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Impact of Rumen Liquor Inoculant Sources on Feed in Vitro Fermentability and Digestibility

Dampak Cairan Rumen Sumber Inokulan pada Fermentabilitas dan Kecernaan Pakan secara *In Vitro*

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ABSTRACT

The aim of this research is to compare alternative inoculant source for in vitro rumen fermentation. In the first experiment, inoculant from fistulated cattle kept in LIPI and IPB (Fis1 and Fis2) and inoculant from Bogor municipality abattoir and IPB abattoir (Abo1 and Abo2) were tested for their pH, total bacterial count, and protozoal number using a complete block design with four replications. In the second experiment, the effect of the inoculant sources was tested on cornmeal (F1), soybean oil meal (F2), Napier grass (F3), and dairy cattle complete ration (F4) fermentability and digestibility including pH, VFA, NH_3, IVDMD and IVOMD parameters. The results showed an unsignificant different protozoal number among inoculant sources. The pH of Fis2 rumen liquor was significantly lower ($p<0.05$) than others. The bacterial population was significantly higher ($p<0.05$) in Fis2 and Abo2 than Abo1, and Fis1. The inoculant pH after feed fermentability was not influenced by feed type but inoculant source with Fis1 was significantly higher ($p<0.05$) than Fis2, Abo2, and Abo1. The ammonia, VFA concentration, IVDMD, and IVOMD were influenced by interaction between inoculant sources and feed types. Although inoculant from cattle close to the laboratory (Fis2 and Abo2) were better in term of higher bacterial population, higher fermentability and digestibility for most type of feeds but other sources can be used in vitro study without differences in average fermentability and digestibility results.

Key words: abattoir, fermentability, fistula, inoculant, in vitro

ABSTRAK

Penelitian untuk mencari sumber inoculant alternative untuk kajian in vitro rumen sudah dilakukan. Pada percobaan pertama, inoculant dari sapi berfistula yang dipelihara di LIPI dan IPB (Fis1 dan Fis2) dan inoculant dari RPH Bogor dan RPH IPB (Abo1 dan Abo2) sudah diuji kualitas pH, total bakteri dan protozoanya menggunakan rancangan acak kelompok dengan empat ulangan. Pada percobaan kedua, efek sumber inoculant diuji pada fermentabilitas dan kecernaan jagung (F1), bungkil kedele (F2), rumput gajah (F3) dan ransum sapi perah (F4) termasuk parameter pH, VFA, NH_3, KCBK, KCBO. Hasil memperlihatkan jumlah protozoa tidak berbeda antar sumber inoculant. Fis2 memiliki pH terendah. Jumlah populasi bakteri lebih tinggi pada Fis2 dibandingkan Abo1, Abo2 dan Fis1. Nilai pH inoculant setelah fermentasi pakan tidak dipengaruhi oleh jenis pakan namun oleh sumber inoculant dimana Fis1 lebih tinggi dibandingkan Fis1, Abo1 dan Abo2. Kandungan NH_3, VFA, KCBK dan KCBO dipengaruhi oleh interaksi sumber inoculant dan jenis pakan. Meskipun inoculant yang berasal dari sapi fistula yang dipelihara dekat laboratorium lebih baik, namun sumber inolukum lain dapat digunakan tanpa perbedaan rataan fermentabilitas dan kecernaan pakan.

Kata kunci: fermentabilitas, fistula, inokulan, *in vitro*, RPH

INTRODUCTION

Feed fermentability and digestibility information can be used as a better prediction of animal performance in comparison to feed chemical composition due to the different biological value of feedstuff for an animal (Despal 2000). However, direct digestibility measurement on the animal is expensive, time-consuming, and need a large number of sample. Therefore, many alternative methods have been developed including the enzymatic method (Dehority and Johnson 1963), gas test method (Menke *et al*. 1979) and two-stage method (Tilley & Terry 1963). According to Despal (2000), crude fiber chemical fraction can be used to estimate in vivo digestibility. Indah *et al*. (2020) explore in more detail the fiber fraction and found that neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose ability to predict digestibility. Although chemical composition can be used in estimating in vivo digestibility, however, according to Aerts *et al*. (1977), better results were obtained with methods using living rumen micro-organisms such as the two-stage technique.

The two-stage technique was developed by Tilley & Terry (1963). The method consisted of incubation with rumen liquor and then with acid pepsin. Several factors influence the results of the technique including fineness of grinding, sample size, pH of original rumen fluid, and size of rumen fluid inoculant (McLeod & Minson 1969). Although many inoculants can be used as microbial sources for the fermentation stage, however, according to Tilley & Terry (1963), an adequate level of efficient digestion was supplied from strained rumen liquor collected from rumen fistulated animal.

In developing countries with many facility restrictions, it is difficult to provide and maintain the fistulated animal. Therefore, an alternative source of inoculant for in vitro study are needed including sharing facilities from other research institution and rumen liquor from an abattoir. According to Li *et al*. (2009), different locations and times for rumen liquor collection influence microbial diversity which may lead to different rumen fermentation activity. Microbial composition was also influenced by feed types, age, and seasons (Lengowski *et al*. 2016). So far, there is still limited information available on the quality of the alternative rumen liquor sources and their effect on different feed fermentability and digestibility. This study was aimed to compare the quality of rumen liquor from a different source and to study their effect on feed fermentabilities and digestibilities.

METHOD

Experiment 1. Rumen Liquor Quality Measurement

Four different inoculant were collected from different sources and compared in the first experiment. They were rumen liquor collected from fistulated cattle kept in Indonesian Institute of Science, Cibinong, Bogor Regency (Fis1), rumen liquor from fistulated cattle kept in Faculty of Animal Science field laboratory, Darmaga, Bogor Regency (Fis2), rumen liquor collected from Bogor municipality abattoir, Bubulak, Bogor municipality (Abo1) and rumen liquor collected from IPB Abattoir, Darmaga Campus, Bogor Regency (Abo2). From each location, rumen liquor collected was transported to laboratory in a pre-warm airtight container. The sources were located 22 km, 0.5 km, 6.5 km and 0.5 km from the laboratory, respectively. The Fis1 was collected from Ongole crossbreed fistulated cattle, while Fis2 was collected from Frisian Holstein fistulated cattle. The Fis2 cattle was fed with 25 kg fresh Napier grass (19.8% DM, 10.68% ash, 9.82% CP, 1.71% EE and 37.68% CF) and 6 kg commercial concentrates (85% DM, 10.3% ash, 17.82% CP, 1.48% Ca and 0.59% P) daily. The Abo1 and Abo2 were mainly local cattle (Bali and Ongole Crossbreed). In Abo1, cattle mainly came from traditional farmers near Bogor, while in Abo2, cattle came from farther distances and larger feedlots. In Fis1 and Fis2 treatments, 1 fistulated cattle was used with 4 different time of rumen liquor collection, while in Abo1 and Abo2, 4 different slaughtered cattle were used.

Parameters observed in this experiment including pH, total bacterial count, and protozoal number. Rumen pH was measured using a digital pH meter (pHep from Hanna Instrument, Romania). The pH meter was pre-calibrated using the standard solution at pH 4 and 7. Total bacterial were counted using Ogimoto and Imai (1981) procedure. BHI media was made by mix BHI powder with other ingredients needed for microbial growth such as glucose, starch, hemin, resazurin, cysteine and CMC 1%. All ingredients were warmed until changing color from red to yellow. The media was cooling down while flown with CO_2 and 5 ml of the media were pipetted into 5 ml tube. The tubes were closed with a rubber prop and sterilized at 21°C for 15 minutes at 1 atm air pressure. Dilution media were made by mixing Na_2CO_3, cysteine, mineral I (0.6 g Na_2CO_3 in 100 ml), mineral II (1.2 g NaCl, 1.2 g $(NH4)_3SO_4$, 0.6 g KH_2PO_4, 0.25 g $MgSO_4$. $7H_2O$, 0.12 g $CaCl_2$ in 100 ml), and resazurin. CO_2 gas was flown into media until it changing color into white clear. The amount of 4.95 ml of the media were put into a Hungate tube, sterilized in 120°C autoclave for 15 minute. The amount of 0.05 ml rumen fluid were put into the Hungate tube. Serial dilution was made four times. The amount of 0.1 ml of each dilution were pipetted into the BIII media. The media were homogenized using a roller tube until the media solid and distributed evenly on the tube wall. The tubes were incubated at 39°C for 24 hours. Total bacterial were count using the following formula: BP (cfu ml-1) = n / (0.05 x 10x x 0.1), where BP

was bacterial population, n was the number of colony-forming unit found in x serial of dilution.

The protozoal population was counted using Ogimoto & Imai (1981) procedure. The amount of 1 ml rumen fluid was pipetted into 1 ml TBFS solution. The mixed solution was dropped into a counting chamber and observed under a microscope with 10x magnification. Protozoal population were counted using formula PP (cell ml^{-1}) = (1 / (0.1 x 0.0025 x 15 x 5)) x 1000 x FP x C) where C is protozoal counted from counting chamber and FP is dilution factor.

Block randomized design was used in this experiment with 4 sources of inoculant as the treatments and 4 replications as a block. Data obtained were analyzed using ANOVA and followed by Duncan multiple rank test.

Experiment II Impact of Inoculant Sources on Feed Fermentability And Digestibility

Four different inoculant sources similar to in Experiment I were used to study effect of four type feeds fermentability and digestibility (Tilley & Terry 1963). The feeds used were corn meal as representation of energy feed source (F1), soybean oil meal as representation of protein feed source (F2), Napier grass as representation of fiber feed sources (F3) and dairy cattle complete ration (F4) consisted of 37% natural grass, 23% Napier grass, 20% corn meal, 4.7% cassava waste, 3% coconut oil meal, 12% soybean oil meal, and 0.3% $CaCO_3$. The F4 contained 64.75% TDN, 13.31% CP, 0.45% Ca, and 0.32% P. Parameters observed in the second experiment were pH, VFA, NH_3, in vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD).

In the stage 1, fermentative digestion was conducted in 100 ml centrifuge tube. The amount of 0.5 g feed was added with 40 ml McDougall buffer and 10 ml rumen liquor. The tube was flown with CO_2 gas for 30 seconds. The tube was sealed with a ventilate rubber prop and incubated in 39°C water shaker bath for 4 hours (Tilley and Terry 1963) . The supernatant was collected after the fermentation were ended by adding 1 ml H_2SO_4 15% and centrifuge at 3000 rpm for 15 minutes. The supernatant were used for pH, VFA, and NH_3 concentration measurement.

The pH value was measured using a similar procedure as in Experiment I. The VFA was measured using the steam distillation method (General Laboratory Procedures 1966). The amount of 5 ml supernatant was pipetted into the distillation tube and added with 1 ml H_2SO_4 15%. The tube was closed. The distillation process was lasted until condensation water collected in Erlenmeyer filled with 5 ml NaOH 0.5 N about 250 ml. The Erlenmeyer was then titrated using HCl 0.5 N. About 2 – 3 drops of phenolphthalein indication were added before the titration process. Titration was stopped if the color changing into light pink. VFA concentration was calculated using the formula: VFA (mmol) = ((a – b) x N HCl x 1000/5) / (sample weight x DM sample), where a is HCl needed for blank titration, b is HCl needed for sample titration, N is the normality of HCl.

Ammonia (NH_3) concentration was measured using Conway and O'Malley (1942). The amount of 1 ml of Na_2CO_3 were pipetted into one section of the Conway chamber. The amount of 1 ml supernatant was put into another section of the chamber. The amount of 1 ml boric acid indicator was put in the middle of the chamber. The chamber was closed and airtight. The supernatant and Na_2CO_3 were mixed inside the chamber by gently shake the chamber. The chamber was left at room temperature for 24 hours. After 24 hours, the boric acid indicator was titrated using H_2SO_4 until the color return to the boric acid initial color. Ammonia concentration were calculated using formula: NH_3 (mmol) = (ml H_2SO_4 x N H_2SO_4 x 1000) / (sample weight in g x % DM sample).

IVDMD and IVOMD followed similar fermentation procedures to collect the supernatant. However, for IVDMD and IVOMD, fermentation lasted for 48 hours. After 48 hours, the fermentation process was terminated by adding 1 ml H_2SO_4 15%. The tubes were centrifuged for 15 minutes at 3000 rpm. The supernatant was removed and replaced with 50 ml acid pepsin solution. The incubation continues for the next 48 hours at 39°C aerobically. After 48 hours, the tubes were filter into a predetermined weight of Whatman paper no 41 with the help of a vacuum pump. The Whatman paper was then folded and dried for 24 hours at 105°C. IVDMD and IVOMD were calculated as the difference between dry matter and organic matter sample weight with dry matter and organic matter residue and represented as a percentage.

This experiment used a factorial block design with inoculant sources as factor A and feed types as factor B. Each replication was repeated four times as a block. Data obtained were analyzed using ANOVA continued with contras orthogonal.

RESULT AND DISCUSSION

Rumen Liquor Quality from Different Sources

Rumen liquor quality as measured from pH, bacterial and protozoal number are shown in Table 1. The table showed that protozoal number were not significantly different in all rumen liquor sources. The value ranged from 5.67 to 5.78 log cell ml^{-1} were comparable to the total protozoal 5.68 log cell ml^{-1} reported by Hungate *et al.* (1971) with comprised of 73% *Entodinium simplex*, 12.4% *E. caudatum*, 3.1% *Dasytricha*, 0.6% *E. vorax*, 1.4% *Eudiplodinium*, 5.8% *Ostracodinium*, 2.7% *Ophryoscolex* and 1% *Polyplastron*. The unsignificant

Table 1 Rumen liquor quality of different sources

Inoculant source	pH	Bacterial count (log CFU ml^{-1})	Protozoal count (log cell ml^{-1})
Fis1	7.15±0.19^{a}	7.78±0.17^{c}	5.78±0.05
Fis2	6.13±0.15^{b}	9.76±0.13^{ab}	5.76±0.37
Abo1	6.91±0.08^{a}	9.69±0.04^{b}	5.67±0.05
Abo2	6.95±0.04^{a}	9.88±0.07^{a}	5.70±0.02

CFU = Colony Forming Unit; different superscripts at similar column are different significantly (p<0.05)

difference in the protozoal number found in this experiment showed that protozoa are more resilient than bacteria.

The bacterial count in Table 1 showed a significant difference (p< 0.05) between rumen liquor sources. Fis2 and Abo2 were significantly higher (p<0.05) than Fis1, while Abo2 was significantly higher (p<0.05) than Abo1. The experiment shows that rumen liquor from fistulated cattle kept far away (22 km) from the laboratory had the lowest bacterial count. It might be caused by the reduction of microbial population during transportation to the laboratory due to difficulty in maintaining an anaerobic condition. Although cellulolytic bacteria and methanogenic archaea are particularly sensitive to declines in pH (Russel 2009), however, due to the rapid process of rumen liquor handling in Fis2 (0.5 km), the bacterial count was still high. The pH value from different inoculant source ranged from 6.13 to 7.15. A lower pH value in Fis2 might also cause by a higher proportion of concentrate used (Dijkstra et al. 2012) to fulfill higher requirement of Frisian Holstein (Fis2) in comparison to Ongole crossbreed (Fis1). The higher proportion of concentrate reduced saliva production which acted as a buffer (Weimer 2015) and drag the pH value further down. According to Erfle et al. (1982), rumen pH can vary from greater than 7 to less than 5 depending on the type of diet fed to an animal. The higher pH found in Fis1 might cause by the higher proportion of forage used in the ration. Erfle et al. (1982) explained that the pH of the rumen contents is near neutrality on a predominantly forage diet and can reach pH near or below 5 when high grain diets are fed.

Impact of Inoculant Sources on Feed Fermentability and Digestibility

The impact of inoculant sources of different feed fermentability and digestibility are shown in Table 2. The table showed that there was no significant difference (p>0.05) of feed type effect on rumen pH after fermentation but source of inoculant. The table shows the pH of Fis1 was higher than Fis2 and Abo2, and Abo1. All inoculant sources have pH after fermentation in the normal range (Aerts *et al.* 1977). The higher pH in Fis1 was related to the initial pH of the rumen liquor. In Fis2 however, the pH increased after feed fermentation due to the effect of artificial saliva was higher than acid produced during the fermentation. In general, fermentation of concentrate reduce rumen pH (Dijkstra *et al.* 2012), however, in F1 and F2 of this experiment, the pH after fermentation was not significantly reduced (p> 0.05). It might cause by the use of corn that is less degradable (Despal *et al.* 2011) and soybean meal that contains several anti-nutrition which might inhibit rapid degradation of the feed (Cheng *et al.* 2019) as a representation of energy and protein feed sources, respectively.

Fermentability and digestibility measured in this experiment show an interaction between factors observed which indicated that inoculant from different sources has different microbial composition depending on the type of feed given to the cattle (Erfle *et al.* 1982). On average, fermentation and digestion activities of all inoculant sources relatively similar. Fis1 has a higher Ammonia concentration on F1, F3, and F4 than Fis2. However, in F2, Fis2 has a higher ammonia concentration. This might indicate a higher proteolytic bacterial activity in Fis2, while Fis1 has higher amylolytic and fibrolytic bacterial activity. This data was supported by a higher VFA concentration in Fis2 for F3 and F4 in comparison to Fis1. Abo2 have higher ammonia concentration in compare to Abo1 for all type of feed which represent the higher fermentation activity. These data were also supported by the VFA concentration of Abo2 that higher than Abo1 in all feeds type. The higher ammonia concentration produced in F2 is caused by the higher crude protein content in the soybean oil meal (Prayitno *et al.* 2018). All ammonia and VFA concentrations in tested feed by all inoculant sources were in the normal range (6 – 21 mmol for Ammonia and 80 – 160 mmol for VFA) according to McDonald *et al.* (2010), except for cornmeal by Fis2 and complete feed by Abo1.

Inoculant impact on digestibility of dry matter and organic matter relatively similar in all feedstuffs. Fis2 and Abo2 produced higher IVDMD and IVOMD. It might cause by the higher microbial population on the inoculant source (Czerkawski 1986) resulted from the closer location (0.5 km) to the laboratory which might reduce the risk of microbial death during transportation. IVDMD and IVOMD found in this experiment were in the normal range for each feed type. Cornmeal and soybean meal had higher digestibility (> 85%) due to low fiber fractions in the feeds. While Napier grass and complete ration had lower IVDMD and IVOMD (< 67%) due to higher fiber fraction in the feed (Despal *et al.* 2017).

Table 2 Impact of inoculant on different feed fermentability and digestibility

Feeds	Fis1	Fis2	Abo1	Abo2	Average
			pH		
F1	7.11±0.35	6.93±0.05	6.56±0.13	6.88±0.06	6.87±0.27
F2	7.15±0.30	6.89±0.09	6.63±0.14	6.89±0.09	6.89±0.25
F3	7.14±0.26	6.86±0.03	6.68±0.06	6.88±0.05	6.89±0.21
F4	7.04±0.28	6.86±0.10	6.66±0.11	6.89±0.05	6.86±0.20
Average	7.11±0.27^{a}	6.88±0.07^{b}	6.63±0.11^{c}	6.88±0.06^{b}	
			Ammonia concentration (mmol)		
F1	8.22±1.33^{Ac}	5.46±0.28^{Bc}	6.14±0.95^{Bc}	7.97±1.41^{Ad}	6.95±1.56
F2	16.51±1.84^{Ca}	20.57±0.76^{Aa}	18.69±0.97^{Ba}	20.21±1.07^{Aa}	19.00±1.98
F3	10.26±2.00^{Ab}	8.68±1.56^{Bb}	9.78±1.20^{Bb}	12.12±2.17^{Ac}	10.21±2.04
F4	10.03±1.58^{Cb}	6.21±1.18^{Dc}	11.03±1.12^{Bb}	13.55±0.53^{Ab}	10.20±2.92
Average	11.26±3.58	10.23±6.36	11.41±4.82	13.46±4.72	
			VFA production (mmol)		
F1	99.68±21.46^{Ab}	73.90±16.36^{Bc}	90.56±8.61^{Bb}	100.03±4.50^{Ab}	91.04±16.86
F2	141.25±18.88^{Aa}	141.20±18.74^{Aa}	129.14±21.77^{Aa}	151.30±8.97^{Aa}	140.72±17.84
F3	103.20±26.26^{Cb}	108.28±9.67^{Cb}	111.69±5.65^{Ba}	133.79±7.33^{Aa}	114.24±17.87
F4	121.04±16.98^{Aa}	123.22±10.17^{Aa}	71.89±14.75^{Cb}	105.25±9.29^{Bb}	105.35±24.26
Average	116.29±25.50	111.65±28.54	100.82±25.61	122.59±22.74	
			IVDMD (%)		
F1	85.52±5.48^{Bb}	92.44±3.38^{Aa}	89.45±2.81^{Ba}	91.24±2.42^{Aa}	89.66±4.29
F2	93.89±0.90^{Aa}	94.88±2.81^{Aa}	92.51±1.85^{Aa}	94.48 ±1.48^{Aa}	93.94±1.93
F3	64.27±3.12^{Ac}	59.94±1.04^{Bc}	56.19±3.16^{Cc}	65.01±2.33^{Ab}	61.35±4.33
F4	66.22±1.30^{Ac}	66.46±1.57^{Ab}	64.07±5.30^{Ab}	66.93±0.61^{Ab}	65.92±2.80
Average	77.47±13.34	78.43±16.08	75.55±16.53	79.41±14.06	
			IVOMD (%)		
F1	86.11±5.44^{Bb}	93.04±2.61^{Aa}	88.85±2.01^{Ba}	91.38±2.43^{Aa}	89.84±4.07
F2	93.73±0.84^{Aa}	95.44±2.41^{Aa}	91.88±1.97^{Aa}	94.45±1.66^{Aa}	93.88±2.12
F3	63.43±3.27^{Ac}	59.42±1.54^{Bc}	53.17±0.93^{Cc}	64.56±2.62^{Ab}	60.15±5.04
F4	65.85±1.15^{Ac}	64.80±3.61^{Ab}	62.10±4.61^{Ab}	66.46±0.54^{Ab}	64.80±3.18
Average	77.28±13.69	78.18±16.90	74.00±17.42	79.21±14.32	

F1 = corn meal, F2 = soybean meal, F3 = Napier grass, F4 = dairy cattle complete feed. Different superscript of regular font on the same column significantly different ($p<0.05$). Different superscript of large capital font on the same row shows a significant different ($p < 0.05$)

CONCLUSION

From this study, it can be concluded that keeping fistulated cattle as a source of rumen liquor for in vitro digestibility is the best for the ability to maintain feeds and rumen liquor quality but sharing rumen liquor with other nearby institution or rumen liquor from abattoir can be used as alternative sources of inoculant without significant difference in average fermentation and digestibility results.

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