Effect of supplementation of various fermentable carbohydrate sources in sorghum straw-gliricidia mixed diet on fermentation kinetics measured using in vitro gas production

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Abstract. Supplementation of fermentable carbohydrate in crop by product-based diet is expected to improve the fermentability and reduced methane production. The study evaluated supplementation of various fermentable carbohydrate in sorghum straw-gliricidia mixture basal diet. Sorghum straw-gliricidia mixture was supplemented either with ground corn grain, rice bran or sorghum grain at 10% of DM basal diet. Treatment diets evaluated were: Sorghum + gliricidia leaf meal (Control); Control + 10% ground corn grain (Corn grain); Control + 10% rice bran (Rice bran); Control + 10% sorghum grain (Sorghum). Sorghum straw was chopped and ground then mixed with gliricidia leaf meal at ratio 60:40% DM. The sample was incubated for 48 hours, gas production was recorded at 4, 8, 12, 16, 24, 36 and 48 hours. Rumen fluid for medium incubation was collected from sheep fed elephant grass basal diet. Supplementation of rice bran lowered (P<0.05) gas production from insoluble fraction of the diet compared to maize. Rice bran and sorghum decreased gas production at 48 h incubation compared to control (P<0.05). Sorghum supplementation decreased CH4 production. It can be concluded that fermentable carbohydrate supplementation from sorghum grain to sorghum straw-gliricidia mixture reduced percentage of methane production.

1. Introduction
Sorghum bicolor L. Moench is suitable to be cultivated in marginal or dry areas in Indonesia. This plant able to adapt in different soil and still productive in water deficit soil [1], more resistance to plant pest than other crops. Sorghum crop is potential to be used as food, feed and fuel. The scarcity of forage available in dry areas or in dry season can be overcome by utilization of sorghum for forage or used sorghum straw/stover as by products from sorghum grain harvest. Sorghum stover has low nutritive value due to its high fibre and lignin contents and low protein and vitamin contents. Sorghum straw contained 2.54% CP, 70.23% NDF, 46.69% ADF and 15.21% lignin [2]. Nutritive value of low-quality roughage such as sorghum straw can be improved through treatment methods (physical, biological, chemical or combination of these treatments) and supplementation.

    Supplementation is the most practical method in small scale farmers particularly by utilization of legume forage. One of legume forage which grows easily in Indonesia is gliricidia (Gliricidia sepium). It contains high crude protein (22.5-29.1%) with high protein degradability (78.5-98.2%) [3], leading to rapid production of ammonia. Ammonia may be used efficiently for microbial protein production
by addition of fermentable energy source [4]. Nitrogen and energy should be simultaneously available to enhance microbial growth rate and nutrient utilization efficiency [5]. Various fermentable energy sources available in Indonesia which can be used such as rice bran, corn or sorghum grain. Rice bran is by-products from rice polishing and abundantly available in rice production area, while corn is the second largest cereals crop which grows for food or animals feed. Sorghum grain mostly available in dry areas where rice could not grow well. Therefore, sorghum grain can be used as alternatives of carbohydrate sources in dry areas. The objective of this study was to evaluate the effect of supplementation of various source of fermentable energy in sorghum straw-gliricidia mixture-based diet on in vitro gas production kinetics and methane production.

2. Materials and methods

2.1. Diet treatments and experimental design
A mixture of sweet sorghum straw (Super-1 varieties) mixed with gliricidia foliage at ratio 60:40% DM was used as control diet. Treatment diets were formulated by supplementing control diet with ground corn grain, rice bran or sorghum grain at the level of 10% as sources of fermentable carbohydrates. The study was conducted in randomized complete design.

2.2. In vitro study
Sample was incubated for 48 hours, gas production was recorded at 4, 8, 12, 16, 24, 36 and 48 hours. The fermentation was conducted for 48 hours under anaerobic condition at 39°C according to procedure of Theodoru and Brooks [6], medium for incubation was prepared according to Menke and Steingass [7]. Fermentation kinetics for gas production was analyzed using the non-linear model [8]. The equation was $P = A + B \left(1 - e^{-ct}\right)$ where $P$ is the amount of gas produced (ml) at time $t$, $A$ is the intercept of the gas production curve at time zero and represent as gas produced from the fermentation of soluble fraction (%), $B$ is the gas produced from fermentation of rumen-insoluble, but slowly degradable fraction (%), $c$ is the rate constant of gas production of the B fraction (%/h) and $t$ is the incubation time (h).

Data collected were analyzed using general linier model procedure in SAS package version 9.1. Means were compared using Duncan’s Multiple Range Test.

3. Results and discussion
Table 1 shows fermentation kinetics of the diet treatments. Gas production from soluble fraction ($A$) was not affected by the treatments. However, corn supplementation yielded higher gas production from the fermentation of insoluble fraction ($B$ values) than rice bran, although these results were not different with the control. Similarly, carbohydrate supplementation did not increase rate of gas production from insoluble fraction. The non significant effect of carbohydrate supplementation to sorghum straw-gliricidia mixture on fermentation kinetics of gas production indicates that carbohydrate contained in the sorghum straw was able to synchronize the available nitrogen from rapidly fermentable protein from gliricidia [9], consequently the diet fermentation was stimulated without fermentable carbohydrate supplementation. The available fermentable carbohydrate in sorghum straw used in the current study originated from the sweet sorghum varieties (sweet sorghum Super-1), which is contained 15.1% sugar in the stalk [10].

Synchronizing available energy and nitrogen in sorghum straw and gliricidia mixture enhanced cellulolytic activity of microbes [11]. The lower gas production in rice bran supplementation was slow down after 8 hours fermentation (Figure 1), this due to 60 % of dry mater in rice bran was water soluble [12]. From this in vitro study the results of fermentation kinetics of gas production indicated that sorghum straw from sweet variety mixed with gliricidia in the diet produced comparable fermentation pattern to other diets, thus, the basal diet did not need fermentable carbohydrate supplementation. However further study using in vivo trial is needed to further evaluation.
Table 1. *In vitro* fermentation kinetics of gas production in 48 hours incubation

| Diet treatments | Fermentation kinetics |   |   |
|-----------------|-----------------------|---|---|
|                 | A<sup>1</sup>         | B<sup>2</sup> | c<sup>3</sup> |
| Control         | -4.02                 | 106.07ab | 0.0473 |
| Corn grain      | -3.74                 | 113.92a  | 0.0452 |
| Rice bran       | -3.28                 | 99.97b   | 0.0461 |
| Sorghum         | -3.63                 | 105.85ab | 0.0477 |

Different letter in the same column indicates significant different (P<0.05)

1 Considered as gas produced from the fermentation of immediately soluble materials
2 Gas produced from the fermentation insoluble fraction, but fermentable
3 Rate of gas production of B fraction

Figure 1. Cumulative gas production of sorghum straw+gliricidia mixed feed supplemented with corn grain rice bran, and sorghum grain incubated for 48 hours.

Methane production following sorghum grain supplementation was lower (P<0.05) than control and corn grain, but it was not different to rice bran supplementation. However, when the methane production was calculated as proportion of total gas production it shows that the highest methane proportion was yielded in rice bran supplementation. The higher methane production in rice bran supplementation could be due higher NDF content in rice bran (21.5 %) [13] compared to corn (11.77-12.7%) [14] and sorghum (7.7-15.9%) [15]. Cell wall carbohydrate (NDF) degradation produced hydrogen which is used by methanogen to produce methane [5]. Although sorghum grain has lower fibre content but its total gas production comparable to rice bran supplementation (Table 2).

Table 2. Total gas and methane production of treatment diets incubated for 48 hours

| Diet treatments | Gas production parameters |   |   |
|-----------------|---------------------------|---|---|
|                 | Total gas (ml) | Total CH₄ (ml) | % CH/total gas |
| Control         | 104.06<sup>a</sup>   | 41.24<sup>a</sup> | 39.55<sup>c</sup> |
| Corn grain      | 93.80<sup>ab</sup>    | 38.56<sup>a</sup> | 41.13<sup>b</sup> |
| Rice bran       | 84.92<sup>b</sup>     | 36.86<sup>ab</sup> | 43.4<sup>a</sup> |
| Sorghum         | 83.96<sup>b</sup>     | 32.25<sup>b</sup> | 38.30<sup>d</sup> |

Different letter in the same column indicates significant different (P<0.05)

The lower gas production in sorghum grain is related to the association of protein matrix with starch granules [16] which caused lower digestibility of sorghum grain. Sorghum grain supplementation produced lower gas production but its proportion of methane production to total gas production was the lowest. The lowest methane production in sorghum could be due to tannin content
in sorghum (0.1-1.35%) [15]. Tannin reduces methane emission because tannin has inhibitory effect on methanogenesis [17].

4. Conclusion
Supplementation of fermentable carbohydrate was not needed in sorghum straw-gliciridia basal diet since the supplementation did not increase fermentation of insoluble fraction of the diet. However, sorghum grain supplementation reduced methane production. In dry area where only sorghum is able to grow, sorghum grain can be used as fermentable carbohydrate source with similar effect to other fermentable carbohydrate sources on fermentation kinetics.

References
[1] Sanchez A C, Subudhi P K, Rosenow D T and Nguyen H T 2002 Plant Mol. Biol. 48 713-26
[2] Akinfemi A, Adu O A and Doherty F 2010 Afr. J. Biotechnol. 9 1706–12
[3] Kabi F and Lutakome P 2013 Nutr. J. Anim. Sci. Adv. 3 320–33
[4] Corbett J L 1987 (CSIRO: Canberra) pp 341–355
[5] Moss A R, Jouany Jean-Pierre and Newbold J 2000 Ann. Zootech. 49 231–53
[6] Theodorou M K and Brooks A E 1990 (Chatham: The Natural Resources Institute)
[7] Menke K H and Steingass H 1988 Anim. Res. Dev. 28 7–55
[8] Orskov E R and McDonald I 1979 J. Agric. Sci. 92 499–503
[9] Avilés-Nieto J N, Valle-Cerdán J L, Castrejón-Pineda F, Angeles-Campos S and Vargas-Bello-Pérez E 2013 Trop. Anim. Health Prod. 45 1357–62
[10] Pabendon M B, Efendi R, Santos O B and Prastowo B 2017 IOP Conf. Ser.: Earth Environ. Sci. 65 1–10
[11] Getachew G, Depeters E J, Robinson P H and Fadel J G 2005 Anim. Feed Sci. Technol. 123-124 547-59
[12] Yulistiani D, Jelan Z A and Liang J B 2008 JITV 13 264-72
[13] Warren B E and Farrel D J 1999 Anim. Feed Sci. Technol. 27 219–28
[14] McCann Melinda C, William A T, Riordan S G, Sorbet R, Bogdanova N N and Sidhu R S 2007 J. Agric. Food Chem. 55 4034-42
[15] Aguerre M, Cajarville C and Repetto J L 2015 Anim. Feed Sci. Technol. 205 75–81
[16] Offner A, Bach A and Sauvant D 2003 Anim. Feed Sci. Technol. 106 81–93
[17] Patra A K and Saxena J 2010 Phytochemistry 71 1198–222