Methane Production and Rumen Fermentation Characteristics of Buffalo Ration Containing Sorghum Silage with Rumen Simulation Technique (RUSITEC) Methods

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ABSTRACT: This study was conducted to evaluate buffalo ration containing samurai 2 sorghum straw silage compared with buffalo ration containing pahat sorghum straw silage using rumen simulation technique (RUSITEC) method. RUSITEC Analysis was conducted for eleven days, consisted of five days for adaptation period and six days another for observation period. Statistics design with 2 treatments and 3 replications was applied in this experiment. The fermentation of responses by time were presented descriptively. Averages of responses were analyzed using student t-test. Two treatments ration were: P1 (50% pahat sorghum straw silage + 50% concentrate) and P2 (50% samurai 2 sorghum straw silage + 50% concentrate). Variables measured were total gas, methane (CH4) production and ruminal fermentation products. Results showed that buffalo ration containing silage of Samurai 2 straw (P2) produced 27.76% lower methane production than buffalo ration containing silage of Pahat straw (P1) (P<0.01). The P2 treatment also produced the highest VFA 24.35% compared to P1, IVDMD (6.22%) and IVOMD (10.56%) (P<0.05). The pH value and total gas production were not significant different. The conclusion showed that buffalo ration containing silage of Samurai 2 straw produced the lowest methane concentration, the highest total VFA, IVDMD and IVOMD.

Keywords: buffalo, CH_4, rumen fermentation, RUSITEC, sorghum silage

INTRODUCTION

Buffalo was a ruminant germplasm in Indonesia which production and conservation needs to be maintained. Although buffalo was also part of the methane (CH_4) emissions contributor from the agricultural and livestock sector. Buffalo population was the second most contributor of enteric methane emission (11.3%) of total enteric methane from livestock meanwhile the largest enteric methane contributor was from cattle (around 74%) (Patra, 2014). In many cases, buffalo productivity should be balanced with good ration to reduce the methane emissions. Methane emissions reflects the loss of some energy that could not be utilized for the production process (Jayanegara *et al.*, 2009).

Sorghum is an alternative roughahe for buffalo to increase the productivity. Pahat and Samurai 2 were sorgum varieties derived from mutation radiation breeding in Indonesia National Nuclear Energy Agency (BATAN). Samurai 2 variety was mutation product from Pahat sorghum (Human, 2013). These two varieties potential to be used as a roughage for dairy buffalo in Indonesia. Combination of concentrate and chopped sorghum bagasse as roughage (50:50 at dry matter/DM) were able to increase Murrah buffalo milk fat content (7.61%) and maintaining milk production above 5 kg/day (Seshaiah *et al.*, 2013). Roughage as fibre source could be made as silage form. Silage treatment on sorghum was to preserve and increase the soluble fiber content as roughage source (Colombo *et al.,* 2007).

Early study to evaluate efficiency of buffalo fed containing sorghum on ruminal fermentation must be done. Rumen simulation technique (RUSITEC) was continous cultures that can be use as tools to evaluate the effects of diets on ruminal fermentation including methane production. The use of this fermenters type may provide useful information before designing and conducting time-consuming in vivo studies (Garcia-Gonzalez *et al*., 2010). The objectives of this study were to evaluate effect of buffalo fed containing sorghum straw silage on methane production and ruminal fermentation product in RUSITEC fermenters.

MATERIAL AND METHODS

Material and treatments. Pahat and Samurai 2 sorghum were harvested at 80 days. Sorghum straw used include leaves and stems in this study. Silage sorghum straw was made by incubation for 21 days. The research material was dried in the oven at 60 °C and milled and screened at a size of 2 mm. Concentrates composition made by grinding various of feed ingredients (% DM) such as soybean meal (9%), pollard (10%), cassava waste (29%), rice bran (28.5%), soy pulp (15%), lacto-mineral (2%), urea (1.5%), salt (1%), lime (1%), molasses (3%) and then mixed into horizontally-mixer. Formula diets were based on nutritional needs for lactating buffaloes (8% CP and 55-56% TDN) (Parakkasi 1999). Feed ingredients were: P1 (50%:50% Pahat sorghum straw silage:concentrate) and P2 (50%:50% Samurai 2 sorghum straw silage:concentrate). Nutrient composition were presented in Table 1.

Table 1. Feed Ingredients and nutrient composition for each treatments

Nutrient composition (% DM)	Treatments	
	P1	P2
Dry Matter (DM)	89.51	88.08
Organic Matter (OM)	85.34	85.34
Crude Protein (CP)	12.15	11.86
Crude Fiber (CF)	22.67	26.26
Ether Extract (EE)	1.14	1.38
Nitrogen Free Extract (NFE)	49.38	45.84

P1 (50%:50% Pahat sorghum straw silage:concentrate); P2 (50%:50% Samurai 2 sorghum straw silage:concentrate); Proximate analyses by animal feed science and technology laboratory (2014).

Semi-continous in vitro system. Cultures of mixed ruminal microorganisms were maintained in Rumen Simulation Technique (RUSITEC). The fermentation equipment included six 800 ml fermentation vessels was usued in this study. The general incubation procedure by Czzerkawski and Breckenridge (1977) and described in detail by Kajikawa *et al.,* (2003). Ruminal inocula was collected from a cannulated buffalo bull (300 kg body weight) fed 50%:50% field grass:concentrate diet two equal meals per day. The fermentation inocula (solid and fluid) were collected through the rumen cannula before morning feeding. Fluid was collected from strained through four layer cheesecloth. The supernatant, diluted 1:1 with buffer (McDougall, 1948), was used as fluid innoculum for the fermentation vessels. Solid digesta were collected from strained digesta.

Around 800 ml of fluid inoculum and 75 g solid inoculum (contained in nylon bag, 50 µm pore size), as well as 15 g feed (Table 1) in separate nylon bag were placed in each vessel. Continual infusion of artificial saliva (McDougall, 1948) was maintained at the rate ± 400 ml through each vessel during study. The experiment in RUSITEC lasted 11 days. To ensure a steady state with in the vessels, an adjustment periods for the first 5 days was allowed. Measurements were on days 6 to11.

Measurements and chemical analyses. Produced gas was collected into gas bag (5 l A SANSHIN®). Gas volumes were measured with a gas meter and methane concentration were analysed in a MRU VarioPlus gas analyzer. A liquid effluent was collected and samples were taken for pH, ammonia (NH_3-N) (Conway, 1962), total VFA (AOAC, 1991), individual VFA (acetate, propionate and butyrate) (Cottyn and Boucque, 1968), In Vitro Dry Matter Degradability (IVDMD) and In Vitro Organic Matter Degradability (IVOMD) (AOAC, 1991).

Statistical analyses. The fermentation of responses by time (6 days) were presented descriptively. The average fermentation parameters would be statistically analyzed using t-test with 18 replications (6th day observation x 3 vessel replications). Analyses of Variance (ANOVA) was performed with SPSS 16.0. (Matjik and Sumertajaya, 2006)

RESULTS AND DISCUSSION

Daily changes in the fermentation parameters of the RUSITEC from the sixth day of the incubation to the eleventh day were showed in Fig 1. The total gas production, pH, IVDMD and IVOMD became steady during incubation while methane production, NH_3-N and total VFA were fluctuate. Methane production on P2 was always lower than P1 during incubation (Figure 1A). Enteric methane production depend on several factor: 1) roughage age and type (Arthington and Brown, 2005) and 2) acetate and propionate ratio on fermentation product (McAllister *et al.,* 1996). The first factor was not cause the different of methane production due to the roughage sources were harvested at the same age. It could be caused by buffalo fed containing silage of Samurai 2 straw could optimize cellulolitic rumen microbes activity such as propionic bacteria (genus Prevotella) to compete methanogenic bacteria. The P2 average propionic product was higher than P1 (Table 2). In Table 2 also seen the production in P1 acetic was higher than P2. In the cycle of acetate and butyrate formation, hydrogen as a raw methane material would be produced, while in the propionic formation required a hydrogen ion. That was the basic lines of hydrogen utilization competition between propionic production and methanogenesis (Li *et al*., 2014).

Table 2. Statistical analyses results of an average ruminal fermentation on RUSITEC

Peubah	Treatment		SEM	Significant
	P1	P2		
Total gas production (ml/d)	1038.33	938.89	30.704	ns
Methane production (ml/d)	67.87^a	49.03^b	0.187	**
pH	6.99	6.97	0.018	ns
NH3-N (mg/100 ml)	21.53^b	25.73^a	0.950	*
Total VFA (mM)	58.44^b	72.67^a	2.102	**
Individual VFA (molar %)				
Acetate	46.86^a	40.58^b	0.745	*
Propionate	34.84b	37.69^a	0.522	*
Butyrate	18.30^b	21.73^a	0.608	*
IVDMD (%)	38.76^b	41.17^a	0.503	*
IVOMD (%)	39.57^b	43.75^a	0.566	*

P1 (50%:50% Pahat sorghum straw silage:concentrate); P2 (50%:50% Samurai 2 sorghum straw silage:concentrate); * significant (P<0.05); ** highly significant (P<0.01); ns (non significant).

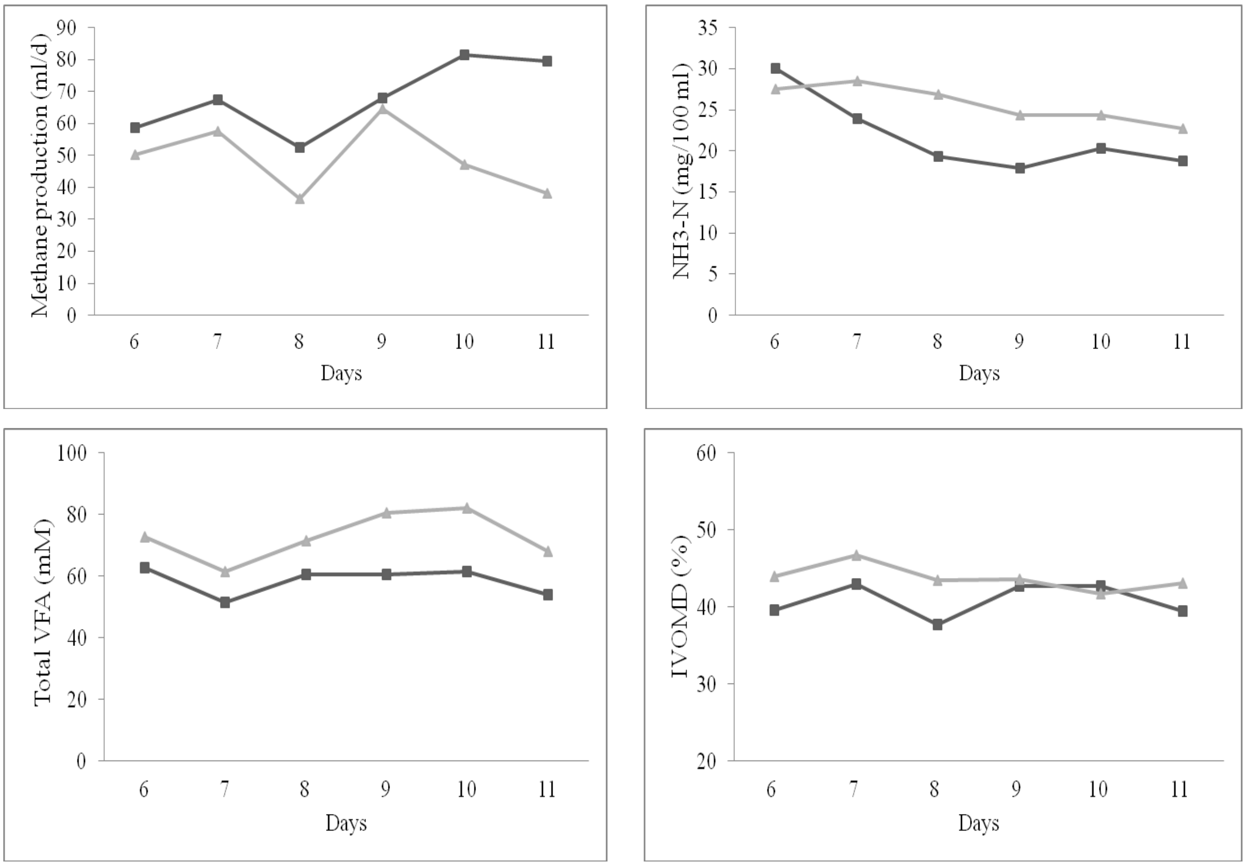

Fig 1. Ruminal Fermentation pattern in RUSITEC. A) methane production (ml/d), B) NH3-N (mg/100 ml), C) total VFA (mM), D) IVOMD. (◆——◆)P1 and (◄——►) P2.

pH values were not significantly different both rations and in neutral range. Kajikawa *et al*., (2003) reported that RUSITEC pH effluent range was usually 6.80 to 7.00. Falling of pH rumen would affect cellulolytic bacteria activity and population, which help the fermentation process (Martinez *et al.,* 2010). P2 was produce an average NH_3-N higher than P1, whereas lower CP content (Table 1). It was thought to be caused by protein solubility of P2 better than P1. Öztürk (2009) reported that in RUSITEC methods, NH_3–N concentration depends on balance between deamination of amino acids and NH_3-N used by rumen microbes. Total VFA production of P2 was much higher than P1 (Table 2). It was prove that soluble fiber content in Samurai 2 variety was higher than Pahat. The mean of P2 IVDMD and IVOMD were higher than P2. That probably caused by fiber structure changes in Samurai 2 sorghum varieties which was result from radiation mutation breeding from Pahat sorghum, but it needs to be studied more deeply. Chatterjee *et al.* (2006) suggested that degradation rate of high feed describes the availability of energy and protein to improve fermentation and microbial population growth.

CONCLUSION

The conclusion showed that buffalo diets containing silage of Samurai 2 straw produced the lowest methane concentration, the highest total VFA, NH_3-N, IVDMD and IVOMD on. It means this ration was the best roughage source for buffalo fed better than buffalo ration containing Pahat straw silage.

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