In Vitro Ruminal VFA Concentrations from Rice Straw and Sugarcane Bagase Based Diets with Different NFC Sources

Joelal Achmadi¹, Eko Pangestu, Surahmanto, Agung Subrata, and M. Ainsyar Harahap
¹ Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia

E-mail: jachmadi@gmail.com

Abstract. Information concerning with ruminal nutrient availability of feed could help in balancing ruminant ration. Rice straw and sugarcane bagase are known as fiber sources, and molasses is known as non fiber carbohydrate (NFC) source for ruminant. The banana plant root meal (BPRM) is postulated to replace the portion of molasses in a ruminant diet because of its NFC content. This study determined the in vitro ruminal concentrations of volatile fatty acids (VFA) from ammoniated rice straw and sugarcane based diets supplemented with molasses and BPRM, respectively, as NFC sources. In the experiment, two diets of rice straw based diets supplemetned with molasses (RSM) and BPRM (RSB) respectively, and two diets of ammoniated sugarcane bagase based diets supplemented with molasses (SGM) and BPRM (SGB) were tested their in vitro ruminal VFA concentrations using a technique of batch culture. Result of the in vitro test showed that there was no significant different in ruminal concentrations of total VFA, acetic, propionic, butyric, isobutyric, isovaleric, and n-valeric among experimental diets. The portions of molasses and BPRM could be replaced each other in the diet, and likewise for rice straw and sugarcane bagase. This is valuable indicative in attempt for sustaining ruminant feed availability.

1. Introduction
Agro-industrial by-product is abundant during harvest season, and this is concurrent with the a high production of forage throughout rainy season. Therefore farmers prefer using forage as feed, and the by-product is neglected. Processing the agro-industrial by-product is necessary to be used during dry season when the availability of roughage feed is low. Thus, ruminant production throughout a year could be sustainable.

Ammoniation of roughage feed using urea is commonly practised to improve its nutrient quality. The ammoniation is also subjected to preserve roughage feed for its storage as a feed block [1]. The use of ammoniated roughage feed in a diet needs the source of readily available carbohydrate, and molasses is utilized to meet the requirement of rumen soluble carbohydrate. The banana plant root meal (BPRM) may be a potent source of NFC in ruminant feed supplemented with urea-limestone mixture [2].

Diversification of feedstuff ingredient is necessary in formulating ruminant diet, especially during dry season when the forage availability is scarcely. The bulkiness of some agro-industrial by-product could be used to replace the forage proportion in a ruminant diet. However, the nutrient quality of agro-industrial by-product is generally low, and ammoniation gives simple and cheap technique to improve nutrient availability of rice straw and sugarcane bagasse. The use of ammoniated roughage feed in a ruminant diet needs a source of readily available carbohydrate.

Sugarcane molasses is commonly utilized as the source of rumen soluble carbohydrate. Ball shape like rhizome beneath stem of the old banana plant is commonly utilized as a source of traditional food in Indonesia, but its use as feed supplement requires a clarification. There is a little information about use of the BPRM as NFC source in formulating ruminant diet. Although the NFC content of banana plant root meal was lower than that of molasses, the banana plant root meal could be used for diversification of readily available carbohydrate source in ruminant diet supplemented with limestone-urea mixture [2].
This experiment studied *in vitro* rumen VFA production from ammoniated rice straw based and ammoniated sugarcane based diets when supplemented with molasses or BPRM. Such information could be benefit in diversification of feedstuffs for balancing ruminant diet.

2. Materials and Methods

In this experiment four fattening goat diets were formulated containing 12% CP; 60% TDN; and 18% NFC (Table 1). Ingredient materials used for diet formulations were purchased from feedstuff supplier in Semarang city. The BRPM was made from part of ball shape like rhizome beneath stem of old banana plant. After peeling this ball shape like rizhome, the rhizome was sliced into 1 cm thin and then was sundried. The sundried matter was then crushed to create the BRPM [2].

2.1. *In Vitro* Technique

Test of *in vitro* rumen fermentability for each experimental diet was conducted using the method of batch culture [3] with correction for blank (without substrate). Rumen fluid for inoculum of was collected from two adult ruminal-cannulated Jawa Randu crossbred goats. Goats were fed twice daily on diet containing 70% elephant grass, 10.7% rice bran, 17% coconut meal, 1.1% cassava waste, 1.0% sugarcane molasses, and 0.2% vitamin-mineral premix (based on dry matter).

McDougall buffer solution and rumen fluid were mixed in a 4:1 ratio to be a rumen inoculum. The *in vitro* rumen incubation was conducted for 3 hours. After the incubation, the fermentation process was ceased by placing fermentor tube in an ice-cold bath, and then was centrifuged (3,000 x g, 15 minutes, 4 °C). The filtrate was divided into 2 parts, one part was stored at -20 °C for volatile fatty acids and ammonia concentrations analysis.

2.2. Chemical and Statistical Analysis

The proximate component contents of diet were determined by using [4]. Neutral detergent fiber was analyzed by using Van Soest method [5], without a heat stable amylase assay. Rumen volatile fatty acids concentration were determined by using gas chromatography (GC-2010, Shimadzu, Tokyo, Japan) as described by Zhang et al. [6]. Rumen NH₃ concentration was assayed using spectrophotometer as described by Chaney and Marbach [7]. Each sample measurement was assayed in duplo. In each dietary experiment, parameter data (total VFA, acetic, propionic, butyric, isobutyric, isovaleric, n-valeric, ammonia) were expressed as mean values of three replicates. The different in parameter among experimental diets were determined using least significance different test.

3. Results and Discussion

The *in vitro* VFA concentrations were determined when ammoniated rice straw based diet and ammoniated sugarcane based diet, respectively supplemented with molasses and BPRM, were incubated using the mixed culture of goat’s rumen fluid. As a ruminal feed fermentation product, VFA is used as intermediary substance in nutrient metabolism. Individual VFA from ruminal degradation of feed carbohydrate, mainly acetic, propionic and butyric are important energy sources for metabolism of host animal. Acetic and butyric are lipogenic nutrients, and propionic is primarily used as glucogenic precursor. Some branched chain fatty acids, namely isobutyric, 2-methylbutyric, isovaleric, and n-valeric are ruminal degradation product of some feed amino acids. These isoacids provide carbon skeletons for some amino acids synthesis. The carbon skeleton of isobutyric and isovaleric are also utilized for biosynthesis of higher branched-chain fatty acids and aldehydes of the lipid fraction of cellulolytic rumen bacteria. Some studies indicated that there is a close correlation between ruminal isoacids concentration and amount of product, especially in lactating dairy ruminant.
Molasses supplementation increased (P<0.01) ruminal butyric concentration in rice straw based diet, but there was no significant different between molasses and BPRM supplementations for sugarcane bagasse based diet (Table 2). Feeding a high sugar diet is often correlated with greater butyric concentration in rumen [8]. Ruminal concentrations of total VFA, acetic, propionic, isobutyric, isovaleric, and ratio of acetic to propionic were similar among experimental diets (Table 2). Gao and Oba [9] clarified that rumen total VFA and propionic concentrations did not change with increasing sugar level in the diet of lactating cows. Ratios of acetic to propionic were similar among experimental diets (Table 2). The rice straw based diet tended to have more higher (P<0.11) ratio of acetic to propionic than that of bagasse based diet in both supplementations. This slight different in ratio of acetic to propionic may be caused by the NDF crude fiber contents in diet (Table 1). Suharlina et al. [10] stated that feed fiber is degraded to become acetic in rumen, and may cause a higher in the ratio of acetic to propionic.

Ruminal concentration of ammonia were higher (P<0.01) in sugarcane bagasse based diets compared with those of rice straw based diets, and there was no significant different between supplementation of molasses and BPRM in roughage based diets (Table 2). Gao and Oba [9] reported that increasing NFC level in diet of lactating dairy cows may decrease ruminal ammonia concentration. The less rumen ammonia concentration may be caused by more higher use of ammonia for microbial protein synthesis [10].

### Table 1. Ingredient and nutrient composition of experimental diets.

| Feedstuff                  | RSM  | RSB  | SGM  | SGB  |
|----------------------------|------|------|------|------|
| Ammoniated rice straw      | 55.00| 55.00| -    | -    |
| Ammoniated sugarcane bagasse| -    | -    | 55.00| 55.00|
| Rice bran                  | 3.00 | 2.00 | 21.00| 20.35|
| Corn meal                  | 25.00| 25.70| 5.00 | 5.00 |
| Cassava waste              | 5.50 | 5.20 | 17.00| 17.00|
| Molasses                   | 2.00 | -    | 2.00 | -    |
| Banana plant root meal     | -    | 2.65 | -    | 2.65 |
| Nutrient content, % of DM  |      |      |      |      |
| Crude fiber                | 23.99| 24.14| 30.49| 30.79|
| Crude protein              | 12.10| 12.08| 12.83| 12.81|
| Neutral detergent fiber    | 68.71| 71.08| 57.98| 60.26|
| Non fiber carbohydrate     | 18.27| 18.07| 18.93| 18.75|
| Total digestible nutrients | 60.00| 60.00| 60.72| 60.57|

1Non fiber carbohydrate calculated as equation of Da-Cheng et al. [8].

RSM = ammoniated rice straw base diet supplemented with molasses,
RSB = ammoniated rice straw base diet supplemented with BPRM,
SGM = ammoniated sugarcane bagasse base diet supplemented with molasses,
SGB = ammoniated sugarcane bagasse base diet supplemented with BPRM
4. Conclusion
Banana plant root meal could be used for diversification in supplying NFC in the goat diet, especially for replacing molasses portion. Further experiment is needed to clarify the use of BRPM in a ruminant diet with different levels of crude protein content.

Acknowledgments
The research was funded by “Selain APBN DPA SUKPA Universitas Diponegoro T. A. 2017”.

Reference
[1] Salman AD, El Shargi KM, Al-Habsi RS and Al-Sadairi T 2017 Livest. Res. Rur. Dev. 29(6) 212-218
[2] Harahap MA, LK Nuswantara, F Wahyono, E Pangestu and J. Achmadi 2019 Livest. Res. Rur. Dev. 31(5) 122-129
[3] Tilley, J. M. A., and R. A. Terry. 1963 J. British Grassland Soc. 18 104-111.
[4] AOAC 1990 Association of Official Agriculture Chemists Inc 1(2) 201-221.
[5] Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991 J. Dairy Sci. 74 : 3583-3597.
[6] Zhang CM, YQ Guo, ZP Yuan, YM Wu, JK Wang, JX Liu and WY Zhu 2008 Anim. Feed Sci. Technol. 146 259–269.
[7] Chaney AL, and EP Marbach 1962 Clin. Chem. 8 130–132.
[8] Da-cheng L, Z Xiangli, Z Pei-ting, G Min, H Hao-qi and H Hong-lian 2013 J. Integr. Agric. 12 319–326.
[9] Gao X and M Oba 2016 J. Dairy Sci 99 291-300.
[10] Suharlina, DA Astuti, Nahrowi, A Jayanegara and L Abdullah 2016 J. Indonesian Trop. Anim. Agric. 41 196-203.