Differences in Drying Method of King Grass (*Pennisetum hybrid*) Silage Samples Prepared for *in Vitro* Digestibility Analysis

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Abstract

In vitro digestibility of silage was influenced sample preparation methods because silage contained volatile compounds had potentially losses during preparation. An experiment had been conducted to evaluate different methods for preparing of a silage sample which was used in vitro digestibility studies. King grass (Pennisetum hybrid) silage were sampled at 21 d incubation for in vitro gas production analysis. Sample preparation was conducted by oven drying at 60°C while freeze drying method at -20°C and both of them conducted during 20 hours. The variables measured were in vitro dry matter and organic matter digestibility (IVDMD and IVOMD), gas production, volatile fatty acids (VFA) production. The experiment was arranged on completely randomized design with t-test analysis. Results showed that either IVDMD or IVOMD from silage dried by oven and freeze drying methods were similar. Production of volatile fatty acid (VFA) and acetate: propionate ratio (C2:C3) had also no differences between silage prepared by oven and freeze drying methods. However, total gas production from silage during 48 hours incubation affected by drying methods significantly (P<0.05). Gas production of freeze dried silage (Y) could be predicted by gas production from oven dried silage (X) as followed the equation was Y=1.0846X+0.7947 (R2=0.997). It was concluded that oven drying method could be used for the sample preparing method of silage at the in vitro digestibility analysis.

Key words: freeze drying, gas production, in vitro digestibility, oven drying, silage

Introduction

Silage is defined as forage that is preserved in the controlled fermentation (McDonald *et al*., 1991). It is a complex routine preparation to determine chemical constitution and characterisation of silage products. Due to silage is a product of

microbial fermentation activity which produce volatile and unstable chemical compound such as ammonia, volatile fatty acids, and lactic acid (Rêgo *et al*., 2010). To overcome the problems, many researchers often use a freeze drying technique for a better sample preparation in future analysis (Grabber, 2009). However, there are limitations applying this method including relatively expensive equipment, more complex sample preparation, etc. On the other hand, there are some relatively simple preparation methods to proceed, such as oven drying (Kamarloiy and Yansari, 2008) or freeze drying (Calabrò *et al*., 2005, Grabber, 2009).

It is necessary to investigate effects of substrate preparation (i.e. freeze versus oven dried) on *in vitro* digestibility of king grass (*Pennisetum hybrid*) on the basis of *in vitro* gas production technique. This is because sample preparation method produced highly significant influence on *in vitro* degradation test. Calabrò *et al*. (2005) indicated that there was significant interaction between sample method preparation and fermentability parameters. Total gas production is known to give a good estimation of digestibility (Beuvink and Kogut, 1993). This method is also able to predict fractions of rumen fermentable organic matter, crude protein and starch escaping rumen degradation as well as *in vitro* organic matter digestibility (DeBoever *et al*., 2005).

Therefore, the objective of this study was to investigate the effect of sample preparation method on king grass silage digestibility based on determination of *in vitro* dry matter and organic matter digestibility, total gas and volatile fatty acids production using gas production technique.

Materials and methods

Silage was made from king grass (*Pennisetum hybrid*). Grass was wilted for 24 hours to increase dry matter (DM) content. Feed materials were chopped with shredded size 1-3 cm. Inoculants 1% (v/w) was added into grasses and water was also added to adjust moisture content up to 75%. After mixing homogenously, all ingredients were packed in plastic bag (5 kg/pack) and incubated for 21 days. Samples were prepared by two methods of drying. The first group was dried by oven-drying (60 °C) for 20 h, and the other one was dried by freeze-drying method using a freeze dryer Leybold-Heraeus GT Lyovac type-2 (Peterswan Ltd., Edinburgh) at -20 °C for 20 h. Samples were then ground with a mortar and sieved by a filter (1.0 mm screening).

Evaluation of silage digestibility, volatile fatty acids (VFA) and ammonia (NH3) productions were measured by the total gas production using Menke *et al*. (1979) that was modified by Jayanegara *et al*. (2009). *In vitro* DM and organic matter (OM) digestibility (IVDMD or IVOMD) were measured according to Blümmel *et al*. (1997) method. *In vitro* digestibility was determined by calculating degradation percentage of DM or OM after incubation for 24 h.

Ground silage samples (380 mg, DM 86.4%) were placed into the syringe to the pre-incubation for 24 h at 39 °C. Rumen fluid (10 ml) and buffer solution (20 ml) were inserted into syringe with saturated CO_2. Composition of buffer solution per 100 ml rumen fluid (Menke *et al*., 1979) consisted of macrominerals (23.7 ml), micro-minerals (0.012 ml), bicarbonate buffer solution (23.7 ml), resazurin 4% (0.122 ml), reducing solution (4.96 ml) and distilled water (47.5 ml). Rumen fluid was taken from fistulated beef cattle (Ongole crossbred) which had been conditioned to feeding standard (feed composition consisted of 60% forage and 40% concentrate). Gas production kinetics was calculated based on exponential equation (Ørskov & McDonald,1979). The estimated value of a, b, c were calculated by fitting curve method using Neway Software (Rowett Research Institute, Aberdeen, UK) installed at Microsoft Office Excel 2007® that was developed by Chen (1997). VFA was analysed using gas chromatography method (Friggens *et al*., 1998) and NH_3 analysis using spectrophotometric method (Broderick & Kang, 1980).

Evaluation of *in vitro* digestibility and fermentability were arranged on completely randomized design with 2 treatments. Each treatment consisted of 3 replications with 2 sub samples. Pangola grass (*Digitaria decumbens*) was used as standard sample and each syringes containing silage, standard samples and blank were randomly allocated in the incubator. Incubation was carried out for 48 h and gas production was observed at 0, 1, 2, 4, 6, 8, 12, 18, 24, 36, and 48 h after incubation. Data of IVDMD and IVOMD, gas production, VFA total, acetate (C2), propionate (C3), butyrate (C4), and NH_3 were analyzed with analysis of variance (ANOVA) and if among the treatments showed significant differences ($P<0.05$) followed by t-test (Gomez & Gomez, 2007).

Results and discussions

Effect of drying methods on *in vitro* IVDMD and IVOMD were shown at Table 1. IVOMD and IVDMD of silages prepared by freeze drying method were 5%-6% higher than those of silages prepared by oven drying method. However, drying methods had no significant influences on those variables. *In vitro* digestibility of silage could be influenced by many factors which were sample preparation methods and chemical component of feedstuff.

Fresh or freeze-dried silages tended to be more fermentable than oven-dried silage (Calabrò *et al*., 2005, Grabber *et al*., 2009), the nutritive value, especially protein and crude fiber in silage, was closely related to *in vitro* degradability (DeBoever *et al*., 2005). In many cases, overheating affected solubility of nutrients. Proteins were binding to NDF content in silage if drying temperature was more than 70 °C (Cone *et al*., 1996). Because of sample was dried at low temperature (60 °C), there was no significant alteration of total nutrient solubility indicated by a similarity in digestibility of oven-dried sample to that of freeze-dried sample.

Table 1. *In vitro* digestibilities, gas production parameters, production of volatile fatty acid (VFA) and amonia (NH_3) from silage prepared by oven and freeze drying

Variable		Drying Methods	
		Oven (60 °C)	Freeze Dry (-20 °C)
In vitro digestibility			
IVDMD[1]	(%)	43.74±3.18	46.29±8.62
IVOMD[2]	(%)	50.61±3.01	53.83±10.55
Gas production[3]			
a	(ml/h)	-0.450±0.401	-0.518±0.479
b	(ml/h)	48.32±2.623	50.746±4.675
a+b	(ml/h)	47.87±2.517	50.227±4.580
c	(ml/h)*	0.032±0.006b	0.025±0.003a
Total VFA	(mM)	161.11±63.74	162.99 ± 79.10
Acetate (C_2)	(mM)	114.11±47.86	109.79 ± 54.89
Propionate (C_3)	(mM)	34.89±11.93	39.25±17.61
Butyrate (C_4)	(mM)	12.11±5.31	13.96 ±7.94
Ratio C_2:C_3		3.20±0.63	2.75±0.39
NH_3	(mg/100 ml)	32.13±3.68	32.08±4.68

[1]IVDMD: *in vitro* Dry Matter Digestibility, [2]IVOMD: *in vitro* Organic Matter Digestibility, [3]Gas Production from Soluble Fraction (a) and Potential Soluble Fraction (b), and Rate of Gas Production (c), * Mean with different superscript at same row showed significant difference (P<0.05).

Calabrò *et al*. (2005) reported that there were no differences in OM degradability (707 vs 708 g/kg) from silage dried by freeze- and oven- drying (65 °C). VFA and ammonia were readily vapor by increasing temperature in drying chamber. Unstable chemical compound (VFA and NH_3) could be exhausted after silo was opened and silage was dried (Rêgo *et al*., 2010). To maintain volatile or unstable compounds, in silage could be conserved by freeze drying (Grabber, 2009). Differences of drying methods were significantly influence kinetics of gas production during 48 h incubation. Gas production was higher for freeze-dried sample than that of oven-dried sample (Figure 1A). This result indicated that gas production from silage was higher when it was prepared by freeze-drying than by oven-drying.

Gas production obtained from freeze-dried silage (Y) could be estimated by that from oven-dried silage (X) following the equation: Y= 1.085X+0.795 (R2= 0.998) (Figure 1B). This equation showed a significant analysis based on consistent increases in gas production from both silages prepared by different methods. Oven-dried silages could be used as estimation of gas production produced by freeze-dried silages. Deinum and Maassen (1994) stated that forage dried at at 70 ^oC produced

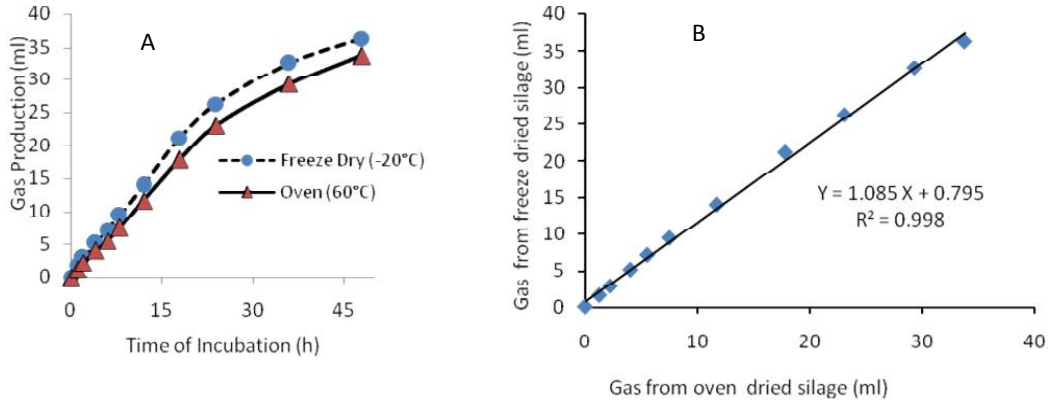

Figure 1. Gas production kinetics (A) and relationship of gas production (B) from silage prepared by freeze and oven drying

less nutrient destruction, but drying up to 105 °C caused more protein bound to NDF fraction and increased other compound losses. This was due to Maillard reaction to occur between amino acid (protein) and glucose that strengthened chemical linkage in those fractions and less fermentable indicated by lowering gas production.

Variables of soluble (a) and insoluble fraction (b) showed non-significant differences between sample prepared by both drying methods. However, gas production rate (c) data was able to show nutrient loses produced by oven-drying method. Fresh silage (200 mg) produced gas (45.6 ml) higher than dried silage (32.4 ml) at 24 h incubation (Calabrò *et al*., 2005). Drying at low temperature was able to keep volatile compound in silage, and this type of silage could be similar to that of fresh silage. Metabolites in silage were dominated by organic acids such as lactic, acetic, propionic, and butyric acids, N-ammonia (McDonald *et al*., 1991).

There were no significant differences between oven- and freeze- drying for silage preparing method on total VFA, acetate, propionate, butyrate and ammonia. Average productions of acetate, propionate and butyrate were 112.0, 37.1, and 13.1 mM, respectively, with average ratio of acetate (C2) and propionate (C3) was 2.98 and ammonia production was around 32.0 mg/100 ml. Calabrò *et al*. (2005) reported that freeze- and oven- drying produced different effects on corn silage, but were not statistically significant. The results showed that total VFA (90.90 vs 82.94 mmol/g OM), acetate (65,60 vs 54.56 mmol/g OM), propionate (23.4 vs 22.26 mmol/g OM), butyrate (1.99 vs 3.03 mmol/g OM). Moreover, those differences value showed that not significant differences. Based on fermentability variables, there were similarity in responses between the two methods. Volatile compound produced by microbes during ensilage was not significantly lost when silage sample was prepared by oven drying (60 °C, 20 h). A higher drying temperature caused many losses of compound in fresh forage decrease in digestion rate, conversely a lower drying temperature (50-70 °C) caused less several loses (Deinum & Maasen, 1994).

Conclusion

There were no differences between oven-dried (60 °C, 20 h) and freeze-dried (-20 °C, 20 h) samples on in vitro digestibility and fermentability. Gas production of freeze-dried silage (Y) could be predicted by that from oven-dried silage (X) using the following equation: Y= 1.085X+0.795 (R2= 0.998). It was concluded that oven-drying (60 °C, 20 h) method can be used for preparing silage samples in *in vitro* digestibility analysis.

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