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Effect of Component and Total Mixed Ration Feeding Systems on *In Situ, In Vitro* Degradability of Ground Filter Paper, and Ruminal pH in Dairy Bulls

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ABSTRACT

The study was to evaluate the effect of feeding systems on the *in situ* and *in vitro* degradability of ground filter paper substrate and ruminal pH level. The experiment used three cannulated dairy bulls. In a 2×2 crossover design, three replications were carried out for every incubated sample. Cattle were fed COMP and TMR feeds twice a day in 2 different treatment groups. The substrate cellulose of Whatman No.1 filter paper was incubated in the rumen fistulated dairy bulls. Rumen fluid samples were collected after 3 hours of morning feeding with a total of 100 mL sampled every 1 h for 24 h. The pH of the rumen fluid was determined using a calibrated pH meter at each sampling interval. The result of the *in situ* ruminal degradation of the DM, NDF, and ADF at 3, 6, 12, 24, 48, and 72 h was not significantly different. The *in vitro* gas production volume and fermentation kinetics at each time set were not significantly different. Rumen fluid pH values were not significantly different. However, the TMR group had a higher determination coefficient of 90% than the COMP group, which had 70%. Overall, COMP and TMR feeding did not affect the ruminal degradability capacity of DM, NDF, and ADF *in situ* techniques. Neither COMP nor TMR feeding affected *in vitro* gas production. However, those feeding systems can influence microbial activity in the rumen, where TMR has an advantage in maintaining and stabilizing pH levels in the fermentation and digestion of feeds in the rumen.

Keywords: *In situ* ruminal degradation, *in vitro* gas production, ruminal pH level, Whatman filter paper

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi pengaruh sistem pemberian pakan terhadap degradasi substrat kertas saring giling secara *in situ* dan *in vitro* serta tingkat pH rumen. Percobaan ini menggunakan 3 ekor sapi perah jantan yang telah dikanulasi. 2×2 rangan silang dilakukan dengan ulangan tiga kali untuk setiap sampel yang diinkubasi. Ternak sapi diberi pakan Komponen (COM) dan pakan lengkat atu total mixed ration (TMR) dua kali sehari dalam 2 kelompok perlakuan yang berbeda. Substrat selulosa dari kertas saring Whatman No.1 diinkubasi dalam rumen sapi perah yang berfistula. Sampel cairan rumen diambil 3 jam setelah pemberian pakan pada pagi hari dengan total 100 mL diambil setiap 1 jam selama 24 jam. Pada setiap pengambilan sampel, pH cairan rumen diukur menggunakan pH meter yang telah dikalibrasi. Hasil degradasi bahan kering (BK), serat deterjen netral (NDF), and serat deterjen asam (ADF) pada *in situ* 3, 6, 12, 24, 48, dan 72 jam tidak berbeda secara signifikan. Volume produksi gas *in vitro* dan kinetika fermentasi pada setiap waktu tidak berbeda nyata. Level pH cairan rumen tidak berbeda secara signifikan. Akan tetapi pada kelompok pakan TMR memiliki koefisien determinasi yang lebih tinggi 90% dibandingkan dengan kelompok COMP sebesar 70%. Secara keseluruhan, pemberian pakan COMP dan TMR tidak mempengaruhi kapasitas degradasi rumen DM, NDF, dan ADF dalam teknik *in situ*. Baik pemberian COMP maupun TMR tidak mempengaruhi produksi gas *in vitro*. Akan tetapi, sistem pemberian pakan dapat mempengaruhi aktivitas mikroba dalam rumen, dimana TMR memiliki keuntungan untuk menjaga dan menstabilkan tingkat pH pada fermentasi dan pencernaan pakan dalam rumen.

Kata kunci: *In situ* degradasi rumen, *in vitro* produksi gas, tingkat pH rumen, whatman No. 1 kertas saring

INTRODUCTION

The development of dairy cows is thought to be essential in order to satisfy consumer needs for milk supply. However, a management of the feeding system, health, reproductive, and environmental problems results in a low capacity for milk production (Gross 2022). The limitations include the digestive system and rumen, feeding sources, tissue mobilization, intermediate metabolism and transport, and the nursing mammary gland's absorption of circulating nutrients. Baris (2023) stated that insufficient nutrition causes stunted growth, reduced milk yield, lower fertility, and higher susceptibility to diseases, so that feed quality can be maximized by appropriate nutrition. Therefore, proper nutrition can maximize feed quality control.

The animals cannot use the nutrients in their feed if they don't eat them. In fact, the amount of feed consumed is very important. In addition, if there are factors that limit voluntary feed intake, or dry matter intake (DMI), the animal will not receive a balanced nutrient, which will negatively impact productivity. There are certain characteristics of feeds that reduce an animal's voluntary intake. Consequently, livestock productivity such as body weight gain is limited by feed consumption and rumen degradability. Feed with low digestible value has low feed degradation, resulting in poor fermentation activity by rumen microbes, leading to low microbial growth in the rumen. This will send a signal for the animal to limit feed intake. If some diet adjustments may improve the fermentation profile of microorganisms (Castillo-González *et al.* 2014).

Rumen is the primary digestive organ of dairy cows, where microorganisms break down and use plant fibers like acid detergent fiber (ADF) and neutral detergent fiber (NDF) for energy. In addition, minerals and dietary sources of crude protein (CP) promote the growth of microorganisms (Grummer *et al.* 2004).

The component (COMP) and total mixed ration (TMR) feeding consist of the feed ingredients combination derived from grasses, legumes, concentrates, vitamins, and minerals, whether given separately (COMP) or in a mixed form (TMR), can stimulate the growth and activity of microorganisms to degradate the crude fiber of the feeds eaten directly and the other subtrate sellulose incubated in the rumen. Palmonari *et al*. (2024) stated that the feeding forage grasses typically boosts ruminal microbial activity. Forage provides the substrate necessary for ruminal microbial growth and activity, particularly for cellulolytic bacteria that breakdown fiber. A diet high in forage supports the rumen's natural microbial community and promotes the growth of beneficial bacteria (Osorio-Doblado *et al.* 2023). Adult ruminants usually eat forages, which provide fiber components mostly are cellulose, hemicellulose, pectins, and lignin, while concentrates are to meet their energy, nitrogen, minerals, and vitamin requirements. Alterations in the chemical composition of the feed or the inclusion of concentrates in the diet may also alter the rumen microbial community's makeup (Gruninger *et al.* 2019 as cited by Palmonari *et al.* 2024). Ruminants that eat a diet high in forage are less likely to develop rumen acidity and are more likely to harbor certain microbes, like cellulolytic bacteria. In contrast, a diet high in concentrates reduces ruminal pH and negatively impacts on bacterial richness and diversity (Sanjorjo *et al.* 2023). Legumes are an excellent protein source, abundant in amino acids, vitamins, and minerals, and serve as effective substrates for cellulolytic microorganisms to support growth and enzymatic activity (Galindo & Marrero 2005).

In situ and *in vitro* methods are used to evaluate the rumen degradation of feeds and digestibility of nutrients by particular microbial fermentation to supply sufficient nutrients for growth and reproductive efficiency. According to Lopez (2005), *in situ* procedures involve conducting digestive investigations in the rumen of live animals instead of mimicking rumen conditions in a laboratory. In addition, López explained that when a tiny quantity of the feedstuffs is suspended in the rumen of the fistulated animal and incubated for a predetermined amount of time, the substrate's disappearance is evaluated. The method makes the assumption that rumen bacteria and enzymes are responsible for the substrates' degradation, which is why they vanish from the bags.

The *in vitro* method needs the use of rumen fluid, which is obtained from cannulated animals, to estimate either digestibility or gas production. The *in vitro* gas production process employs buffers, chemical solvents, rumen fluid, and enzymes that are either extracted from the rumen's contents or purchased commercially (Mohammed and Chaudhry 2008). Tilley and Terry (1963) method, as cited by Olivo *et al.* (2017), simulates *in vitro* the digestion processes that take place in the gastrointestinal tract of animals. Nevertheless, the *in vitro* gas production technique allows estimating the digestibility, the fermentation rate of the different feed fractions, and the ruminal microbial activity. This method also makes it possible to determine the food's nutritional value by measuring the amount of short-chain fatty acids (SCFA) and the gases CO_2 (carbon dioxide) and CH_4 (methane), which are produced from the fermentation of the substrate (Oliveira *et al*. 2014, as cited by Olivo *et al*. 2017). Such information provides a rapid estimate of the digestibility of feed for the cattle (Theodorou *et al*. 1998, as cited by Olivo *et al*. 2017). The in vitro gas production method has an advantage over other digestibility methods because it yields two different feed data in a single incubation: the volume of gas produced indicates the feed's apparent digestibility, while the residue produced indicates the feed's actual digestibility.

The technique of *in vitro* gas production can be complemented with the determination of sample residue to obtain the residual nutritional value of the feed. The residue indicates the portion of the substrate that remained unused during fermentation, unlike the measurement of gas production, which solely represents the production of SCFAs and gases (Blummel & Becker 1997, as cited by Olivo *et al*. 2017). Measuring the gases produced can assist in estimating the voluntary feed intake of ruminants.

Rumen degradation of feeds must be assessed in order to measure the nutritional condition of ruminant animals. A good nutritional status is one in which an animal's daily

nutrient intake matches its daily demands (Mohamed and Chaudhry 2008). In order to formulate animal rations that offer required levels of rumen degraded and undegraded nutrients, a systematic approach for predicting nutrient degradation of feeds in the rumen is required. *In situ* and *in vitro* studies show that cellulose digestion can be greatly inhibited even with a slight decrease in rumen pH (Russell and Wilson 1996). Furthermore, Russel stated that the influence of ruminal pH on cellulose digestibility is often complicated by variations in feed intake or the fiber content of the diet. The rumen pH relies on saliva production, the generation and absorption of short-chain fatty acids (SCFA), the type and amount of feed consumed, and bicarbonate and phosphate exchange through the luminal epithelium (Aschenbach *et al*. 2011). Thus, in the reticule ruminal environment, these factors impact both pH and buffering capacity. Because saliva production is a continuous process that provides bicarbonate and phosphate into the rumen, the pH is constantly changing (Russell & Strobel 1989), but it normally stays in the range of 5.5 to 7.0 (Krause & Oetzel 2006), based on the feed and saliva's capacity to act as a buffer. Furthermore, reticulospinal secretions include buffering properties, so this environment is not just reliant on saliva's buffering capacity (Krause & Oetzel 2006).

Microbial enzymes are sensitive to pH fluctuations, as seen by the reduction of bacterial growth at acidic pH. It's probable that a hydrogen ion imbalance within the cell is to blame (Russell & Wilson 1996). The ions and molecules in the rumen produce gas tension, which determines the osmotic pressure (Lodemann & Martens 2006). Unmixed feeding systems (COMP), the main components of cattle feeds, are roughage and concentrate. Among the straw cereals used for roughage are wheat, rice, corn, and sorghum. Napier is a popular fodder grass that is very nutrient-dense but has lower levels of proteins and minerals. The types of diet taken can alter ruminal fermentation processes, affecting the rumen's osmotic pressure. The presence of volatile fatty acids (VFAs) generated by fermentation processes increases osmotic pressure, which is directly related to pH in feeds of high carbohydrates (Lodemann & Martens 2006).

Rumen pH fluctuates throughout the day and could have an important effect on the fermentation and digestion. Hagg (2007) stated that various rumen microbes are active at different pH levels. The rumen pH condition ranged from 6.0 to 6.9 is ideal for the growth of bacteria that break down fiber, while the pH ranged from 5.5 to 6.0 is ideal for the growth of bacteria that break down starch (Hutjens 2002). Thus, for the optimal bacterial development, a high-producing cow must maintain a pH of approximately 6.0.

MATERIAL AND METHODS

In Situ Ruminal Degradation

Three (3) cannulated HF×Jersey bulls with an average body weight of 531.7±40.1 kg were used in the *in situ* degradability experiment. A 2×2 crossover design was performed. Therefore, there were a total of 3 replications per incubated sample. Cattle were fed COMP and TMR feeds in 2 different treatment groups. Whatman No. 1 filter paper was cut into 2 mm, utilized as the substrate that represents the fibrous fraction of the cattle diet. The feed ingredients used in COMP and TMR feeding, including filter paper, were analyzed for the chemical composition of DM, CP, NDF, and ADF prior to feeding trials.

The adjustment period was 10 days. Approximately 5 g of DM sample of Whatman paper grade 1, cycle, with diameter of 25 mm, pack of 100, thickness of 180 µm, and pore size of 11 µm (particle retention) were put into each polyester bag in duplicate and inserted into the rumen of 3 cannulated bulls. The nylon bags were incubated in the rumen for 3, 6, 12, 24, 48, and 72 h at different times and pulled out at the same time. To stop fermentation, the nylon bags were immediately rinsed underwater after being taken out of the rumen. Tap water was used to wash the nylon bags until the water flowed clear. The bags were also oven-dried at 65°C for three days in order to preserve their weight. They were placed in a desiccator and kept at room temperature.

The bags were weighed at room temperature and the percent digestion of ingredients was calculated. The sample fractions that were removed during the incubation were then oven-drying in order to determine the DM, NDF, and ADF (Van Soest method). The determination follows the methods of analysis developed by AOAC (2016). The NDF and ADF were analyzed following the procedure of the ANKOM200 fiber analyzer. To calculate the percentage of DM, NDF, and ADF degradability was used the formula as follows:

$$\% \text{ DM degraded} = \frac{(\text{Wt before incubation} - \text{Wt after incubation})}{\text{Wt before incubation}} \times 100$$

$$\% \text{ NDF/ADF degraded} = \frac{100 \times (W_3 - (W_1 \times C_1)}{W_2}$$

Where: W1 = Bag tare weight, W2 = Sample weight, W3 = Dried weight of filter bag with fiber after extraction process, and C1 = Blank bag correction (running average of final oven-dried weight divided by original blank bag weight).

In Situ Gas Production Method

In vitro gas production method: Rumen fluid was extracted from three cannulated dairy bulls following three hours of morning feeding as part of the in vitro gas generation process. A calibrated pH meter was used to measure the pH after collecting and filtering approximately 300 milliliters per animal using three layers of cheesecloth. The collected rumen fluid was placed in a zip-lock bag. The anaerobic condition was maintained inside the zip-lock by removing the air from the bag. The bags were then kept inside an ice box with a thermometer sticking out and hot water inside to maintain an internal temperature of 39 to 40 ^{o}C.

Feed sample was ground in Whatman No. 1 filter paper, the same as used in the *in situ* nylon bag technique, and was replicated in 3 replications. The feed samples were milled in a 1 mm sieve and weighed about 200 mg and were put into 50 ml disposable syringes. The preparation of 0.154 M Magnesium sulfate ($MgSO_4$) weighed 37.88 g hydrated to 1 L of distilled water, and mixed disodium hydrogen orthophosphate (Na_2HPO_4) weighed 3.7 g, and

sodium dihydrogen orthophosphate (NaH_2PO_4 weighed 3.5 g dissolved in 1 L of distilled water. The buffer (5 mL 0.05 M PO_4 and 1 mL 0.154 M $MgSO_4$) with a pH maintained at 6.9, was put into the syringes. The collected rumen fluid was immediately transferred to the syringes contained substrate with buffer solution to attain a final volume of 20 mL (Guadayo *et al*. 2019).

Following 3, 6, 12, 24, 48, and 72 h of incubation, the gas volume was measured. Before the samples were incubated, the initial volume of material in each syringe was noted. Each inoculation period, the gas production volume was estimated using the following equation, which was fitted to the non-linear equation model (Ørskov & McDonald 1979):

$$A = b\ (1 - e\text{-}c\ (t - L))$$

Where: A = Volume of gas produced at time t, b = Gas produced from the insoluble fraction, a+b = Total gas produced, c = Fractional rate of gas production (/h) from the slowly fermentable of fraction b, L = Discrete lag time prior to gas production**.**

The effective gas production (EP) in the rumen was calculated from the equation of Denham *et al*. (1989):

$$EP = a + [b \times c /(c + k)]$$

Where: EP = effectivity of gas production, k = rumen outflow rate of 2% per h, the recommended rate for animals at maintanance level from the equation developed by NRC (2001). To calculate the gas production kinetics were using the non-linear procedure of GraphPad Prism 8.0.1.

Ruminal pH

Rumen fluid was collected 3 hours after morning feedings from 3 cannulated HFxJersey bulls with an average body weight of 531.7±40.1 kg. Cattle were fed the daily amount of COMP and TMR feeds of 3% DM per body weight for 7 days. Ruminal samples were collected for pH analysis using the portable pH brand YSI, model number pH10A.

The rumen fluid 100 mL was sampled every 1 h for 24 h. The samples were manually taken from the dorsal, ventral, and medial regions inside the rumen of bulls. At each sampling time set, the pH was measured immediately after collection using a calibrated pH meter. The ruminal pH value was fitted with a regression equation of y = a + bx + e, where y = dependent variable, x = independent variable, a = intercept, and b = slope of the line, and e = error term.

Data Analysis

The data of *in situ* ruminal degradation and *in vitro* gas production were subjected to a T-test procedure of SAS (Studio) Statistical Software with a significant level of $P<0.05$. A linear regression model was used to evaluate the rate of ruminal pH and GraphPad Prims 8.0.1 was used to compute the gas production kinetics and fit the curves.

RESULTS AND DISCUSSION

Chemical Composition of Filter Paper Substrate and Feed Ingredients

Whatman No. 1 filter paper is a Whatman qualitative filter paper, grade 1, cycle, with diameter of 25 mm, pack of 100, thickness of 180 µm, and pore size of 11 µm (particle retention). It was used as a substrate for the selulose *in situ* nylon bag technique and *in vitro* gas production method.

Feedstuffs such as Napir grass, corn silage, *Leucaena leucocephala, Gliricedia sepium, Moringa olifera,* concentrate mash were analyzed for their chemical compositions at the inception of feeding trials.The Whatman No. 1 filter was used as a component cellulose substrate representing the true feed ingredients in both methods of *in situ* and *in vitro* gas production techniques, which assumes that the substrate of filter paper can be digested by rumen microorganisms.

According to the chemical composition, the ground Whatman No. 1 filter paper utilized as a sample during the incubation process had higher levels of DM, NDF, and ADF (Table 1) than the real feed ingredients that were given to bulls using *in situ* nylon bag techniques and *in vitro* gas production method.

Table 1. Chemical composition of filter paper substrate and feedstuffs offered to the cattle

Substrate/ Feedstuffs	DM, %	CP, %	NDF, %	ADF, %
Whatman No. 1 filter paper	93.6	0.20	98.60	95.30
Napier grass	22.60	7.60	69.00	48.50
Corn silage	31.70	8.60	60.70	42.80
Leucaena leucocephala	40.71	27.40	29.60	19.90
Gliricidia sepium	34.47	26.40	24.50	19.50
Moringa oleifera	30.05	29.30	39.90	28.10
Concentrate mash	90.20	26.00	27.60	13.10

*DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber

In Situ Ruminal Degradation

In situ ruminal degradation rate of DM, NDF, and ADF during 72 h of incubation is shown in Table 2. The percentage disappearance of the DM, NDF, and ADF of ground Whatman No. 1 filter paper incubated in the rumen fistulated of the HF×Jersey bulls fed COMP and TMR feeding the means of the degradation at 3, 6, 12, 24, 48, and 72 h incubation were not significantly different ($P>0.05$) among 2 dietary treatment groups.

The degradation rates of DM, NDF, and ADF in the rumen environment have been evaluated using *in situ* method. The NDF degradation is used as an indicator of forage quality and intake potential (Varga *et al.* 1983) and the ADF degradability is measured to evaluate the forages digestibility and energy intake (Schick 2023).

Table 2. Percentage of DM, NDF, and ADF disappearance from in situ degradation

Parameters	Incubation Time (h)	Treatments		SEM	P-value
		COMP1	TMR2		
DM Degradation, %	3	2.54±1.34	1.38±0.37	0.71	0.1925
	6	2.91±1.70	2.91±0.54	0.98	0.9976
	12	5.66±1.25	5.75±0.93	1.30	0.9520
	24	17.91±1.26	18.61±4.42	0.73	0.8059
	48	50.71±12.41	46.97±14.64	7.16	0.7525
	72	70.95±17.36	73.07±10.39	10.02	0.8648
NDF degradation, %	3	2.00±1.00	2.00±1.00	0.58	10.000
	6	4.61±1.32	2.83±0.33	0.76	0.0867
	12	5.58±1.94	4.21±0.50	1.12	0.3027
	24	8.55±2.54	7.82±0.27	1.47	0.6484
	48	22.30±3.34	16.63±4.10	2.38	0.2068
	72	52.69±11.71	48.80±14.98	14.97	0.7414
ADF degradation, %	3	3.28±1.32	2.18±0.47	0.76	0.2460
	6	3.93±2.50	3.43±1.16	1.44	0.7719
	12	8.28±0.77	8.35±1.02	0.58	0.9288
	24	20.33±2.94	16.62±7.02	4.05	0.4454
	48	53.62±11.45	49.68±15.27	8.81	0.7387
	72	72.66±17.10	74.58±10.55	6.1	0.8770

*COMP: component feed, TMR: total mixed ration, DM: dry matter, NDF: neutral detergent fiber, ADF: acid detergent fiber, and SEM: standard error of mean

The substrate cellulose of Whatman No.1 filter paper incubated in the rumen showed the rate of DM, NDF, and ADF disappearance following the incubation time points at 3, 6, 12, 24, 48, and 72 h.

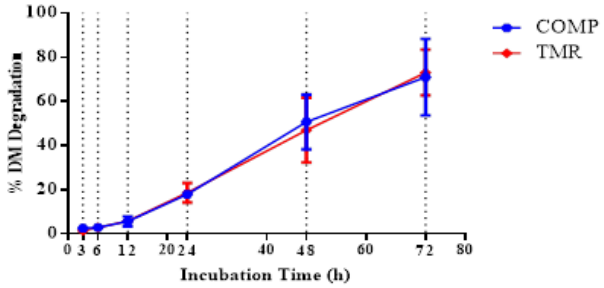

Figure 1. Curves of *in situ* DM degradation (%) of ground Whatman No. 1 filter paper in two dietary treatment groups

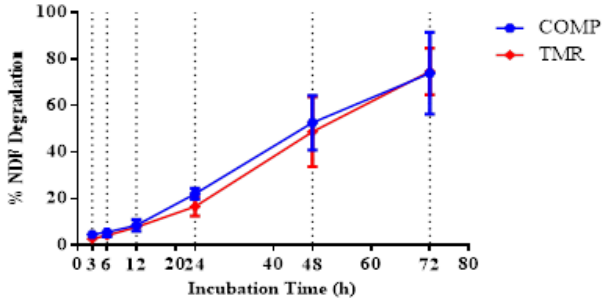

Figure 2. Curves of *in situ* NDF degradation (%) of ground Whatman No. 1 filter paper in two dietary treatment groups

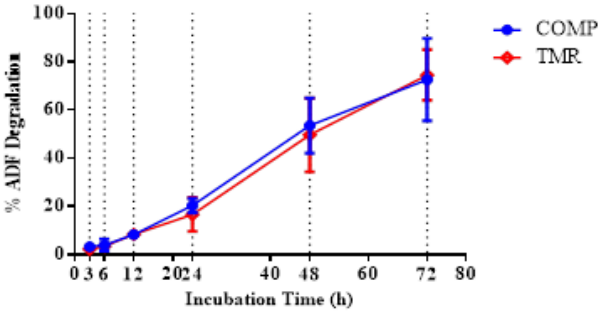

Figure 3. Curves of *in situ* ADF degradation (%) of ground Whatman No. 1 filter paper in two dietary treatment groups

The rumen degradation rates of the DM, NDF, and ADF were slightly closer to one another between COMP and TMR feeding (Graph 1, 2, and 3), where the degradation rate was extremely slow at 3, 6, and 12 h in both COMP and TMR groups. DM degradation was very slow in both the COMP and TMR groups. The cattle that received TMR only showed a percentage degradation of 1.38%, which was lower than the COMP group. However, at 24 and 72 h, TMR was slightly higher than COMP feeding, with percentages of 18.61 and 73.07%, respectively (Table 2). NDF degradation in bulls fed a TMR was lower at each incubation time point compared to the COMP feeding. This indicates that both the ADF and NDF components of Whatman No. 1 filter paper were degraded slowly in bulls fed TMR.

The ruminal degradation was observed slowly as a result of the high cellulose of Whatman No. 1 filter

paper in each incubation time in animals fed a COMP and a TMR feeding group. Although it is high cellulose, it can be assumed that the amount of the cellulose was degraded in the rumen bulls when compared to the initial chemical composition of Whatman No. 1 filter paper (Table 1) as an illustration of the nutrient degradation capacity of the true feed in the rumen. The degradation was associated with how much NDF and ADF contained in the substrate and the capacity of the microorganisms to break down the cellulose, hemicellulose and lignin. Figures 1, 2, and 3 indicate the percentage of the DM, NDF, and ADF degraded in the rumen bulls fed a COMP and a TMR feeding was very slow degradability of soluble and insoluble fractions at 3 to 6 h incubation where the point was very close to the 0 (Figures 1, 2, and 3) either in Treatment 1 and Treatment 2. In addition, the high degradation of cellulose was at 48 to 72 h. This made the graph linearly increase and did not become a plateau, indicating DM, NDF, and ADF degradation by microbial rumen still continued to increase even after 72 h incubation. The assumption is that microorganism's activities and fiber digestion in the rumen are continuous process that goes beyond this time period. This was because the Whatman No. 1 filter paper substrate used has high cellulose content, especially NDF and ADF, so that it can make microorganisms active in breaking down cellulose. Ma *et al*. (2021), who stated that the the degree of lignification in fodder and the rumen breakdown of cellulose have been identified as key indicators of degradation complexity. As the time spent in the rumen degradation of DM, NDF, and ADF increases and eventually stabilizes. This indicated that the levels of DM, NDF, and ADF disappearing in each incubation time were close to each other in the two dietary treatments.

The degradation was influenced by the concentration of pH and temperature where rumen pH levels in the two dietary treatments were slightly acidic and also at normal pH for the activity of microorganisms to degrade cellulose. The initial pH ranged from 5.9 to 6.8 at the time the experiment started, with the rumen temperature ranging from 33 to 38 °C. The rate of substrate degradation at a given time point can be inhibited by low pH and temperature. This had to do with the decrease in the fibrolytic microbial population, which had been linked to either a decrease in the fibrolytic bacteria's capacity to adhere to feed particles or a decrease in the digestion of fiber at low pH (Cheng *et al.* 1980). Additionally, Calsamiglia *et al.* (2008) found that a decrease in pH is associated with a decrease in the amount of digestible organic matter consumed and the formation of volatile fatty acids (VFAs). This could be a component of the rumen ecosystem's self-regulatory mechanisms against ruminal acidosis.

In Vitro Gas Production Technique

The results of the gas production volume from the *in vitro* fermentation were presented in Table 3. The volume of the gas production was observed at 3, 6, 12, 24, 48, and 72 h was found no significant difference (P>0.05) in the two treatments. However, gas production was higher in the cattle fed COMP feeding. Gas production kinetics was not significantly different (P>0.05) among the two dietary treatment groups.Potential gas production from the fermentable fraction of ground Whatman No. 1 filter paper, total gas production, and effectivity of gas production at 2% per h of rumen outflow rate observed was higher in the cattle received COMP feeding. However, the gas production rate was observed higher in the TMR feeding group.

The high amount of gas production was attributed to the high fermentation process of the substrate by microbial rumen with adequate pH conditions so that the microorganisms were able to digest the cellulose substrate. The means of the initial rumen fluid pH value measured in this trial was 6.60. In the cattle fed a COMP feeding slightly with cattle fed a TMR feeding, the pH value was 6.50 (ranging from 6.25 to 6.95). When Amanzougarene and Fondevila (2020) assessed the "Fitting of the *in vitro*

Table 3. Means of in vitro gas production and fermentation kinetics of ground Whatman No. 1 filter paper

Parameters	Treatments		SEM	P- value
	COMP1	TMR2		
Gas Production, mL				
3 h	1.14±0.56	1.33±0.60	0.32	0.6998
6 h	2.01±0.79	1.75±0.66	0.46	0.6852
12 h	3.88±0.96	3.00±1.73	0.55	0.4826
24 h	6.67±3.32	5.73±3.31	1.92	0.7452
48 h	10.51±5.27	8.00±5.32	3.04	0.5926
72 h	11.93±5.83	10.50±4.59	3.36	0.7558
Fermentation Kinetics				
a, mL	-3.89±0.63	-3.21±1.59	0.36	0.5250
b, mL	9.48±4.88	7.66±4.57	2.81	0.6612
c, mL	0.15±0.04	0.18±0.09	0.02	0.63
a+b, mL	5.59±4.35	4.45±4.41	2.51	0.765
EP0.02, mL	4.34±3.48	3.47±3.67	2.01	0.7805

1component, 2total mixed ration, a: gas production from the immediately soluble fraction, b: gas production from insoluble fraction, c: gas production rate constant, a+b: total gas production, and EP0.02: effectivity of gas production at 2% rumen outflow rate per h

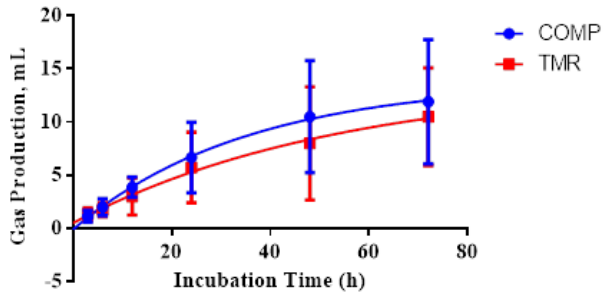

Figure 4. *In vitro* gas production curves of ruminal fermentation of ground Whatman No. 1 filter paper in two diatary treatment groups

gas production technique for the concentration diet study, they found that this was similar to the 6.50 medium pH. In addition, *in vitro* gas production is highly correlated with the medium pH, either in terms of indirect gas, which results from the medium's buffering capacity, or direct gas, which comes from the microbial fermentation of substrates and is therefore dependent on the medium conditions for microbial activity. The amount of indirect gas generated per unit of acid produced should be determined by the initial pH (Amanzougarene & Fondevila 2020).

Ruminal pH

The means of rumen fluid pH values measured every hour for 24 hours were not significantly different (P>0.05) at every time point. The values ranged from 5.86 to 6.72 in the COMP feeding group and 6.05 to 6.83 in the TMR group.

At the start of morning feeding, it was probable that the cattle were selected for the feeds and more consumed the concentrate that was given separately in COMP feeding. In accordance with Ramos *et al.* (2021), the decline in the average minimum rumen is associated with the provision of high concentrate feeds. Additionally, due to the high concentration of feeds, this species alters its metabolism to produce lactic acid as the end product, which lowers the pH to 5.5 and is harmful to the ruminant (Russell & Hino 1985). The pH level of the rumen is also kept somewhat constant and steady with TMR feeding. According to Nocek *et al.* (1985) and McGilliard *et al.* (1983), the TMR feeding system contributes to maintaining rumen pH by providing a better balanced diet with a constant supply of concentrate and roughage, which increases DM intake. The ruminal environments are required to ensure the growth and maintenance of cellulolytic bacteria. The ideal pH for fiber digestion in the rumen is 6 to 9, while the pH less than 5.5, can negatively affect the fiber digestion (Weimer 1996).

Table 4. The average of the ruminal pH value of dairy bulls fed COMP and TMR feeding was measured every hour for 24-hour period

Parameters	Time (h)	Treatments		SEM	P-value
		COMP1	TMR2		
pH value	1	6.61±0.20	6.52±0.02	0.12	0.4665
	2	6.60±0.15	6.45±0.09	0.09	0.2061
	3	6.46±0.23	6.38±0.15	0.14	0.6328
	4	6.40±0.21	6.20±0.16	0.13	0.2735
	5	6.33±0.22	6.20±0.20	0.13	0.5085
	6	6.37±0.20	6.15±0.26	0.12	0.3178
	7	6.59±0.18	6.27±0.19	0.11	0.1078
	8	6.02±0.38	6.25±0.18	0.22	0.4000
	9	5.86±0.10	6.15±0.25	0.06	0.1425
	10	6.05±0.35	6.06±30	0.20	0.9716
	11	6.30±0.54	6.05±0.32	0.31	0.5197
	12	6.25±0.51	6.16±0.34	0.29	0.8103
	13	5.97±0.13	6.12±0.36	0.06	0.5221
	14	5.97±0.09	6.27±0.39	0.05	0.2762
	15	6.20±0.06	6.34±0.26	0.03	0.4360
	16	6.18±0.04	6.38±0.29	0.02	0.3068
	17	6.33±0.06	6.46±0.23	0.03	0.3779
	18	6.32±0.10	6.55±0.16	0.06	0.1010
	19	6.42±0.14	6.61±0.13	0.08	0.1614
	20	6.53±0.21	6.63±0.16	0.12	0.5626
	21	6.69±0.19	6.75±0.17	0.11	0.7048
	22	6.71±0.12	6.83±0.06	0.07	0.2138
	23	6.72±0.09	6.84±0.08	0.05	0.1708
	24	6.72±0.09	6.82±0.05	0.05	0.1877

1component, 2total mixed ration, pH: potential hydrogen, h: hour

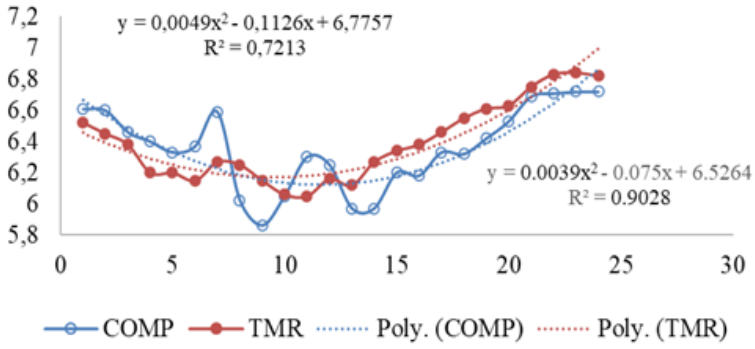

Figure 5. Rumen pH curves of growing dairy cattle fed COMP and TMR

The decreased pH level was highly dependent on saliva production and rumen microorganism activities. Castillo-Lopez *et al*. (2020) described that reduced saliva can result in a drop of ruminal pH because saliva includes bicarbonate, an essential buffer that aids in the neutralization of acids generated during rumen fermentation. Reduced salivary flow reduces the rumen's buffering capacity, which permits acids to build up and bring the pH down.

The lowest value of ruminal pH was observed in the cattle fed COMP diet at 9 h after feeding was reached of 5.86. In both COMP and TMR groups, the pH levels were gradually upward after 12 h and reached a maximum of 6.82. In the TMR group, soon after the next morning, feeding was offered in the last 22 to 24 h reached the normal. The normal pH for the optimum cellulolytic activity is 6.2 to 7.4 and rumen pH of less than 6.2 would seriously inhibit the growth of cellulolytic bacteria (Ørskov 1982; Lana *et al.* 1998; Pamungkas *et al.* 2005).

The decreased ruminal pH may also be influenced by the degree of digestibility and the rate of fermentation (Schroeder *et al*. 2003). Kaufmann (1976), who reported that the high forage ration has a longer rumination time compared to the high concentration rations, which have a shorter rumination time, because they are highly digestible. The shorter time of rumination resulted in less saliva production, increased acid production, and lower pH. Grant and Colenbrander (1990), and Allen (1997) also noted that the forages with smaller particle sizes are highly digestible, rapidly fermented, and lead to a reduction in rumen pH.

Statistically, there were no significant differences in means, but practically, the feeding system affects the rumen pH fluctuation in both groups of the cattle fed COMP and TMR feeding. Figure 5 indicates that the coefficient determination was that 90% of the cattle received TMR and 70% of the COMP group were under the feed condition, digestion, and rumen fermentation by microbial rumen following the retention time. A greater coefficient of determination suggests a strong correlation between the TMR feeding system and improved rumen pH. In addition, the deviation of rumen pH level in the cattle that received TMR feeding with an average of 0.07 was smaller than the cattle received COMP feeding with an average of 0.09. This showed the trend of the rumen pH level from 1 to 24 h, where the cattle fed TMR had less fluctuation. This was in line with the findings of Coppock *et al.* (1981), who compared TMR feeding versus conventional feeding on dairy cattle. Their results suggest that TMR facilitates the improvement of the rumen condition, especially keeping fewer rumen pH fluctuations and making a favorable environment for rumen pH because TMR could provide a more balanced ration with a uniform rate of roughage and concentrate feed (McGilliard *et al*. 1983 and Nocek *et al*. 1985, as cited by Li *et al*. 2003). For dairy cattle, TMR feeding can enhance ruminal digestion, passage rate, and ultimately dry matter intake (Bargo *et al.* 2001; Soriano *et al*. 2001; Kolver *et al.* 1998).

CONCLUSION

The component (COMP) and total mixed ration (TMR) feeding systems did not affect the ruminal degradability capacity of DM, NDF, and ADF in the *in situ* technique. In addition, both COMP and TMR feeding did not affect *in vitro* gas production either. However, this feeding system can influence microbial activity in the rumen, a vital part of a ruminant's digestive system, where TMR feeding has an advantage for maintaining and stabilizing pH levels in the fermentation and digestion of feeds in the rumen. Overall, *in situ* and *in vitro* gas production methods contribute to a better understanding of nutrient utilization and describe digestibility in animals on feed ingredients.

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