

The effect of inoculant to substrate ratio on feedstuffs' in vitro fermentability and digestibility

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Abstract. Environment influences feedstuffs available for cattle, which later impact the inoculant to substrate ratio and digestion process in the rumen. This study aimed to compare inoculant to substrate ratio in several in vitro methods regarding local cattle conditions. The first experiment used five local cattle rumen from Bogor Regency abattoir to measure inoculant to substrate ratio (T0). The ratio compared to the proportion used in vitro by Tilley & Terry (T1), Theodorou (T2), and Sutardi (T3) methods using a completely randomized design. The proportions used in T1, T2, and T3 were tested for in vitro fermentability and digestibility of Napier grass (F1), dry ground corn (F2), and soybean (F2). The pH, VFA, and NH₃ concentrations, IVDMD, and IVOMD, were measured using a block randomized factorial design (3 x 3). It was found that the inoculant to substrate ratio used T3 was 40, closer to the ratio found in local cattle. There is no interaction in the second experiment. Feedstuff influenced fermentability and digestibility parameters with F3>F2>F1. The effect of inoculant to substrate ratio was only found in NH₃ concentration with T3>T2>T1. It is concluded that in vitro method modified by Sutardi is more relevant to local cattle conditions.

Keywords: fermentability, fistula, in vitro, inoculant, substrate ratio

1. Introduction

Animal environment influences feeds availability, which impacts the feed to solid ratio and digestion in the rumen [1]. Feedstuffs' fermentability and digestibility information is needed to improve ration formulation accuracy [2] because it tells the biological process subjected to in the animal body. This digestibility describes the proportion of reserved feedstuffs that are not excreted via feces [3]. Moreover, the number and type of microbes influence feedstuffs fermentation and digestibility in ruminants. Other influencing factors include fermentation condition, digesta passage rate, and solid to liquid ratio in the rumen, which depend on the feed type, and particle size used [1]. The feedstuffs' fermentability and digestibility are measured using in vivo [2,3,4], in vitro [5,6,7], or in sacco techniques. The in vivo measurement requires at least three

animals and three weeks, while it also consists of preliminary and collecting phases that may vary between species [2,3,4]. This is time-consuming, and needs a large number of animals, workers, and samples, provided total collecting methods are primarily used.

In vitro method which can be further grouped into simple batch methods [7] or continuous culture has been developed to overcome the in vivo problems. Among popular simple batch methods are Tilley & Terry [5], cellulase technique, Hohenheim gas test developed by Menke *et al.*, [6], and Pressure transducer by Theodorou [7]. In vitro method imitates the fermentation and digestion process in an animal body. The Tilley & Terry [5] and Theodorou [7] are frequently used in the laboratory, where the first involves incubation with rumen liquor and acid pepsin. Theodorou [7] is principally similar to other in vitro methods, but its fermentation is conducted in gas-tight culture bottles, which enable measuring gases accumulation and fermentation kinetics of ruminant feeds. Besides, both methods differ in principle and the rumen's liquid to solid feed sample ratios.

The in vitro results have been compared on their ability to estimate in vivo parameters in different animal species. However, most of the studies were conducted in a temperate environment which might be different from the tropical conditions, namely both feed types and animal conditions. Sutardi [8] tried to modify this method and adapted it to local conditions, but the results have not been compared to other in vitro methods. Therefore, this current study aimed to observe the impact of different solid to liquid ratios used in several in vitro methods on their suitability to local cattle condition and feedstuffs' fermentability and digestibility.

2. Materials and methods

The study conducted consisted of two experiments and lasted from February 2019 to May 2020. In the first experiment, the liquid to solid ratio of local cattle rumen conditions was measured and compared with the ratio used in the in vitro methods. In the second experiment, several liquid to solid ratios used in the in vitro methods were compared for their feedstuffs' fermentability and digestibility results.

2.1 Solid to liquid ratio in local cattle

Rumen liquor of local cattle was collected from Pratama Pangan Utama Abbatoir. Five rumens were opened in separate plastic containers, and 1 L of the content of each was sampled. The weight of each liter of the rumen content was measured before being filtered using a predetermined weight of two-fold cheesecloth. Solid and liquid fractions were weighed to determine their proportion to the total rumen content sample. The solid fraction was then dried in an Eyela NDO 400 (Made in Japan) oven at 60°C until its weight was constant to determine the dry matter weight. The ratio of solid to liquid found in the local cattle rumen (T0) was compared to the ratio used in Tilley and Terry (T1), Theodorou (T2), and Sutardi (T3) in vitro methods.

2.2. *In vitro* feeds' fermentability and digestibility measurements

In the second experiment, the solid to liquid ratio used in Tilley & Terry (T1), Theodorou (T2), and Sutardi (T3) methods were compared in terms of their fermentability and digestibility on three feedstuffs; F1 = Napier grass; F2 = Dry ground corn; F3 = Soybean oil meal. Parameters observed in this experiment were fermentability involving pH, ammonia, & VFA concentration and digestibility consisting of dry matter (DMD) & organic matter (OMD). Rumen fluid from similar cattle used in the first experiment was applied as an inoculant, while the fermentation process was conducted in 100 ml tubes. The amount of sample, rumen liquid, and buffer used in each method can be seen in Table 1.

Table 1. The amount of sample, rumen liquid, and buffer used in different *in vitro* methods

Parameters	T1	T2	T3
Sample (g)	0.5	0.75	0.5
Rumen liquid (ml)	10	25	8
Buffer (ml)	40	50	12

T1 = Tilley & Terry, T2 = Theodorou, T3 = Sutardi

2.3. *Fermentative study*

The fermentative study measured pH, ammonia (NH₃), and VFA concentration after 4 hours of fermentation with different methods and samples. Here, samples were incubated in a tube fermentor, into which rumen liquor and buffer were added, afterward, the tubes were put inside a 39°C water shaker bath. Initially, the fermentor was aerated with CO₂ for 30 seconds to build an anaerobic condition, then it was closed with a ventilated rubber stopper. The fermentation lasted for 4 h after which two drops of HgCl₂ were added to stop the fermentation. The tubes were then centrifuged for 15 minutes at 3000 rpm to separate supernatant from the feed residues. The supernatant was collected and stored chill (4 °C) until it was applied for pH, ammonia, and VFA concentration determination. The pH was measured using the Hanna HI98191 pH meter (made in China), then ammonia concentration was determined using the micro diffusion Conway method. VFA concentration was measured through the steam distillation method, while fermentability measurement employed a method similar to Riestanti et al. [9].

2.4. *Digestibility Study*

Digestibility study measured DMD & OMD using different methods and samples after fermentative and enzymatic digestion of feedstuffs. Furthermore, samples were incubated with a similar procedure as in the fermentative study. However, the fermentative digestion lasted for 48 h after which two drops of HgCl₂ were added to stop the fermentation activity. The tubes were centrifuged for 15 minutes at 3000 rpm, and the supernatant was removed, then 50 ml of 2% HCl-pepsin solution were added and incubated aerobically for 48 h in a 39°C water shaker bath. After 48 h enzymatic digestion, the tubes were filtered using a predetermined weight of Whatman paper no 41 using a vacuum pump. The residue was dried in a 105 °C oven for 24 h to determine dry matter residues and then incinerated in a 600°C furnace oven for 4 h to ascertain ash residue. The DMD and OMD were calculated by subtracting DM and OM residue from samples.

2.5. *Statistical analysis*

In this study, a completely randomized design was employed for the first experiment and a block factorial design 3 x 3 for the second. The data were analyzed using ANOVA and continued with the Tukey test, then statistical analyses were conducted using SPSS version 20.

3. Results and Discussion

3.1. The solid to liquid ratio in local cattle

The ratio of solid to liquid content of local cattle rumen can be seen in Table 2. The table shows that the solid to liquid ratio in rumen local cattle varies from 1:29 to 1:87, with an average of 1:46. The ratios are closer to the value of T3 than T1 and T2. High differences between local cattle rumen condition (T0) compared to T1 and T2 is caused by the high liquid ratio used in T1 and T2 which subsequently increases fermentation activity from rumen fluid, such as in T2. However, fermentation activity is not influenced significantly when it comes from a buffer such as in T1. The considerable influence of rumen fluid on fermentation activity is due to the high microbial colony content such as bacteria, protozoa, and fungi [10]. Rumen microbes were found higher in the liquid than solid fraction [1]. However, a ratio of rumen fluid to buffer of about 1:2, 1:3, and 1:4 from tropical environment cattle and buffaloes have been reported to have insignificantly different effects on DMD and OMD [11].

Table 2. The ratio of solid to liquid content of local cattle and in vitro methods

Parameters	T0	T1	T2	T3
Solid (g)	21.44±9.61	0.5	0.75	0.5
Liquid (ml)	1000	(50)	(75)	(20)
Rumen fluid (ml)		10	25	8
Buffer (ml)		40	50	12
Rumen fluid: buffer ratio		(1:4)	(1:2)	(1:1.5)
Solid : Liquid ratio	1:46 (1:29 – 1: 87)	1:100	1:100	1:40

T0 = local cattle, T1 = Tilley and Terry, T2 = Theodorou, T3= Sutardi

3.2. Impact of different solid to liquid ratio in vitro methods on feedstuffs' fermentability and digestibility

The effect of different solid and liquid ratios on feedstuffs' fermentability and digestibility can be seen in Table 3. The table shows that the ratio of solid to liquid tested did not significantly influence pH, VFA, DMD, and OMD but influenced NH₃ concentration. The type of feeds used influenced all parameters, while no interaction occurred between the entire factors in this study.

Based on the results, the pH value was 6.4 – 6.7, which is in the normal range for optimal rumen microbial growth [1]. The values were influenced by the type of feeds used [2], where forage used in F1 produced higher pH than the concentrate (F2 and F3). Rapid degradation of concentrates in rumen produced higher volatile fatty acids, which decreased rumen pH [1] however, forage degradation required more time and produced fewer acids [10]. Both factors influenced the NH₃ concentration, but no interaction between the two factors was found. The T3 methods produced higher NH₃ concentrations in comparison to T1 and T2 due to high ratio of rumen liquid used in this method. The high ratio of rumen liquid as inoculant source increases rumen activity in degradation of feeds protein by bacteria to produce NH₃ [1]. High microbial population increase protein degradation in the rumen by increasing hydrolysis of proteins by proteinases [12]. This breakdown protein into to peptides and amino acids that can be used directly by the microflora or degraded further by peptidases and deaminating enzymes into VFA and NH₃ [1]. The type of feed also influenced the NH₃ concentration detected. Soybean, which contained high protein, produced higher NH₃ because of high availability of substrate to be degraded [2].

Table 3. Impact of solid to liquid ratio on feedstuffs' fermentability and digestibility

Parameters	Solid to liquid ratio			Average
	T1	T2	T3	
Fermentability				
pH				
F1	6.68±0.10	6.66±0.05	6.64±0.04	6.66±0.02 ^a
F2	6.52±0.08	6.58±0.07	6.53±0.16	6.54±0.03 ^b
F3	6.49±0.04	6.52±0.02	6.52±0.03	6.51±0.01 ^b
Average	6.56±0.01	6.58±0.07	6.56±0.06	
NH ₃ (mM)				
F1	10.35±2.35	10.55±2.70	12.70±0.8	11.2±1.30 ^b
F2	8.10±2.02	7.6±2.14	9.25±3.00	8.35±0.81 ^c
F3	18.71±2.86	16.11±3.41	21.00±2.00	18.61±2.45 ^a
Average	12.39±5.59 ^b	11.45±4.28 ^c	14.32±6.04 ^a	
VFA (mM)				
F1	70.84±11.69	73.55±15.29	91.38±18.9	78.59±11.16 ^b
F2	90.26±33.77	76.1±14.62	95.20±32.47	88.21±9.46 ^b
F3	125.98±27.57	104.55±12.01	134.46±78.04	121.66±15.42 ^a
Average	95.70±33.78	85.00±17.01	107.02±23.84	
Digestibility				
DMD				
F1	61.62±8.56	61.58±7.22	59.28±9.21	60.83±1.34 ^c
F2	84.95±6.14	75.09±11.01	77.00±10.77	79.01±5.23 ^b
F3	89.82±3.77	88.22±7.13	89.55±4.46	89.20±0.86 ^a
Average	78.80±15.07	74.96±13.32	75.28±15.21	
OMD				
F1	60.81±9.25	61.16±5.63	57.72±10.36	59.90±1.86 ^c
F2	83.28±7.58	73.70±12.10	74.03±12.75	77.00±5.43 ^b
F3	88.95±4.61	88.26±5.63	88.73±4.27	88.65±0.35 ^a
Average	77.68±14.88	74.37±13.56	73.49±15.51	

T1 = Tilley & Terry, T2 = Theodorou, T3 = Sutardi, NH₃ = ammonia, VFA = volatile fatty acids, DMD = dry matter digestibility, OMD = organic matter digestibility, F1=Napier grass, F2= dry ground corn, F3= soybean, Bold numbers show a statistically significant correlation (P < 0.05)

The VFA value was not significantly influenced by the solid to liquid ratio due to considerable variation in the data discovered within the treatment, but it was influenced by feed types. Soybean produced higher VFA in comparison to Napier grass and dry ground corn. Despite being the primary energy source in many dairy rations, corn produces low VFA due to the processing of dry ground corn that may change some components' chemical structure [13]. Furthermore, dry ground corn has been shown to have low water solubility [14], and steam-flaked corn processing exhibited greater ruminal starch degradability than dry ground corn [13].

The feedstuffs' digestibility was also not significantly influenced by the solid to liquid ratio but by the feed types. Concentrates have higher digestibility (DMD and OMD) in comparison to forage (F1), while soybean oil meal with higher protein content digested better than dry ground corn. The high fermentability of carbohydrates and protein in soybean oil meal produced higher NH₃ and VFA that microbes can use for

microbial protein synthesis. Also, the high microbial population led to even higher fermentation activity, especially fibre fraction degradation, which is related to higher digestibility [1].

4. Conclusion

It can be concluded that for the tropical environment, the solid to liquid ratio used by the Sutardi method is closer to the rumen condition of local cattle. According to the fermentability studies, rumen microbial activity on protein degradation in Sutardi methods is higher for all feeds used. Meanwhile, digestibility studies showed that all methods produced similar results. The solid to liquid ratio of Sutardi's method is further suggested for conduction of the in vitro fermentability and digestibility study using local cattle.

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References

- [1] Czerkawski J W 1986 *An Introduction to Rumen Study* (Oxford: Robert Maxwell, M.C.) p. 236.
- [2] Hasanah U, Permana I G and Despal 2017. *Pakistan J. Nutr.* **16** 577–87.
- [3] Despal, Mubarak, Ridla M, Permana I G and Toharmat T 2017. *Pakistan J. Nutr.* **16** 435–43.
- [4] Zahera R, Permana I G and Despal 2015. *Media Peternak.* **38** 123–31
- [5] Tilley J M A and Terry R A 1963. *Grass Forage Sci.* **18** 104–11.
- [6] Menke K H, Raab L, Salewski A, Steingass H, Fritz D and Schneider W 1979. *J. Agric. Sci.* **93** 217–22.
- [7] Theodorou M K, Williams B A, Dhanoa M S, McAllan A B and France J. A 1994. *Anim. Feed Sci. Technol.* **48** 185–97.
- [8] Sutardi T 1980 *Landasan Ilmu Nutrisi* (Bogor: Fakultas Peternakan, IPB).
- [9] Riestanti L U, Despal and Retnani Y.2021. *Am. J. Anim. Vet. Sci.* **16** 172–84.
- [10] Theodorou M and France J 2005. Rumen microorganisms and their interactions. In: Dijkstra J, Forbes J and France J, editors. *Quantitative Aspect for Ruminant Digestion and Metabolism 2nd Ed.* (London: CABI).
- [11] Suningsih N, Novianti S and Andayani J 2017. *J. Sain Peternak. Indones.* **12** 341–52.
- [12] Brock F M, Forsberg C W and Buchanan-Smith J G 1982. *Appl. Environ. Microbiol.* **44** 561–9.
- [13] Shen J S, Song L J, Sun H Z, Wang B, Chai Z, Chacher B and Liu J X 2015. *Asian-Australasian J. Anim. Sci.* **28** 351–9.
- [14] Despal, Permana I G, Safarina S N, Tatra A J 2011. *Media Peternak.* **34** 69–76.