) that was collected at pasture grass situated in Pango, Banda Aceh, Aceh Province, Indonesia. Before using it as a substrate in rumen fermentation, its' size was reduced into 0.2 ± 0.1 cm. Tofu pulp used for the experiment was taken from the tofu-processing industry located at Punge Blang Cut, Banda Aceh. Concentrate used for the current experiment was a commercial concentrate used for cattle feed, which was obtained from cattle feed store at Banda Aceh. Rice bran and corn bran used for this rumen fermentation were commercial bran, typically used for cattle and livestock feeds, and those were obtained from cattle and livestock feed store at Tungkop, Darussalam, Banda Aceh.

2.3. Experimental Design and Procedures

2.3.1. Investigation of various biomass substrates fermented in rumen culture

The present study consisted of a sequence of batch processing tests with various biomass substrates. The batch tests were carried out in a thermostatic water bath for 48 h to have complete digestion of the substrates and to allow the rumen microbes to adjust to the environment and the substrates loaded to the reactor. To evaluate the characteristics of the typical ruminant's feeds fermented in rumen culture *in vitro*, various types of the feeds used for the current tests included grass (S1), rice bran (S2), concentrate (S3), and tofu pulp (S4). The control test (S0) was a blank digester in which the reactor was filled with merely fresh rumen fluid. Each batch digester was loaded with 10 g of the substrate and topped-up with 100 mL of fresh rumen fluid. During the fermentation, the operational temperature was maintained at 38 ± 0.5°C. The working volume applied was 100 mL, and the rumen culture was anaerobically mixed. In these trials, there were no alkaline and/or acid solutions added to the rumen culture.

2.3.2. Rumen fermentation of the mixtures of substrates

To assess the effects of various compositions of grass and other substrates, such as concentrate, tofu pulp, rice, and corn bran on the potential of acid build-up, fermentation of the mixtures of grass and other substrates was conducted in batch mode. In the current trial, each digester was inoculated with fresh rumen culture. The first fermentor (CS1) was added with the mixture of 50% grass, 25% rice bran, and 25% corn bran. The second fermentor was loaded with the mixture of 50% grass, 25% rice bran, and 25% tofu pulp. The third fermentor was filled with the mixture of 50% grass, 25% rice bran, and 25% concentrate. For this test, the blank reactor (CS0) was filled only rumen fluid without the addition of substrates. All procedures applied in these tests were the same as those applied to the rumen fermentation of various biomass substrates. During the rumen fermentation process, pH was not adjusted and/or not maintained in a certain level. Hence, no basic solution, acid solution, and other chemicals introduced to the digester during the rumen fermentation [21].

2.4. Analytical Methods

The influent and effluent samples taken from each trial of the rumen fermentation processes were periodically analyzed for its pH utilizing a Laboratory Benchtop pH Meter Multifunction Complete Probe. The rumen fluid used for the experiment was also measured for its alkalinity to assess the effectiveness of rumen for buffering the fermentation culture from the organic acid build-up and/or ammonia accumulation [22]. Ammonia concentration of each rumen fermentation sample was measured using a manual colorimetric procedure of NH_3 test kit and reagent [23]. Each sample was measured for total solid and moisture content in which the sample was dried for 24 h at the temperature of 105 ± 0.5°C using a laboratory drying oven [24-26].

To characterize organic acids production as the metabolite fermentation, the titratable acidity was measured to assess the total acids generated during the rumen fermentation process of various biomass substrates. The analysis of titratable acidity of organic acids was carried out using a Laboratory Benchtop Standard pH meter with two decimal precision. The titrant filled in the stand-up burette tube was 0.1 M sodium hydroxide standard solution. Before the titration analysis, the analyte was added a few drops of phenolphthalein solution as a pH indicator [17]. To ensure an accuracy of the data collected, each sample analyzed was conducted in replicate.

3. RESULTS AND DISCUSSION

3.1. Rumen Fermentation on Single Substrates

To evaluate the characterization of various biomass substrates fermented in the rumen culture, a series of the batch test had been carried out. As presented in Table 1, the pH of the rumen fluid used for the experiments was somewhat optimal for performing rumen digestion in which the pH was at a neutral level (pH 7.13). This suggested that the culture was not acidified previously, and indicated that no organic acids accumulated in the rumen. This is also in agreement with some studies revealing that the pH of the healthy rumen should be at the neutral level, which was above 6.5 and/or around 7.2 [27]. Some studies reported that pH below 6.5 may slow down the performance of microbes for digesting fiber substrates [28-30]. Furthermore, the low level of pH may cause the rumen to perform less efficiently in the digestion of feeds supplied.

The present study revealed that the addition of non-fiber substrates (i.e., rice bran and concentrate) to the rumen culture may cause a significant drop in pH [Figure 1]. This present study showed that merely within

Table 1: Physicochemical properties of rumen fluid and substrates used for the batch experiments.

Parameters	Unit	Rumen	Grass	Rice bran	Tofu pulp	Concentrate
Total solids	%	9.55	16.78	97.01	24.78	93.63
Moisture content	%	90.50	83.20	3.00	75.20	6.40
pH	-	7.15	-	-	-	-
Total ammonia	mg/100 mL	9.5	-	-	-	-
Titratable acidity	%	0.26	-	-	-	-
Alkalinity	mmol/L	6.57	-	-	-	-

2 h of incubation, the pH of the rumen culture fermenting rice bran and concentrate dropped dramatically from 7.0 to 5.67, leading to ruminal acidosis. This is different from the rumen culture fed with grass and tofu pulp in which within that period (2 h of incubation), their pH decreased slightly from 7.0 to 6.85, suggesting that no ruminal acidosis occurred. This agreed with the study by Calsamiglia *et al*. [31] reporting that subacute ruminal acidosis is a generic digestive disorder in ruminants fed with high-concentrate diets, and normally occurs when ruminal pH drops below 6.0.

In this present study, acidosis occurred since rice bran and concentrates were rich in rapidly degradable carbohydrates (i.e., starch and soluble sugars) and thereby would easily be degraded and converted into organic acids during the rumen fermentation. This suggested that rice bran and concentrate would not be suitable to be added to rumen culture as the main substrates for prolonged digestion. This result was in agreement with some studies that revealed that a dramatic drop of rumen pH causing ruminal acidosis typically occurred when the rumen was supplied with high-concentrate feeds and/or a large amount of non-fiber carbohydrates [32,33].

As depicted in Figure 2, the rumen culture fed with rice bran and concentrate produced a lot of organic acids. After 48 h of incubation, the rumen culture loaded with rice bran produced a higher amount of acetic acid (12%) in comparison to other substrates (i.e., grass and tofu pulp) producing the acid between 2% and 7% of acetic acid. Results also revealed that within 2 h of incubation, the rumen culture added with rice bran and concentrate experienced the organic acid build-up represented in acetic acid accumulated in the digester between 8% and 10% of the broth. This suggested that rice bran and concentrate may have the potential to generate acetic acid build-up in the rumen culture that may lead to acidosis. The result of the present study was in agreement with the study finding that ruminal acidosis could be initiated with acetic acid build-up resulting in a drop of pH in the rumen culture [17].

The present study showed that lactic acid build-up occurred in the rumen culture supplied with rice bran and concentrate [Figure 3]. Merely within 2 h of incubation, the lactic acid build-up (12% of lactic acid) occurred in the rumen culture digesting rice bran and concentrate. This indicated that only within 2 h of incubation, rumen culture fermenting rice bran and/or concentrate would experience subacute ruminal acidosis. An acute acidosis occurred within 8 h of incubation in which within that period, lactic acid was accumulated in the digester (14–15% of lactic acid). The results somewhat agree with some studies reporting that when pH drops below 5.5, lactic acid would be accumulated in the rumen culture [34]. This occurred because the rumen was supplied with rapidly degradable carbohydrates and/or forage that are less ineffective fiber [28].

3.2. Mixture of Substrates

The feeds for ruminants comprised a mixture of various types of substrates including lignocellulosic and non-lignocellulosic biomass. To evaluate whether the above-mentioned results occurred as well in the mixtures of feed, a series of batch rumen fermentation tests applying different ratios of substrates added were carried out [Figure 4]. Results of the present study showed that mixtures of 50% grass, 25% rice bran, and 25% corn bran (CS1) had the lowest pH among other mixtures. This suggested that the mixture of grass, rice bran, and corn bran fed to the rumen culture would cause an acute ruminal acidosis in which pH decreased significantly from 6.8 to 5.0. This result agrees with a study by Jaramillo-López *et al*. [3] reporting that acute acidosis may occur

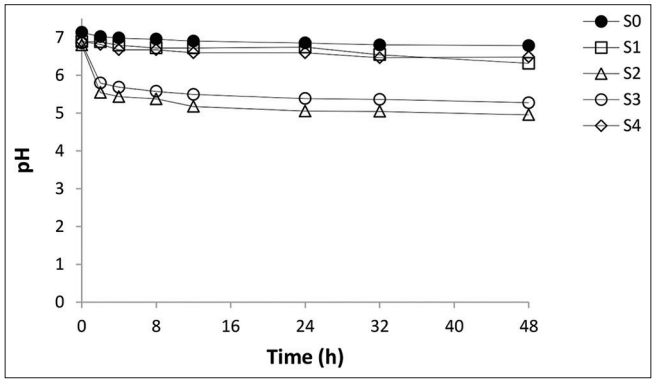

Figure 1: The pH of various types of substrates fermented in the rumen culture.

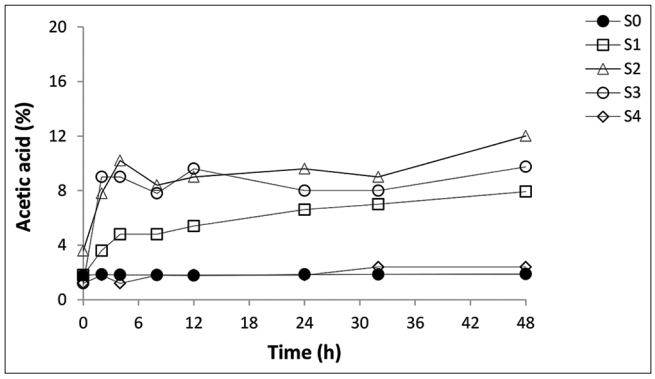

Figure 2: Profile of acetic acid formation during the rumen fermentation of various substrates.

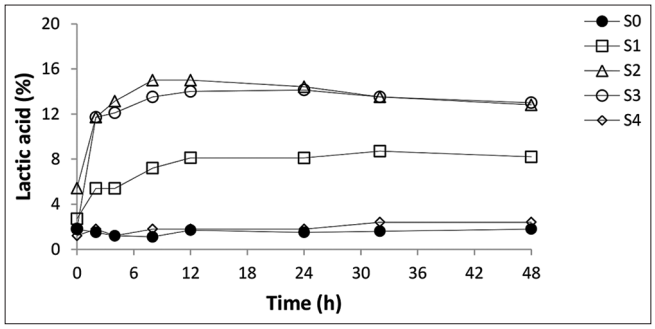

Figure 3: Profile of lactic acid formation during the rumen fermentation of various substrates.

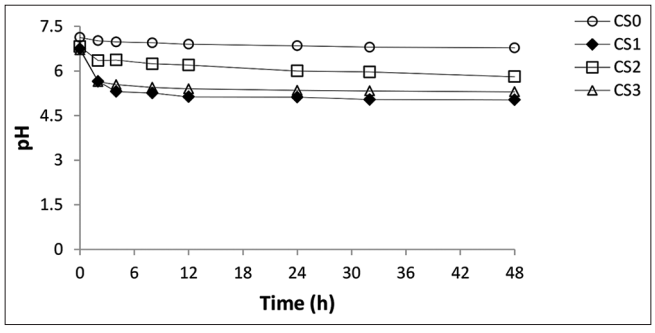

Figure 4: The pH of different mixtures of substrates fermented in the rumen culture.

to the rumen with prolonged exposure to an extremely acidic condition or at low pH (≤5.0).

The current experiments also revealed that the mixture of 50% grass, 25% rice bran, and 25% concentrate (CS3) also experienced serious acidosis in which pH dropped dramatically from 6.77 to 5.30. It is interesting to note that a dramatic drop in pH occurred in both CS1 and CS3 samples. Only within 2 h of the incubation period, those trials experienced serious acidosis in which their pH dropped dramatically from 6.8 to 5.6. However, the mixture of 50% grass, 25% rice bran, and 25% tofu pulp (CS2) did not experience a significant drop of pH in which the pH decreased slightly from 6.82 to 6.4. The results suggested that the mixture consisting of concentrate and starch-based materials tends to have ruminal acidosis. The result is in agreement with the study revealed that the feeds containing a significant amount of soluble (i.e., sugar) as well as insoluble carbohydrates (i.e., starch) would easily have acidosis in the early stage of rumen fermentation [18,29].

As depicted in Figure 5, both CS1 and CS3 produced a lot of acetic acids in which the average production was about 6.4% of acetic acid. After 2 h of incubation, the rumen culture supplied with the mixture of grass, rice bran, corn bran (CS1), and/or the mixture of grass, rice bran, and concentrate (CS3) produced a significant amount of organic acid (6–7% of acetic acid). However, within that period (2 h of incubation), the rumen culture fed with the mixture of grass, rice bran, and tofu pulp (CS1) produced a small amount of acetic acid (4%). This suggested that the rumen culture fermenting substrates

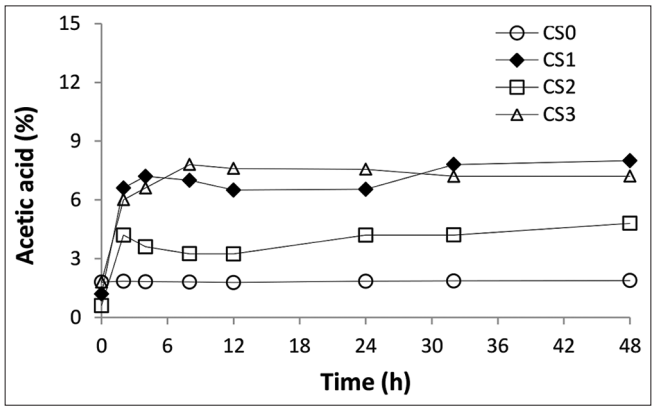

Figure 5: Profile of acetic acid formation during the rumen fermentation of a different mixture of substrates.

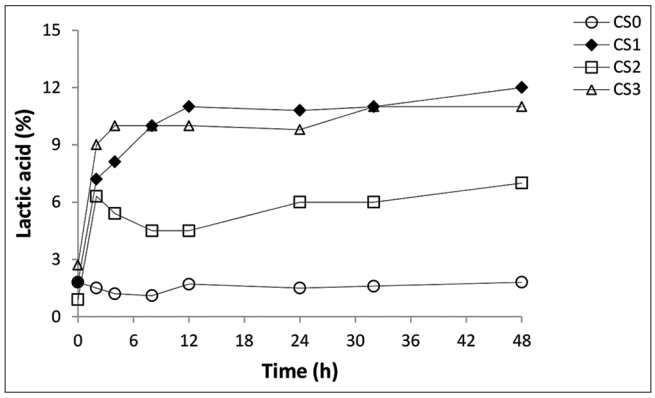

Figure 6: Profile of lactic acid formation during the rumen fermentation of different mixture of substrates.

containing many biodegradable carbohydrates may potentially generate organic acid build-up leading to ruminal acidosis. The results were in agreement with the study revealing that providing of providing feeds rich in ruminal fermentable carbohydrates may result in the generation of a high amount of volatile fatty acids, such as acetic acid [35].

The present study found that within 4 h of incubation, the rumen culture supplied with the mixture of grass, rice bran, and concentrate (CS3) had organic acids build-up in which lactic acid accumulated in the digester about 10% [Figure 6]. After 12 h of incubation, the lactic acid produced in the digester of CS1 had surpassed the production of lactic acid in the digester of CS3 in which its lactic acid production reached about 11%. This is different from the rumen culture fermenting the mixture of grass, rice bran, and tofu pulp (CS2) in which the production of lactic acid was quite low, which was only 5%. This result reaffirmed the previous results revealing that ruminal acidosis affected by an accumulation of lactic acid may occur when the rumen culture digested substrates that contain a lot of carbohydrates. This was in agreement with some studies revealing that lactic acid build-up causing acute ruminal acidosis occurred as a result of carbohydrate overload in the rumen [35,36]. The studies reported that shifting the feeds of ruminants from roughage to concentrates consisting of grains such as wheat, barley, and corn would potentially induce lactic acid accumulation, which causes serious acidosis.

4. CONCLUSION

The present study showed that fermentation of rice bran, corn bran, and concentrate in rumen culture could induce pH drop (pH <6.0) that may lead to ruminal acidosis. Acidosis in the rumen culture fermenting rice bran and concentrate occurred merely within 2 h of incubation in which the pH dropped drastically from about 7.0 to 5.5. This is different from the rumen fermentation of grass and tofu pulp in which the pH of both samples did not experience pH depression. The results reveal that within 12 h of the incubation process, fermentation of grass and tofu pulp in rumen culture did not depict any significant drop of pH in which the pH was somewhat stable at the level between 7.0 and 6.7.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on human and/or animals.

10. DATA AVAILABILITY

All data for publication were presented in this article.

11. PUBLISHER'S NOTE

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