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The Potential of Synbiotics Supplementation using Gembili Inulin as a Prebiotic in Improving Fermentability and Productivity of Lambs Fed Diet of Different Fibre Content

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Abstract | This study was carried out to examine the potential of Gembili inulin as a prebiotic source in synbiotic supplementation on nutrient utilization, fermentability and productivity of lambs fed diet of different fibre content. A completely randomized design was used in experiments 1 with 4 treatments. There were 2 steps in experiment 1. 1) Inulin extract from Gembili, buffalo rumen fluid, and a diet were used in vitro 1. 2) Synbiotic (Sin) supplementation, sheep rumen fluid, and a diet were used in in vitro 2. In experiment 2, the study used a completely randomized design (CRD) in 3x2 Factorial structure with 4 replications. The twenty four lambs divided into 3 groups of Pakchong grass: concentrate ratio and each diet conducted in 2 level of synbiotic supplementation. The parameters observed were the fermentability, digestibility of the diet and productivity of lambs. The data obtained were analysed by ANOVA, and if there was a significant effect among treatments, it was followed by Duncan's multiple range test. The study showed that fibrous feed supplemented GI up to 15mg had higher volatile fatty acids and ammonia concentrations ($P<0.05$) and increased microbial protein production ($P<0.05$). Fibrous feed supplemented synbiotic up to 3 % had highest digestibility ($P<0.05$) and increased butyric acid ($P<0.05$). In experiment II, Lambs fed 100% grass and supplemented synbiotic up to 3% had lowest acetic acid and methane production ($P<0.05$). Supplementation of synbiotic did not affect feed intake, digestibility and productivity of lambs ($P>0.05$). The feed intake, nutrient utilization, and productivity of lambs were highly affected by fibre and protein content in the diet ($P<0.05$). In conclusion, The use of Inulin Gembili as a prebiotic source up to 3% in various types of dietary fibre can improve the rumen profile in vitro. Feed intake, nutrient utilization and sheep productivity in vivo did not change with synbiotic supplementation at different levels of fibre and concentrate in the diet. However, Inulin from Gembili as a prebiotic source in synbiotic supplementation reduces the production of acetic acid and methane simultaneously. This kept the ammonia concentration stable and prevents the risk of bloating when protein is increased and dietary fibre sources are reduced.

Keywords | Gembili inulin, Synbiotic, Fermentability, Digestibility, Productivity, Lambs

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INTRODUCTION

Fibrous feed plays an important role in ruminant production. In addition to maintain the life of microorganisms in the rumen, feed with high fibre content has consequences in decreasing digestibility and indirectly have negative impact in livestock productivity and resulted in high methane production (Galyean and Hubbert, 2014; Luthfi *et al*., 2023). Several studies found that the ability to digest fibre in feed and livestock productivity can be optimized by administering probiotics, prebiotics or a combination of both. Probiotics and prebiotics can increase the number of microbes, the number of enzymes and rumen microbial activity (Riswandi *et al*., 2015; Hartono *et al*., 2016). The combination of probiotics with prebiotics are called synbiotics which have great potential to improve conditions in the rumen (Markowiak and Śliżewska, 2018). Increasing the rumen microbial population will increase microbial activity, secretion and concentration of cellulolytic enzymes, thereby increasing the digestibility of fibre in feed and protein and VFA (Irwanto *et al*., 2019; Muhtarudin *et al*., 2020).

Commonly, buffalo rumen fluid was used as a source of probiotic. Buffalo rumen fluid contains bacteria, fungi, yeast and cellulolytic protozoa with the highest ability to degrade fibre in feed. Adult buffalo rumen fluid contains a total of 18.45 x 108 CFU/mL bacteria, total cellulolytic bacteria 6.86 x 108- 3.3 x 109 CFU/mL, cellulolytic activity 43.2%/day, VFA production rate is faster and higher than cow rumen fluid (Aminah *et al*., 2020). On the other hand, a combination of probiotic and prebiotic sources is required to form synbiotic. Prebiotics with high fibre are fermented by rumen bacteria to be used as an energy source (Samanta *et al*., 2013). One of the substances that functions as a prebiotic is inulin. Inulin is a compound that plays an indirect role as a prebiotic by forming short-chain fatty acids in the large intestine after fermentation (Tarini and Wolever, 2010; Ríos-Covián *et al*., 2016) and can increase the number of *Bifidobacterium* and *Lactobacillus* in the large intestine and increase their activity (Samanta *et al*., 2012). Inulin can be easily found in Gembili (*Dioscorea esculenta L.*). The previous study showed that Gembili contains inulin ranging from 10.53% to 14.80%; (Bahlawan *et al*., 2020; Hilman *et al*., 2021). Gembili is also a source of carbohydrate (Bahlawan *et al*., 2020). Gembili contains 59.06–60.52% carbohydrates, including amylose of 9.22–11.4% and amylopectin of 49.84–50.24% (Afoakwa *et al*., 2013; Retnowati *et al*., 2019; Riley *et al*., 2014). The production of Gembili in Indonesia is not recorded. However, this stuff is found abundant in many places in Indonesia, such as Papua (60 – 70 ton/ha/year; Sabda *et al*., 2019), Moluccas (43,02 – 67,56 ton/ha/year; Pesireron *et al*., 2021), Java (6,1-7,1 ton/ha/year; Fitria *et al*., 2024).

Studies of the potential synbiotic supplementation that use Gembili inulin as a source of prebiotic in the fibrous diet of lambs are still limited. It is necessary a study to examine the effect of synbiotic derived from the combination of Gembili inulin as a source of prebiotic to stimulate fermentability and digestibility in fibrous diet of lambs. This study was aimed to determine the potential of Gembili inulin as prebiotic source in synbiotic supplementation on nutrient utilization, fermentability and productivity of lambs fed different fibrous diet.

MATERIALS AND METHODS

There were 2 experiments in this study. The first was an in vitro experiment that consisted of two stages (In vitro 1 and 2). The experiment 1 was to examine the inulin levels of Gembili as a source of prebiotic in forming synbiotics supplementation in the next study. The flow chart of protocol of experiment 1 is presented in Figure 1. The experiment II was conducted in vivo to evaluate the nutrient utilization, fermentability and productivity of lambs. The flow chart of protocol of experiment 1 is presented in Figure 1.

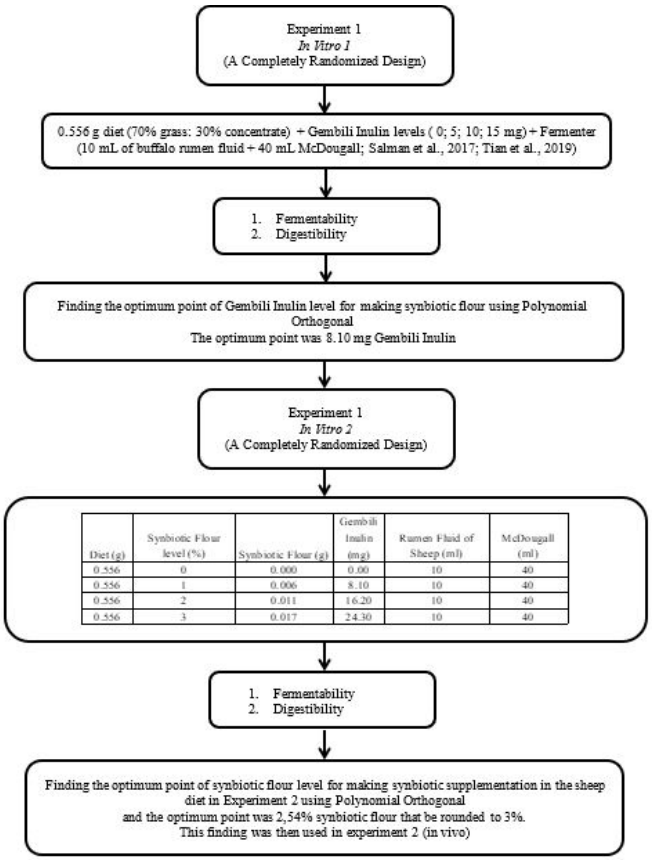

Figure 1: Flow Chart of Protocol of Experiment 1 (In vitro).

Experiment 1

In vitro 1: Experiment I, in vitro 1 and 2 used a Completely Randomized Design (CRD) with 4 treatments and 4 replications. The first step aimed to determine the best

Table 1: Nutritional content of the in vitro diet.

Feed stuff	Moisture (%)	DM (%)	OM (%)	Nutrient Contents (%)					TDN (%)
				Ash	CP	EE	CF	NFE	
				100% DM					
Napier Grass	28.80	71.20	51.20	20.00	9.34	1.26	40.52	28.88	43.99
Concentrate	13.12	86.88	78.30	8.57	18.25	1.44	11.14	60.60	67.89
Total	24.10	75.90	59.33	16.57	12.01	31.71	1.32	38.39	51.16

Abbreviations: DM: Dry Matter; **OM:** Organic Matter; **CP:** Crude Protein; **EE:** Ether Extract; **CF:** Crude Fibre; **NFE:** Nitrogen Free Extract. TDN was calculated based on the equation proposed by Harris *et al.* (1972), as presented in Hartadi *et al.* (1980).

Table 2: Nutritional content of the in vivo diet.

Diet	DM (%)	OM (%)	Nutritional content					TDN (%)	TDN/CP	Energy (kal/g)
			Ash	CP	EE	Cfi	NFE			
			----------100%----------							
D1S0	90.05	77.00	13.05	6.29	0.97	28.95	50.74	49.43	7.86	3900.29
D1S1	92.61	79.49	13.11	6.33	1.00	28.97	53.58	52.06	8.22	4018.01
D2S0	90.35	79.34	11.01	9.68	2.81	25.87	50.62	59.30	6.12	4022.70
D2S1	92.91	81.83	11.08	9.73	2.84	25.89	53.47	61.93	6.37	4140.42
D3S0	90.75	82.45	8.30	14.21	5.27	21.76	50.47	72.45	5.10	4185.91
D3S1	93.31	84.95	8.36	14.25	5.29	21.78	53.31	75.08	5.27	4303.63

Legend: DM: Dry Matter; **OM:** Organic Matter; **CP:** Crude Protein; **Cfi:** Crude Fibre; **EE:** extra ether, **NFE:** Nitrogen free extra; **TDN:** *Total digestible nutrients.*

Gembili level to make synbiotic flour. The treatment applied in experiment 1, in vitro 1 was the level of Gembili inulin (GI) in 50 mL of fermenter (10 mL of buffalo rumen fluid + 40 mL of McDougall + 0.556 g of diet), namely:

G1 = 0 mg GI + 50 mL fermenter
G2 = 5 mg GI + 50 mL fermenter
G3 = 10 mg GI + 50 mL fermenter
G4 = 15 mg GI + 50 mL fermenter

The concentrate contained 67.89% TDN and 18.25% CP. The Napier grass contained 43.99% TDN and 9.34% CP. The nutritional content of diet is presented in Table 1.

Each treatment was conducted in a 100 mL capacity of fermenter tube consisting of a 100-mL fermenter tube filled with 0.556 g of diet + levels of GI + fermenter (40 mL of McDougall's solution + 10 mL of rumen fluid), then supplied with CO gas for 30 seconds and closed with a ventilated rubber cover. The tube was put into a shaker water bath at 39°C, then incubated for 3 hours for volatile fatty acids (VFAs), rumen ammonia, microbial protein; 6 hours for dry matter digestibility (DMD), organic matter digestibility (OMD), crude fibre digestibility (CFD), neutral detergent fibre digestibility (NDFD), and acid detergent fibre digestibility (ADFD). Those incubation were centrifuged to obtain the supernatant and the solid residue. The supernatant liquid was used for testing rumen ammonia, rumen VFAs concentration, DMD, OMD, CFD, NDFD, and ADFD. The solid residue was used for testing rumen microbial protein (Aminah *et al.*, 2020; Nuswantara *et al.*, 2020).

Synbiotic test (In vitro 2): The best results from in vitro 1 were then made into synbiotic flour and continued with the test stage. The treatment applied in experiment 1, in vitro 2 study was Synbiotic (Sin) supplementation in 0.556 g of diet and 50 mL of fermenter (10 mL of sheep rumen fluid (SRF) + 40 mL of McDougall) (Salman *et al*, 2017; Tian *et al.*, 2019), namely:

S1 = 0% Sin + 50 mL fermenter
S2 = 1% Sin + 50 mL fermenter
S3 = 2% Sin + 50 mL fermenter
S4 = 3% Sin + 50 mL fermenter

Making of symbiotic: Cassava flour was sterilized by autoclaving at a temperature of 121°C, a pressure of 1 ATM for 15 minutes. Liquid synbiotics were mixed with cassava flour as much as 1.5 times the weight of the liquid synbiotic until homogeneous and smooth. The dough was placed on a sterile tray and ovened at a temperature of 40°C for 24-48 hours. The synbiotics were then ground, sieved, and tested for the total number of bacteria (Total Plate Count method). A 100 mL capacity fermenter tube was filled with 0.556 g of ration (70% Napier Grass + 30% Concentrate), 0-3%

synbiotic flour from the ration and 50 mL of fermenter (10 mL CRF + 40 mL McDougall's solution) (Salman *et al*., 2017; Tian *et al*., 2019), then flowed with CO gas for 30 seconds and closed with a ventilated rubber cover. The tube was inserted into an shaker water bath at a temperature of 39°C, then incubated for 3 hours (VFA, rumen ammonia, microbial protein); 6 hours (DMD, OMD, CfiD, NDFD, ADFD) and incubated for 6 days to measure cellulolytic enzyme activity (Aminah *et al*., 2020; Nuswantara *et al*., 2020).

Fermentability and digestibility measurements: The parameters observed in experiment I were rumen ammonia concentration, microbial protein concentration, rumen volatile fatty acid (VFA) concentration, dry matter digestibility (DMD), organic matter digestibility (OMD), and crude fibre digestibility (CfiD). Rumen ammonia concentration and microbial protein concentration were analysed using the Conway method (Putri *et al*., 2021); rumen VFA concentration (acetic acid, propionic acid, and butyric acid) was analysed using chromatography method (Jiang *et al*., 2013); dry matter digestibility (DMD), organic matter digestibility (OMD), and crude fibre digestibility (CFD) were analysed using methods employed by Evitayani *et al*. (2023) and Saleem *et al*. (2019), neutral detergent fibre digestibility (NDFD), and acid detergent fibre digestibility (ADFD) were analysed using AOAC method (AOAC, 2016; method 2002.04) as used by Ran *et al*. (2021).

Experiment 2

The best liquid synbiotic (polynomial orthogonal test results) was made into synbiotic flour using the method used by Tampangallo *et al*. (2018) which was modified. Orthogonal polynomial analysis showed the highest value in the cubic phase (R = 0.906) with a regression equation of $Y = 0.816X^3 - 4.507X^2 + 9.230X + 61.485$, resulting in an optimal digestibility value of 69.06% at a synbiotic level of 2.46%, then the result was rounded to 3%. The second experiment was conducted based on the ethical feasibility of the study protocol with No. 58-09/A-9/KEP-FPP. This study used 24 lambs aged 3 months and weighed 12.10 ± 0,67 kg. The lambs were divided into 6 groups of treatments and reared for 3 months. The grouping of lambs based on Completely Randomized Design (CRD) 3x2 Factorial with 4 replications. The first factor was Pakchong grass (PG): concentrate ratio (C), namely 100% PG and 0% C (D1); 70% PG and 30% C (D2); 30% PG and 70% C (D3). The second factor was synbiotic supplementation (S) namely without synbiotic supplementation (S0); synbiotic supplementation 3% of the diet (S1). The combination of the diet and synbiotic supplementation were as follows:

D1S0 = 100% PG: 0% C + 0% synbiotic
D1S1 = 100% PG: 0% C + 3% synbiotic
D2S0 = 70% PG: 30% C + 0% synbiotic
D2S1 = 70% PG: 30% C + 3% synbiotic
D3S0 = 30% PG: 70% C + 0% synbiotic
D3S1 = 30% PG: 70% C + 3% synbiotic

The diet was given in the form of mash. Lambs were fed and watered ad libitum. The parameters observed in Experiment 2 were: feed intake, digestibility, fermentability in rumen and lambs productivity. Feed intake was recorded daily from the calculation of the provision minus the remaining rations every morning. The lambs were weighed every week to obtain body weight data as a basis for feeding (measured ad libitum).

A measurement of feed digestibility and fibre fractions and estimation of body protein production through purine derivatives were carried out through total collection of feces and urine accordance by Luthfi *et al*. (2024). Total collection was carried out in the 3rd week for 7x24 hours, with rations given as much as 4% of body weight. Analysis of dry matter of fresh feces and urine was carried out every day for a week. Feces and urine were collected using a metabolic cage connected to a jerrycan filled with 20% H_2SO_4 as much as 20 mL to keep the nitrogen in the urine from evaporating. The results of the 24-hour feces collection were then weighed every morning and samples of 10% of the total collection were taken for analysis of feces DM levels. The remaining feces samples were then mixed until homogeneous and dried, then ground. The feces samples were then analysed proximately (DM, ash, CP, EE, Cfi, and NFE), energy and Van Soest fibre fraction analysis (NDF ADF, hemicellulose, cellulose and lignin). The results of the 24-hour urine collection were then weighed every morning and samples of 10% of the total collection were taken for analysis of CP levels, and energy and purine derivatives.

Measurement of feed fermentability was carried out by taking rumen fluid at 0; 3 and 6 hours after morning feeding in the 12th week. Rumen fluid was taken by inserting a plastic tube into the lamb's mouth, until it passed through the esophagus into the rumen cavity. The next step was to connect the plastic tube to a stemmed Erlenmeyer flask connected to a vacuum pump and then the vacuum pump was turned on to suck out the rumen fluid until it entered the Erlenmeyer flask as much as ± 50 mL (Luthfi *et al*., 2024). The rumen fluid that had been placed in the Erlenmeyer flask was then filtered using gauze and its pH was measured with a pH meter and H_2SO_4 was added to a pH value of ± 3 then put into a plastic bottle so that could be stored in the freezer and then taken to the laboratory to be analysed for VFA, NH_3 and rumen microbial protein. Measurement of VFA production was carried out using the Gas Chromatography method according to Susilo *et al*. (2019). Measurement of NH_3 production was carried out using the Conway microdiffusion method as used by Suharti *et al*. (2018). Calculation of CH_4 production was

carried out based on the concentration of acetate, propionate and butyrate, according to the method used by Susilo *et al.* (2019). The flowchart of experiment 2 is presented in Figure 2.

Parameters in experiments 2 were measured as follows:

a	**Nutrients intake**	:	**Feed nutrients provided – Remaining feed nutrients**
b	Nutrient digestibility (%)	:	$\frac{Nutrient\ intakes\ (g) - nutrients\ in\ Feces\ (g)}{Nutrient\ intakes(g)} \times 100\%$
Fermentability			
a	Rumen pH Value	:	pH meter
b	Total VFA of Rumen fluid	:	$\frac{(mL\ blank\ titrant - mL\ sample\ titrant) \times N\ HCl \times 1000/_5 mM}{g\ sample \times DM\ sample}$
c	A partial VFA (mM)	:	$\frac{Sample\ area \times standard\ concentration \times 1000}{Standard\ area \times molecular\ weight*}$ * molecular weight of acetate, propionate, and butyrate
d	Ammonia (NH_3) of Rumen fluid	:	$\frac{mL\ H_2SO_4 \times N\ H_2SO_4 \times 1000g}{g\ sample \times DM\ sample}$
e	Methane production (mM)	:	0,5 A - 0,25 P + 0,5 B A: mol acetate; P: mol propionate; B: mol butirate
Feed Utilization			
a	Retained Protein (g)	:	Protein intake - Fecal Protein - Urine Protein
b	Retained Energy (MJ)	:	Energy intake - Fecal energy - Urine Energy
c	Derivate purine	:	Allantoin, uric acid, xanthine-hypoxanthine levels (mmol/L) Allantoin, uric acid, xanthine-hypoxanthine volume (mmol/day) Percentage of allantoin, uric acid, xanthine-hypoxanthine 9%)
d	Microbial protein production	:	Y = 0,84X+(0,15$BW^{0,75}$ $e^{-0,25X}$); BW = Body Weight $Microbial\ nitrogen\ efficiency\ \left(\frac{g}{d}\right) = \frac{X \times 70}{0,83 \times 0,116 \times 1000}$ = 0,727 × X
Lambs Productivity			
e	Average Daily Gain (ADG;g)	:	$\frac{Final\ weight\ (g) - Initial\ weight\ (g)}{rearing\ period\ (days)}$
f	Feed conversion ratio (FCR)		$\frac{Feed\ intake\ (g)}{ADG\ (g)}$

Data Analysis

All data obtained from experiment 1 and 2 were analysed using analysis of variance (F test) with 5% accuracy to determine the effect of treatment. If there was a significant effect. The duncan test was carried out to determine the effect of synbiotic supplementation.

Table 3: Fibre fraction content of the diet in vivo.

Diet	NDF	ADF	Hemicellulose
	.....%		
D1S0	65.45	42.38	23.07
D1S1	65.92	42.47	23.46
D2S0	58.24	35.55	22.68
D2S1	58.71	35.64	23.07
D3S0	48.62	26.45	22.17
D3S1	49.09	26.54	22.55

RESULTS AND DISCUSSIONS

Experiment 1

In vitro 1: The Gembili inulin significantly increased ruminal ammonia, microbial protein production, propionic, butyric acid and acetic/propionic ratio (P<0.05) while acetic acid concentration was significantly decreased (P<0.05). However, total VFA and methane energy were similar among the treatments (P>0.05). The fermentability profile of the diet is presented in Table 4.

The treatment G3 resulted in the highest ruminal ammonia production, while G0 was the lowest one (P<0.05). This study showed that higher inulin supplementation led to higher ruminal ammonia production. This study also showed treatment G3 resulted in the highest microbial protein production (P<0.05). It indicated that the supplementation of inulin encourage the microbial synthesize so that can improve nitrogen utilization. Salman *et al.* (2017) stated that inulin had the potential to improve nitrogen utilization. Wang *et al.* (2020) claimed that inulin fermentation improved nitrogen metabolism in the rumen. Rumen ammonia concentration is determined by protein breakdown by rumen microbes. Inulin provides extra energy for the growth of rumen microorganisms and balances the ratio of carbohydrates to nitrogen. which can contribute to microbial protein synthesis in the rumen.

The increase of inulin stimulated the microbial growth and lead the microbes to digest fibre of the diet to be better. This study agrees with Zhao *et al.* (2016) that ammonia concentration was determined by protein breakdown of rumen microorganisms. Inulin was more effective in promoting bacterial growth when rumen ammonia was sufficient. This finding was also in parallel with the study of Wang *et al.* (2021) that inulin increased the diversity and richness of rumen microbes, the elevated synthesis of microbial protein in rumen.

This study showed that the supplementation of GI up to 15mg (G4) in the fibrous diet increased the proportion of propionic and butyric while decreased the concentration of acetic (P<0.05). Therefore, the acetic/propionic ratio in this

Table 4: Fermentability and digestibility with Gembili inulin (In Vitro 1) .

In Vitro 1	Level of Gembili Inulin				P Value
	G1	G2	G3	G4	
Rumen Fluid Profile					
Ammonia (mg/100mL)	9.27^{c}	11.62^{b}	12.35^{ab}	12.79^{a}	0.00
Microbial Protein Production (mg/100mL)	19.99^{bc}	22.64^{ab}	18.88^{c}	24.27^{a}	0.02
VFA (mMol)	10.49	9.35	12.04	13.15	0.64
Acetic (%)	75.83^{a}	75.44^{a}	73.82^{b}	73.77^{b}	0.00
Propionic (%)	16.87^{c}	17.36^{bc}	18.11^{a}	18.02^{ab}	0.01
Butyric (%)	7.30^{b}	7.20^{b}	8.08^{a}	8.21^{a}	0.00
Acetic/Propionic Ratio	4.50^{a}	4.35^{a}	4.08^{b}	4.10^{b}	0.01
Methane energy (MJ)	0.06	0.05	0.06	0.07	0.35
Digestibility					
DM	39.87	43.23	40.27	40.76	0.29
OM	47.36	51.35	48.34	48.48	0.21
Cfi	43.81	48.65	44.89	47.27	0.07
NDF	37.39	38.00	39.29	40.63	0.93
ADF	37.51	37.11	40.26	43.57	0.47

Abbreviations: VFA: Volatile Fatty Acid; **DM:** Dry Matter; **OM:** Organic matter; **CF:** Crude Fibre; **NDF:** Neutral Detrgent Fibre; **ADF:** Acid Detergent Fibre; $^{a, b, c)}$ different superscripts on the same line shows significant differences (P<0.05).

study decreased, parallel with the increase of GI. In feedlot system, this result was highly expected as indicator of deposition of protein in the body tissue. This was due to inulin can alter the fermentation pattern of rumen microbes without changing the total VFA production. It also indicated that the activity of ruminal microbes lead to a more efficient fermentation pathway for producing propionic and butyric acids, which has the potential to enhance energy efficiency in digestion. Changes in the diversity of rumen microbes or fermentation substrates (fibre and carbohydrates) can alter the composition of VFA without affecting the total amount of VFA produced. Zhao *et al.* (2016) and Zhang *et al.* (2021) stated that properties of inulin as a prebiotic containing fructose, which is easily and quickly fermented by rumen microbes, producing propionic and butyric acids. Inulin supports the growth of non-cellulolytic bacteria (*Prevotella* spp), where the production of propionate through the succinate pathway efficiently utilizes carbon and hydrogen, thereby reducing the availability of hydrogen for methanogenesis. Additionally, the increase in rumen ammonia concentration, which supports microbial protein synthesis, can enhance fermentation efficiency towards propionate production (Misra *et al*., 2022).

Methane energy in this study was similar among the treatments (averaged 0.06 MJ; P>0.05). It indicated that there was no significant change in fermentation efficiency. When the total VFA remains stable (Table 6), there was not enough change in the energy flow produced by fermentation to affect methane production or methane energy. Although there were changes in the proportion of propionate, these changes were not significant enough to reduce methane production. The change of concentration of acetate, propionate, and butyrate was not significant enough to drastically change hydrogen flow or methanogen activity. Therefore, even though there were changes in the VFA composition, the effects on methane emissions and methane energy were not visible.

The digestibility of fibrous diet supplemented GI is presented in Table 5. The supplementation of GI in fibrous diet did not change the DMD, OMD, NDFD, and ADFD of the fibrous diet (P>0.05).

The average of DMD was 41.03%; OMD was 48.88%; CfiD was 46.16%, NDFD was 38.83%; and ADFD was 39.61%. This indicated that inulin in first stage only worked in the rumen fermentation, but did not change the digesta absorption in the digestive tract after the rumen. Inulin was more appropriate to the fermentation substrate composition to promote more efficient alterations in rumen microorganisms than digestion. The DMD and OMD values in this study were lower than the finding of Salman *et al.* (2017), which showed that the inclusion inulin up to 100 mg/l in the rumen fluid fermenter of Karayaka sheep resulted in 66.69-73.21% DMD, 68.55-75.32% OMD, and 20.06-35.70% NDFD. According to Jayanegara *et al.* (2019), the in vitro digestibility value for DMD was 34.0% and OMD was 30.9%.

Table 5: Fermentability and digestibility of the diet supplemented synbiotic in vitro 2.

In Vitro 2	Synbiotic				
	S1	S2	S3	S4	P value
Rumen Fluid Profile					
Ammonia (mg/100mL)	17,20	18,05	18,21	18,41	0,61
Microbial Protein Production (mg/100mL)	25,96	26,15	28,46	30,42	0,31
VFA (mMol)	12,22	12,14	11,18	8,81	0,26
Acetic (%)	75,23	71,97	71,07	67,73	0,06
Propionic (%)	16,16	15,55	15,31	14,65	0,15
Butyric (%)	8,60^{c}	12,48^{bc}	13,63^{ab}	17,63^{a}	0,01
Acetic/Propionic Ratio	4,67	4,64	4,64	4,63	0,99
Methane energy (MJ)	0,07	0,07	0,06	0,05	0,39
Digestibility					
DM	58,81^{c}	63,99^{b}	65,38^{ab}	67,90^{a}	0,00
OM	64,16^{c}	70,05^{b}	71,43^{ab}	73,37^{a}	0,00
Cfi	61,48^{c}	67,02^{b}	68,41^{ab}	70,63^{a}	0,00
NDF	40,45	42,69	44,13	44,21	0,09
ADF	21,17	21,23	23,66	24,01	0,52

Abbreviations: VFA: Volatile Fatty Acid; **DM:** Dry Matter; **OM:** Organic matter; **CF:** Crude Fibre; **NDF:** Neutral Detrgent Fibre;**ADF:** Acid Detergent Fibre;$^{a,b,c)}$ different superscripts on the same line shows significant differences (P<0.05).

Table 6: Nutrient intake of lambs fed different Pakcong grass: concentrate ratio and different synbiotic levels .

Intake	Synbiotics	Diet			average	P value	
		R1	R2	R3			
DM (g/d)	S0	509,27	623,70	739,23	624,07	Diet	0,00
	S1	499,84	693,08	799,83	664,25	Synbiotics	0,16
	average	504,56^{c}	658,39^{b}	769,53^{a}	644,16	Interaction	0,45
OM (g/d)	S0	392,14	494,83	609,52	498,83^{y}	Diet	0,00
	S1	397,34	567,15	679,42	547,97^{x}	Synbiotics	0,04
	Average	394,74^{c}	530,99^{b}	644,47^{a}	541,36	Interaction	0,38
CP (g/d)	S0	32,03	60,40	105,04	65,82	Diet	0,00
	S1	31,65	67,41	113,99	71,02	Synbiotics	0,07
	Average	31,842^{c}	63,91^{b}	109,52^{a}	68,42	Interaction	0,34
Cfi (g/d)	S0	147,44	161,33	160,84	156,53	Diet	0,03
	S1	144,82	179,44	174,20	166,15	Synbiotics	0,20
	Average	146,13^{b}	170,39^{a}	167,52^{a}	161,34	Interaction	0,48
TDN (g/d)	S0	251,75	369,84	536,54	385,71^{y}	Diet	0,00
	S1	260,23	429,20	600,49	429,97^{x}	Synbiotics	0,01
	Average	255,99^{c}	399,52^{b}	568,01^{a}	407,84	Interaction	0,32
Energy (MJ/d)	S0	8,31	10,50	12,95	10,59^{y}	Diet	0,00
	S1	8,40	12,01	14,40	11,60^{x}	Synbiotics	0,04
	Average	8,36^{c}	11,25^{b}	13,68^{a}	10,60	Interaction	0,38

Explanation: ^{a,b,c} Different superscripts in the same row indicate significantly different feed ratio factors (P<0.05; Duncan's Test); ^{x,y} Different superscripts in the same column indicates significant differences between synbiotic supplementation levels (P<0.05).

In vitro 2: Based on the first stage, the results of the Gembili inulin level from the previous stages were then made into synbiotic flour and continued with stage 2. Fermentability of fibrous diet is presented in Table 6. The study showed that there was significant effect on butyrate (P<0.05).

Table 7: Nutrient digestibility of lambs fed different Pakcong grass: Concentrate ratio and different synbiotic levels.

Digestibility (%)	Synbiotic	Diet			Average	P value	
		R1	R2	R3			
DM	S0	54,20	61,03	66,93	60,72	Diet	0,00
	S1	52,45	68,11	69,18	60,79	Synbiotics	0,45
	Average	53,32^{c}	60,28^{b}	68,85^{a}	60,78	Interaction	0,77
OM	S0	54,19	61,03	66,93	60,72	Diet	0,00
	S1	54,18	62,32	63,80	60,79	Synbiotics	0,25
	Average	54,18^{c}	61,48^{b}	65,37^{a}	60,40	Interaction	0,67
CP	S0	54,40	66,31	66,47	68,71	Diet	0,00
	S1	58,84	67,36	68,35	76,87	Synbiotics	0,45
	Average	56,62^{c}	66,83^{b}	67,41^{a}	72,79	Interaction	0,98
Cfi	S0	34,09	43,00	42,09	39,73	Diet	0,33
	S1	43,88	43,10	50,52	45,83	Synbiotics	0,14
	Average	39,98	43,05	46,30	42,78	Interaction	0,56
Retained							
Digested protein (g/d)	S0	14,04	33,09	71,67	39,60	Diet	0,00
	S1	11,89	34,51	75,65	40,68	Synbiotic	0,65
	Average	12,96^{c}	33,80^{b}	73,66^{a}	40,18	Interaction	0,58
Retained Protein (g/d)	S0	6,90	27,35	57,61	30,62	Diet	0,00
	S1	7,84	27,00	65,82	33,54	Synbiotic	0,20
	Average	7,37^{c}	27,16^{b}	61,723^{a}	32,08	Interaction	0,25
Digested energy (MJ/d)	S0	3,34	5,16	7,80	5,55	Diet	0,00
	S1	3,67	6,18	9,38	6,41	Synbiotic	0,10
	Average	3,50^{c}	5,85^{b}	8,59^{a}	5,98	Interaction	0,57
Retained energy (MJ/d)	S0	2,29	4,83	6,48	4,53	Diet	0,00
	S1	3,13	4,78	8,44	5,45	Synbiotic	0,10
	Average	2,71^{c}	4,81^{b}	7,46^{a}	5,49	Interaction	0,20

Explanation: a,b,c Different superscripts on the same row indicate significantly different feed ratio factors (P<0.05); x,y,z Different superscripts in the same column indicate significantly different synbiotic supplementation factors (P<0.05).

The higher supplementation synbiotic up to 3% increased concentration of butyric acid. It indicated that the increase in butyrate was also supported by synbiotic, which aids the growth of butyrate-producing bacteria (*Butyrivibrio fibrisolvens*), and provided a substrate that is easily and quickly fermented through the butyrogenic pathway, especially under conditions of high rumen ammonia concentration. This study was similar to the finding of Zhao *et al.* (2016) that the inclusion of inulin in the low crude protein (CP) 8% and high crude protein (CP) 16% in the diet decreased the concentration of acetic acid, AP ratio, and methane concentration and increased the concentration of propionate and butyrate. Zhao *et al.* (2016) claimed that inulin was likely to suppress rumen microbes involved in fibre fermentation, resulting in a reduction in acetic production. The percentage of acetic, propionic, and butyric acids was higher than the finding of Umucallilar *et al.* (2010), which showed that inulin supplementation of 0–4% in rations with concentrate forage ratios of 20/80, 40/60, and 60/40 analysis in vitro using rumen fluid of male Holstein sires resulted in acetic acid percentages of 52.84–55.67%, propionic acid 18.27–20.07%, and butyric acid 25.05–28.54%.

The digestibility of fibrous diet supplemented synbiotic is presented in Table 7. The supplementation of synbiotic in fibrous diet had significant effect in nutrient digestibility (P<0.05).

It indicated that the digestibility of fibrous diet can be optimized by supplementation of synbiotics. This was due to synbiotics up to 3% increased the population of fermentative microbes in the rumen, which supports feed degradation, especially through the role of inulin as a prebiotic that stimulates the growth of *Fibrobacter succinogenes* and *Ruminococcus albus*, the main bacteria in the breakdown of crude fibre (Amin and Mao, 2021). Buffalo rumen fluid in synbiotics increased the population of more adaptive microbes in the sheep rumen, thereby increasing the activity of

cellulase and protease enzymes, accelerating fibre hydrolysis, increasing nutrient availability, and optimizing fermentation efficiency and feed utilization (Zhao *et al*., 2022).

Experiment 2

Feed intake: Based on the results of Experiment I, the study continued to in vivo treatments. The nutrient intake of lambs is presented in Table 6. The study showed that nutrient intakes had significantly different among treatments (P<0.05). Although there were significant impacts on feed intakes in each treatment factor, there were no interaction between synbiotics and different diet quality (P>0.05). Lambs fed R3 (high concentrate) had highest nutrient intake than the others. The highest DMI intakes was in line with OM, CP, Cfi, TDN and Energy intakes of lambs fed R3. The higher the DM intake, the higher the intakes of OM, CP, Cfi, TDN and energy. This was parallel with the studies of Luthfi *et al*. (2023) and Luthfi *et al*. (2024) that the nutrient intake was influenced by the amount of dry matter intake; the higher the dry matter consumed, the higher the nutrient intake.

The highest nutrient intake in R3 was due to high fibre sources feed such as R1 and R2 have bulky feed characteristics. Increasing concentrate and decreasing grass in feed has consequences for decreasing NDF. The higher the grass used in this study, the bulkier the feed. Bulky feed easily fills the rumen cavity of sheep, so that it physically pushes on the rumen wall and causes faster physical satiety. Increasing concentrate and decreasing grass in feed has consequences for decreasing NDF. Karimazadeh *et al*. (2017) claimed that feed with low volume and high density of nutrient content increases voluntary intake of animals. This study was similar to the finding by Quang *et al*. (2015) and Truong (2021), which found that feed intake enhanced as the amount of concentrate consumed increased in Brahman cattle. On the other hand, supplementation of Synbiotics had significant effects on OM, TDN and energy intakes (P<0.05) but did not on DM, CP and Cfi (P>0.05). Lambs fed S1 had higher intakes than those of S0. It indicated that feed supplemented by Synbiotics improve the organic consumption and total energy.

Digestibility and utilization: The feed digestibility is presented in Table 7. The study showed that the different nutrient quality of feed have significant effect on nutrient digestibility (P<0.05). However, synbiotic supplementation up to 3% in the diet did not change the digestibility (P>0.05). There were no interaction among treatments in nutrient digestibility. Lambs fed R3 had highest digestibility than those of R1 and R2 (P>0.05).

This study indicated that the lower grass and the higher concentrate composition in the diet caused the low fibre content in the diet (Table 3) and it increase the nutrient digestibility. Mirzaei-Alamouti *et al*. (2021) found that the lower NDF resulted in high digestibility. The concentration of NDF was corelated to the cell wall of fibre sources and has the highest impact on feed intake and thus on nutrient intake between diet containing different fibre (Tedeschi *et al*., 2019; McDonald *et al*., 2021).

Supplementation of synbiotic up to 3% in the diet have no effect on the digestibility of DM, OM, CP and Cfi. The digestibility of DM, OM, CP and Cfi was 43.56%, 45.86%, 53.05% and 42.78%, respectively. This study indicated that for ruminants, the addition of synbiotics in the diet of lambs was not strong enough to improve overall digestibility. In this study, there was no influence of synbiotic supplementation on the digestibility value of sheep feed, resulting in no difference in protein and energy utilization in lambs (P>0.05). This finding was in line with the study by Hamasalim (2016), that the concept of synbiotics in supplementation was to change the composition of the gut microbiota with beneficial organisms and non-absorbable organism substrates. The non-significant results on the synbiotic effects of nutrient utilization in sheep were due to improved feed quality, thus improving microbiota and digestibility values. the previous study by Arowolo and He (2018), found that the high digestibility of feed supplemented by synbiotic- mannooligosaccharides (similar to inulin) was largely due to the high digestibility of NDF on the diet. This is thought to be due to the basal diet (different fibre content) may also be a contributing factor and the type and level of probiotics/prebiotics used in synbiotic supplementation on fibre digestion was not yet large enough. This finding was similar to the study by Shrama *et al*. (2023) that supplementation of synbiotic did not affect DM digestibility in the diet of calve. The study by Kazemi-Bonchenar, (2013) dry matter digestibility, crude protein and organic matter were not affected by the use of synbiotics.

The digestion and retention of feed were presented in Table 8. As the results of feed intake and digestibility, this study showed that lowering fibre content in the diet improved the digested and retained nutrients (P<0.05). There was no significant impact on digested and retained nutrient of lambs fed the diet supplemented synbiotic levels (P>0.05) and also there was no interaction among factor treatments (P<0.05). The higher quality diet, the higher the digested and retained nutrient intake of lambs. Lambs fed R3 had the highest digested-retained protein and energy than those of the others. It was due to the significant and the highest nutrient intake of Lambs fed R3 with high digestibility. Therefore, a high feed intakes resulted in high nutrients being digested and metabolized. Luthfi *et al*. (2022) and Luthfi *et al*. (2024) found that digested nutrient of feed was highly affected the degradability and the amount of feed intake. This is in line with this study, where

Table 8: Rumen fluid profile of lambs fed different Pakchong grass: concentrate ratio and different synbiotic levels.

Parameters	Hour	Synbiotic	Diet			average	P value	
			R1	R2	R3			
pH	0	S0	7,55	7,71	7,76	7,67	Interaction	0,83
		S1	7,36	7,68	7,73	7,59	Diet	0,14
		Average	7,46	7,70	7,74	7,63	Synbiotic	0,50
	3	S0	7,57	7,59	7,37	7,51	Interaction	0,99
		S1	7,56	7,56	7,31	7,47	Diet	0,46
		Average	7,56	7,57	7,34	7,49	Synbiotic	0,84
Acetic acid (mmol/L)	0	S0	13,90	19,80	14,78	16,16	Interaction	0,77
		S1	15,40	20,63	22,08	19,37	Diet	0,53
		Average	14,65	20,21	18,43	17,76	Synbiotic	0,43
	3	S0	40,26	16,34	19,98	25,52	Interaction	0,02
		S1	11,04	18,13	26,39	18,52	Diet	0,18
		Average	25,65	17,23	23,18	22,02	Synbiotic	0,07
Propionic acid (mmol/L)	0	S0	2,49	3,68	2,82	3,00	Interaction	0,97
		S1	4,31	5,60	5,36	5,09	Diet	0,71
		Average	3,40	4,64	4,09	4,04	Synbiotic	0,11
	3	S0	8,76	3,72	4,61	5,70	Interaction	0,00
		S1	2,35	3,37	7,64	4,45	Diet	0,04
		Average	5,56[a]	3,55[b]	6,12[a]	5,08	Synbiotic	0,13
Butyric acid (mmol/L)	0	S0	0,79	1,28	1,40	1,15	Interaction	0,91
		S1	1,54	2,44	1,99	1,99	Diet	0,58
		Average	1,16	1,86	1,69	1,57	Synbiotic	0,15
	3	S0	2,66	1,20	1,80	1,89	Interaction	0,02
		S1	0,72	1,19	3,23	1,71	Diet	0,08
		Average	1,69	1,19	2,51	1,80	Synbiotic	0,71
Acetic/Propionic Ratio	0	S0	5,46	5,67	5,30	5,48	Interaction	0,42
		S1	3,73	4,24	4,82	4,26	Diet	0,61
		Average	4,60	4,95	5,06	4,87	Synbiotic	0,12
	3	S0	4,61	4,38	4,42	4,47	Interaction	0,18
		S1	5,26	5,17	3,54	4,65	Diet	0,13
		Average	4,93	4,77	3,98	4,56	Synbiotic	0,63
VFA (mmol/L)	0	S0	17,18	24,75	18,99	20,31	Interaction	0,87
		S1	21,24	28,67	29,43	26,45	Diet	0,55
		Average	19,21	26,71	24,21	23,38	Synbiotic	0,29
	3	S0	51,67	21,26	26,39	33,10	Interaction	0,00
		S1	14,11	22,69	37,26	24,69	Diet	0,14
		Average	32,89	21,97	31,82	28,89	Synbiotic	0,09
Methane (mmol/L)	0	S0	6,73	9,62	7,38	7,91	Interaction	0,81
		S1	7,39	10,14	10,70	9,41	Diet	0,50
		Average	7,06	9,88	9,04	8,66	Synbiotic	0,45
	3	S0	19,26	7,84	9,74	12,28	Interaction	0,00
		S1	5,30	8,82	12,90	9,00	Diet	0,21
		Average	12,28	8,33	11,32	10,64	Synbiotic	0,09
NH_3 (mg/100 mL)	0	S0	6,42	6,72	6,15	6,43	Interaction	0,88
		S1	6,35	6,59	6,22	6,38	Diet	0,07

		Average	6,38	6,66	6,18	6,41	Synbiotic	0,77
	3	S0	6,63	6,71	6,75	6,70	Interaction	0,91
		S1	6,60	6,88	6,82	6,77	Diet	0,66
		Average	6,61	6,79	6,79	6,74	Synbiotic	0,70

Table 9: Protein microbial production and efficiency of microbial protein synthesize of lambs.

Microbial Nitrogen Production (g/d)	S0	0,71	0,91	1,19	0,93	Interaction	0,50
	S1	0,68	1,05	1,36	1,03	Diet	0,00
	Average	0,69^{c}	0,98^{b}	1,27^{a}	0,98	Synbiotic	0,20
Microbial protein production (g/d)	S0	4,41	5,67	7,42	5,83	Interaction	0,50
	S1	4,25	6,55	8,48	6,43	Diet	0,00
	Average	4,33^{c}	6,11^{b}	7,95^{a}	6.13	Synbiotic	0,20
OMD (g/d)	S0	82,78	149,39	218,87	150,35	Interaction	0,54
	S1	96,97	167,54	270,31	178,27	Diet	0,00
	Average	88,88^{c}	158,46^{b}	244,59^{a}	164,31	Synbiotic	0,08
Efficiency of microbial protein synthesize (g/kg OMD)	S0	59,36	39,81	34,18	44,45	Interaction	0,27
	S1	45,44	39,19	31,34	38,66	Diet	0,00
	Average	52,40^{a}	39,50^{b}	32,76^{b}	41,56	Synbiotic	0,11

Explanation: a,b,c Different superscripts on the same row indicate significantly different feed ratio factors (P<0.05); x,y,z Different superscripts in the same column indicate significantly different synbiotic supplementation factors (P<0.05).

probiotic/prebiotic supplementation did not affect NDF digestion (Jin *et al*., 2014; Nunez-Benitez *et al*., 2021). Mc Donald *et al*. (2021) found that low fibrous diet (low NDF) and high dry matter intake resulted in high nutrient digestibility and promote high composition of nutrient that can be metabolized.

Rumen fluid profile: The effect of Synbiotic supplementation in the different quality feed is presented in Table 8. There were no different significant effect on pH rumen fluid among factor treatments and interaction (P>0.05). The average pH value at 0 h and 3 h after feeding was 7.34 and 7.49, respectively. It indicated that pH rumen fluid of lambs was stable even they fed different quality of diet and supplemented different synbiotics.

In general, there was no significant effect among treatments on volatile fatty acids and methane production (P<0.05). However, there was an interaction between treatment at 3 h after feeding. The study showed that increasing supplementation synbiotics in low quality diet was able to reduce acetic acid concentration, and methane production simultaneously. It can be seen that the addition up to 3% in 100% Pakchong grass was able to reduce acetic acid concentration from 40.26 mmol/L to 11.04 (decreased about 28 mmol/l) which also resulted in a decrease in methane from 19.26 mmol to 5.30 (decreased about 14 mmol/l). When the diet quality was improved, the addition of synbiotics actually increased acetic acid and methane production. On the other hand, the propionic acid increased along with the increase of diet quality. Therefore, the acetic/propionic ratio in this study was stable in 4.56. The previous study showed that the combination of prebiotics inulin and *Enterococcus faecium* stimulates postnatal rumen development and improves its function (Arne and Ilgaza, 2021). Commercial synbiotic supplementation in dairy cattle diet has been shown to enhance rumen fermentation and allow cattle to consume feed more efficiently without affecting blood parameters (Turkhachev *et al*., 2022). Other studies have shown that inulin and *S. cerevisiae* supplementation improved rumen and gastrointestinal development of Holstein crossbred calves (Jonova *et al*., 2021). Rumen VFA production is highly correlated with the rumen OM digestion rate, and in turn, the rate and extent of rumen fibre digestion. A study by Arne and Algaza (2021), showed that the addition of inulin as a synbiotic combination in the diet can significantly increase the development of rumen papillae and muscle layer thickness. It was evaluated that the growth of the Saccusventralis muscle and longer Saccusventralis papillae improved in calves fed medium and high doses of inulin and its combination with *E. faecium*. Inulin has increased the development of these tissues because the rumen requires a longer breakdown process for fibrous feed. On the other hand, prebiotic inulin enhances the growth of lactic acid bacteria such as Bifidobacterium and Lactobacillus, which have a positive impact on the microflora and increase the availability and absorption of nutrients (Singh *et al*., 2017).

The ammonia rumen fluid production is presented in Table 9. Ammonia rumen fluid in this study was not significantly different (P>0.05) and there were not interactions among

Table 10: Productivity and FCR of lambs fed different quality diet and supplemented Synbiotic.

Parameters	Synbiotic	Diet			Average	P value	
		R1	R2	R3			
Initial weight (kg)	S0	10,72	11,96	12,91	11,86	Interaction	0,98
	S1	10,96	12,48	13,56	12,33	Diet	0,14
	Average	10,84	12,22	13,24	12.10	Synbiotic	0,63
Final weight (kg)	S0	13,21	17,78	20,98	17,32	Interaction	0,33
	S1	12,30	18,65	24,03	18,32	Diet	0,00
	Average	12,75[c]	18,21[b]	22,50[a]	17,82	Synbiotic	0,36
ADG (g/hari)	S0	29,64	69,23	96,04	64,97	Interaction	0,18
	S1	16,00	73,45	124,56	71,34	Diet	0,00
	Average	22,82[c]	71,34[b]	110,30[a]	68,16	Synbiotic	0,48
FCR	S0	24,01	9,09	8,77	13,97	Interaction	0,31
	S1	34,02	9,72	6,55	16,76	Diet	0,00
	Average	29,04[b]	9,40[a]	7,66[a]	15.37	Synbiotic	0,41

the treatments (P>0.05). The ammonia rumen in this study was in 6.74 mg/100 mL. It indicated that supplementation synbiotics up to 3% kept the ammonia rumen to be stable. By increasing synbiotic supplementation in the diet, it could reducing the risk bloat in animals fed high protein - low fibre diets.

Microbial protein production and efficiency of microbial protein synthesize are presented in Table 9. Different quality diet had significant effect on protein microbial production and microbial protein synthesize (P<0.05). However, synbiotic did not change the protein microbial production, OM digested and microbial protein synthesize (P>0.05). This study also implicated that there was no interaction among factor treatments. The higher the quality diet, the higher protein microbial production and OM digested. Lambs fed R3 had highest protein microbial production and lowest efficiency protein microbial rumen compared to lambs fed R1 and R2. It indicated that increasing of concentrate and reducing the grass composition in the diet drive the high protein content in the diet. The higher protein content in the diet, the higher nitrogen source for synthesize the microbial protein in the rumen. Sharma *et al.* (2023) claimed the microbial protein production was highly associated with increased microbial protein synthesis. Both conditions depend on reduction of ruminal breakdown of dietary N and or increases on microbial protein synthesis. In this study, synbiotics did not significantly affect the amount of N and OM degraded. Therefore, ammonia concentration in the rumen did not change the even the diet were in high protein and low fibre.

Productivity

The productivity lambs fed different fibre content supplemented synbiotics is presented in Table 10. The different of fibre content in feed change the productivity of lambs (P<0.05). However, levelling of synbiotic did not affect productivity and there was no interaction between the treatment on the lambs productivity (P>0.05).

The lower fibre content of the diet, the better the productivity of lambs. The lambs fed R3 had the highest ADG, highest final weight, and lowest FCR (110.30 g/d; 22.50 kg; 7.66) and the lambs fed R2 had the lowest ADG, lowest final weight and highest FCR (22.82 g/d) than the others. It was due to the higher DMI and nutrient utilization of lambs fed R3. Therefore, increasing feed quality resulted in good performance. The lowest FCR of lambs fed R3, indicated that enhancing feed fibre was able to reduce DM requirements for increasing weight gain. Luthfi *et al.* (2022) and Luthfi *et al.* (2024) found that the higher nutrient intake and utilization resulted in high body weight gain and low feed conversion ratio. In this study also showed that no significant effect on lambs productivity in synbiotic supplementation was due to there were no significant effect of synbiotic addition in feed digestibility and utilization.

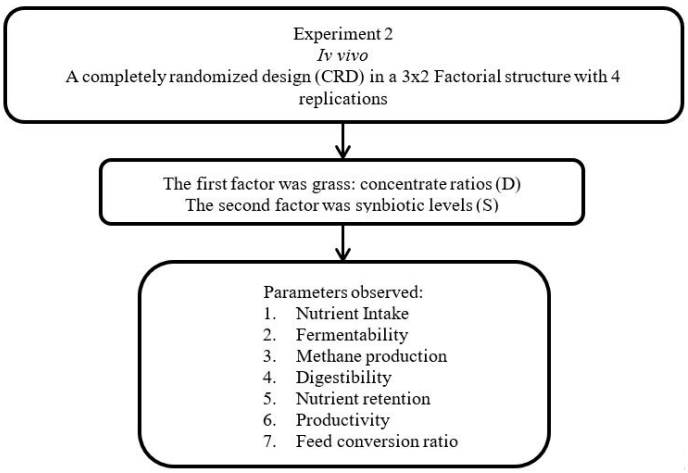

Figure 2: Flow chart of protocol of experiment 2.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results, it can be concluded that Gembili Inulin can be used to be a prebiotic source up to 15 mg. The level of synbiotic up to 3% can be supplemented into the diet. A synbiotic supplementation has a good impact on the fermentability of a diet with high fibre and low protein content. Inulin from Gembili as prebiotic source in synbiotic supplementation decreased acetic acid and methane production simultaneously. It kept the ammonia concentration to be stable and prevented the risk of bloat when protein was increased and fibre source of the diet was reduced.

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NOVELTY STATEMENTS

The author stated that study on the topic presented in this paper is very limited.

AUTHOR'S CONTRIBUTIONS

Conceptualization: EDR, EWP, and ANM; methodology: EDR, ANM, EWP and HDA; validation: EDR.; formal analysis: HDA; investigation: HDA, and EDR. Data curation, EWP and ANM; writing—original draft preparation: HDA and EDR; writing—review and editing: EWP and ANM. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors have declared no conflict of interest.

REFERENCES

Afoakwa EO, Polycarp D, Budu A, Mensah-Brown H, Otoo E (2013). Variability in biochemical composition and cell wall constituents among seven varieties in Ghanaian Yam (*Dioscorea Sp.*) Germplasm. Afr. J. Food Agric. Nutr. Dev., 13(4): 8107-8127. https://doi.org/10.18697/ajfand.59.13280

Aluns MS, Luthfi N (2018). The Productivity of Male Thin-Tailed Lambs and Sheep Fed Complete Feed. OP Conf. Ser. Earth Environ. Sci., 119: 012047. https://doi.org/10.1088/1755-1315/119/1/012047

Aminah, S., L.K. Nuswantara, B.I.M. Tampoebolon dan S. Sunarso. 2020. Peningkatan kualitas sabut kelapa melalui teknologi fermentasi menggunakan mikroba pencerna serat terseleksi dari mikroba rumen kerbau. Jurnal Sains Peternakan, 16 (2): 44–52. DOI: http://dx.doi.org/10.20961/sainspet.v%vi%i.35976

Amin A.B., S. Mao. (2021). Influence of yeast on rumen fermentation, growth performance and quality of products in ruminants: A review: Anim. Nutrit. 7 (1): 31-41. https://doi.org/10.1016/j.aninu.2020.10.005

AOAC. 2016. Official Methods of Analysis As-soc. Off. Anal. Chem., Arlington, VA. Washington DC, USA.

Arne A, Ilgaza A (2021). Prebiotic and synbiotic effect on rumen papilla length development and rumen pH in 12-week-old calves. Vet. World, 14:2883–2888. https://doi.org/10.14202/vetworld.2021.2883-2888

Arowolo MA, He J (2018). Use of probiotics and botanical extract to improve ruminant production in the tropics: A review. Anim. Nutr., 4: 241-249. https://doi.org/10.1016/j.aninu.2018.04.010

Bahlawan ZAS, Damayanti A, Majid NA, Herstyawan A, Hapsari RA (2020). Gembili (*Dioscorea esculenta*) tube modification via hydrogen peroxide oxidation. The 8th Engineering International Conference 2019. Journal of Physics: Conference Series J. Phys. Conf. Ser. 1444. https://doi.org/10.1088/1742-6596/1444/1/012007

Evitayani L Warly A, Fariani B, Putra A, Amir A (2023). The digestibility of *Pennisetum purpureum Cv. Thailand* concerning cowdung supplementation and *Arbuscula Mycorrhizal* Inoculation. Am. J. Anim. Vet. Sci., https://doi.org/10.3844/ajavsp.2023.166.172

Fitria, A., Helbawanti, O., Yuliyani, L., Nuryaman, H., Hakim, A., Benatar, G. (2024). Sub-Optimal Dryland Management: Potential for Local Food Cultivation of Dioscorea sp. (Gembili) to Mitigate Food Security in Sukoharjo Regency, Central Java. PLANTROPICA: Journal of Agricultural Science. https://doi.org/10.21776/ub.jpt.2024.009.1.3

Galyean MI, Hubbert ME (2014). A review: Traditional and alternative sources of fiber-roughage values, effectiveness, and levels in starting and finishing diets11Substantial portions of this paper were presented at the 2012 Plains Nutrition Council Spring Conference and published in the conference proceedings (AREC 2012-26, Texas AgriLife Research and Extension Center, Amarillo). Prof. Anim. Sci., 30 (6): 571-584. https://doi.org/10.15232/pas.2014-01329

Hamasalim H (2016). Synbiotic as Feed Additives Relating to Animal Health and Performance. Adv. Microb., 6: 288-302. https://doi.org/10.4236/aim.2016.64028

Hartono, E.F., N. Iriyanti dan S. Suhermiyati. 2016. Efek penggunaan sinbiotik terhadap kondisi miklofora dan histologi usus ayam sentul jantan. Jurnal Agripet, 16(2): 97–105 . DOI: https://doi.org/10.17969/agripet.v16i2.5179

Hilman A, Harmayani E, Cahyanto MN (2021). The potential of Gembili (*Dioscorea esculenta L.*) and Dahlia (*Dahlia spp L.*) from Indonesia as prebiotic compound. International Conference on Agriculture, Environment and Food Security:2020. IOP Conf. Ser. Earth Environ. Sci., 782 (2021): 032109 IOP Publishing. https://doi.org/10.1088/1755-1315/782/3/032109

Irwanto, Sunarso dan A. Muktiani. 2019. Pengaruh pemberian probiotik isi rumen dari berbagai ternak ruminansia terhadap penampilan produksi domba Batibul. Bulletin of Applied Animal Research, 1(2): 15–20. https://doi.org/10.36423/BAAR.V1I2.267

Jayanegara A, Ardhisty NF, Dewi SP, Antonius, Ridwan R, Laconi EB, Nahrowi, Ridla M (2019). Enhancing nutritional qualityof oil palm empty fruit bunch for animal feed by using fiber cracking technology. Adv. Anim. Vet. Sci. 7(3): 157-163. http://dx.doi.org/10.17582/journal.aavs/2019/7.3.157.163

Jiang, J., Y. Zhang, K. Li, Q. Wang, C. Gong, M. Li. 2013. Volatile

fatty acids production from food waste: effects of pH, temperature, and organic loading rate Bioresour. Technol., 143: 525-530. https://doi.org10.1016j.biortech.2013.06.025

Jin L, Dong G, Lei C, Zhou J, Zhang S (2014). Effects of dietary supplementation of glutamine and mannan oligosaccharides on plasma endotoxin and acute phase protein concentrations and nutrient digestibility in finishing steers. J. Appl. Anim. Res., 42: 160-165. https://doi.org/10.1080/09712119.2013.823863

Jonova S, Ilgaza A, Zolovs M (2021). The impact of inulin and a novel synbiotic (yeast Saccharomyces cerevisiae strain 1026 and inulin) on the development and functional state of the gastrointestinal canal of calves. Vet. Med. Int., 201:8848441. https://doi.org/10.1155/2021/8848441

Karimizadeh E, Chaji M, Mohammadabadi T (2017). Effects of physical form of diet on nutrient digestibility, rumen fermentation, rumination, growth performance and protozoa population of finishing lambs. Anim. Nutr., 3 (2): 139-144. https://doi.org/10.1016/j.aninu.2017.01.004

Kazemi-Bonchenar M, Ghasemi HA, Khodaei-Motlagh M, Khaltabadi-Farahani AH, Ilani M (2013). Influence of Feeding Synbiotic Containing Enterococcus faecium and Inulin on Blood Metabolites, Nutrient Digestibility and Growth Performance in Sheep Fed Alfalfa-Based Diet. Sci. Res. Essays, 8: 853-857. https://doi.org/10.5897/SRE2013.5402

Luthfi N, Rianto E, Purbowati E, Lestari CMS, Purnomoadi A, Mukminah N (2024). Rumen fluid profile, methane emission and nitrogen excretion of young and mature kacang goats under different feeding levels. J. Anim. Health Prod., 12(3): 420-428. https://dx.doi.org/10.17582/journal.jahp/2024/12.3.420.428

Luthfi N, Lestari CMS, Purnomoadi A (2014). Ruminal fermentation and blood glucose at low and high level intake of growing and mature Kacang goat. J. Indonesian Trop. Anim. Agric., 39(3): 152-158. https://doi.org/10.14710/jitaa.39.3.152-158

Luthfi N, Adiwinarti R, Purnomoadi A, Rianto E (2022). Effect of feeding level on growth rate, carcass characteristics and meat quality of thin tailed lambs. J. Indones. Trop. Anim. Agric., 47(4):290-300. https://doi.org/10.14710/jitaa.47.4.290-300

Luthfi N, Solkhan M, Suryani HF, Hindratiningrum N (2023). The Determination of Nutrient Intake on Productivity and Potential Methane Emission of Fat-Tailed Sheep Fed Odot Grass as a Source of Crude Fibre. J. Sain Peternakan Indones., 18(2): 88–92. https://doi.org/10.31186/jspi.id.18.2.88-92

Luthfi N, Restitrisnani V, Umar M (2018). The Optimation of Crude Fiber Content of Diet for Fattening Madura Beef Cattle to Achieve Good A:P Ratio and Low Methane Production. IOP Conf. Ser. Earth Environ. Sci., 119: 012056. https://doi.org/10.1088/1755-1315/119/1/012056

Macdonald JF, Einkamerer OB, Hugo A, Lepori A, Josling GC, Fair MD (2021). Effects of fibre source on nutrient digestibility of diets for finishing lambs. S. Afr. J. Anim. Sci., 51(1): 98-103. https://doi.org/10.4314/sajas.v51i1.11

Markowiak, P., Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathog 10, 21. https://doi.org/10.1186/s13099-018-0250-0

Mirzaei-Alamouti H, Beiranvand A, Abdollahi A, Amanlou H, Patra AK, Aschenbach JR (2021). Growth Performance, Eating Behavior, Digestibility, Blood Metabolites, and Carcass Traits in Growing-Finishing Fat-Tailed Lambs Fed Different Levels of Dietary Neutral Detergent Fiber with High Rumen Undegradable Protein. Agriculture, 11: 1101. https://doi.org/10.3390/agriculture1111110

Misra AK, Singh P, Pathak NN (2022). Effects of dietary prebiotics on rumen fermentation and microbial activity. Anim. Feed Sci. Technol., 297: 114889. https://doi.org/10.1016/j.anifeedsci.2022.114889

Muhtarudin, dan Agung Kusuma Wijaya, M. A. R. F. E. (2020). Pengaruh multi nutrient sauce (MNS) dengan dosis yang berbeda dalam ransum terhadap kecernaan protein kasar dan serat kasar pada domba. Jurnal Riset Dan Inovasi Peternakan (Journal of Research and Innovation of Animals), 4(1), 14-20. Retrieved from https://jrip.fp.unila.ac.id/index.php/JRIP/article/view/81

Núñez-Benítez VH, Barreras A, Estrada-Angulo A, Castro-Pérez BI, Urías-Estrada JD, Zinn RA, Leyva-Morales JB, Plascencia A (2021). Evaluation of a standardized mixture of synbiotic-glyconutrients as a feed additive in steers fed a finishing diet: Site and extent of digestion, ruminal fermentation, and microbial protein synthesis, Livestock Sci., 243:104373. https://doi.org/10.1016/j.livsci.2020.104373

Nuswantara, L. K., Sunarso, M. Arifin dan A. Setiadi. 2020. Komponen serat sabut kelapa yang difermentasi menggunakan mikroba pencerna serat dari rumen kerbau. Jurnal Agripet, 20(1): 1–8. https://doi.org/10.17969/agripet.v20i1.15545

Pesireron, M., R.E. Senewe, A. Gaffar, E.D. Waas, and S. Kaihatu. 2021. Morphology characterization of gembili (Dioscorea esculenta L.) Tanimbar, Maluku Province. E3S Web of Conferences, 306, 01017. https://doi.org/10.1051/e3sconf/202130601017

Putri EM, Zain M, Warly L and Hermon H, 2021. Effects of rumen-degradable-to-undegradable protein ratio in ruminant diet on in vitro digestibility, rumen fermentation, and microbial protein synthesis. Veterinary World 14: 640–648. https://doi.org/10.14202/VETWORLD.2021.640-648

Quang DV, Ba NX, Doyle PT, Hai DV, Lane PA, Malau-Aduli AEO, Van NH, Parsons D (2015). Effect of concentratesupplementation on nutrient digestibility and growth of Brahman crossbred cattle fed a basal diet of grass and rice straw. J. Anim. Sci. Technol., 57(35): (2015).

Ran T, Saleem A, Beauchemin K, Penner G, Yang W (2021). Processing index of barley grain and dietary undigested neutral detergent fiber concentration affected chewing behavior, ruminal pH, and total tract nutrient digestibility of heifers fed a high-grain diet. J. Anim. Sci., 99:1. https://doi.org/10.1093/jas/skab011

Retnowati DS, Ratnawati R, Kumoro AC (2019). Nutritional characteristics and potential applications of flour prepared from Indonesian Wild White Yam (*Dioscorea esculenta L.*). Reaktor; 19 (2): 43-48. https://ejournal.undip.ac.id/index.php/reaktor/article/view/23182

Riley CK, Bahado-Singh PS, Wheatley AO, Asemota HN (2014). Physicochemical properties of low-amylose yam (*Dioscorea spp.)* starches and its impact on α- amylase degradation *in vitro*. Int. J. Nutr. Food Sci., 3(5): 458-464. https://doi.org/10.11648/j.ijnfs.20140305.23

Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, Salazar N (2016). Intestinal short chain fatty acids and their link with diet and human health. Front. Microbiol., 7: 185-185. https://doi.org/10.3389/fmicb.2016.00185

Riswandi, Muhakka dan M. Lehan. 2015. Evaluasi nilai kecernaan secara in vitro ransum ternak sapi Bali yang disuplementasi dengan probiotik Bioplus. Jurnal Peternakan Sriwijaya, 4:

35-46

Sabda, M., H.C. Wulanningtyas, M. Ondikelew and Y. Baliadi. 2019. Characterization of potential local gembili (Dioscorea esculenta L) from Papua as alternative of staple food. Bul. Plasma Nutfah 25(1):25–32. https://doi.org/10.21082/blpn.v25n1.2019.p25-32

Saleem AM, Ribeiro GO, Sanderson H, Alipour D, Brand T, Hünerberg M, Yang WZ, Santos LV, McAllister TA (2019). Effect of exogenous fibrolytic enzymes and ammonia fiber expansion on the fermentation of wheat straw in an artificial rumen system (RUSITEC). J. Anim. Sci., https://doi.org/10.1093/jas/skz224

Salman M, Cetinkaya N, Selcuk Z, Genc B, Acici M (2017). Effects of various inulin levels on in vitro digestibility of corn silage. perennial *Ryegrass (Lolium perenne L.*) and common *Vetch (Vicia sativa L.)*/*Oat (Avena sativa L.)* hay. S. Afr. J. Anim. Sci., 47(5): 723–29. https://doi.org/10.4314/sajas.v47i5.16

Samanta, A.K., N. Jayapa, S. Senani, A.P. Kolte and M. Sridar. 2013. Prebiotic inulin: useful dietary adjuncts to manipulate the livestock gut microflora. Brazilian Journal of Microbiology, 44 (1): 1–14. https://doi.org/10.1590/S1517-83822013005000023

Samanta A, Senani S, Kolte AP, Sridhar M, Bhatta R, Jayapal N (2012). Effect of prebiotic on digestibility of total mixed ration. Indian Vet. J., 89(1): 41-42.

Sharma AN, Chaudhary P, Kumar S, Grover CR, Mondal G (2023). Effect of synbiotics on growth performance, gut health, and immunity status in pre-ruminant buffalo calves. Sci. Rep., 13(1):10184. https://doi.org/10.1038/s41598-023-37002-6

Singh AK, Kerketta S, Yogi RK, Kumar A, Ojha L (2017). Prebiotics: The new feed supplement for dairy calf. Int. J. Livestock Res., 7:1–17. https://doi.org/10.5455/ijlr.20170610051314.

Suharti, S., D.N. Aliyah dan S. Suryahadi. 2018. Karakteristik fermentasi rumen in vitro dengan penambahan sabun kalsium minyak nabati pada buffer yang berbeda. Jurnal Ilmu Nutrisi dan Teknologi Pakan, 16(3): 56–64. https://doi.org/10.29244/jintp.16.3.56-64

Susilo, E., L.K. Nuswantara dan E. Pangestu. 2019. Evaluasi bahan pakan hasil samping industri pertanian berdasarkan parameter fermentabilitas ruminal secara in vitro. Jurnal Sain Peternakan Indonesia, 14(2): 128–136. https://doi.org/10.31186/jspi.id.14.2.128-136

Tampangallo, B.R., I. Trismawanti dan Muliani. 2018. Kelayakan penggunaan probiotik RICA kemasan serbuk untuk pemeliharaan pasca larva udang Windu penaeus Monodon dalam bak terkontrol. Media Akuakultur, 13(2): 91–98. http://dx.doi.org/10.15578/ma.13.2.2018.91-98

Tarini, J. and T.M. Wolever, 2010. The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. Applied Physiology, Nutrition, and Metabolism, 35(1): 9-16. https://doi.org/10.1139/h09-119

Tedeschi LO, Molle G, Menendez HM, Cannas A, Fonseca MA (2019). The assessment of supplementation requirements of grazing ruminants using nutrition models. Transl. Anim. Sci., (3): 812-828. https://doi.org/10.1093/tas/txy140

Tian, K., J. Liu, Y. Sun, Y. Wu, J. Chen, R. Zhang, T. He and G. Dong. 2019. Effects of dietary supplementation of inulin on rumen fermentation and bacterial microbiota. inflammatory response and growth performance in finishing beef steers fed high or low-concentrate diet. Animal Feed Science and Technology.258:1–12. http://dx.doi.org/10.1016/j.anifeedsci.2019.114299.

Trukhachev VI, Buryakov NP, Shapovalov SO, Shvydkov AN, Buryakova MA, Khardik IV, Fathala MM, Komarova OE, Aleshin DE (2022). Impact of inclusion of multicomponent synbiotic Russian Holstein dairy cow's rations on milk yield, rumen fermentation, and some blood biochemical parameters. Front. Vet. Sci., 9:884177. https://doi.org/10.3389/fvets.2022.884177

Truong NB (2021). Effects of dietary concentration levels on feed intake and nutrients digestibility in crossbred beef cattle. Online J. Anim. Feed Res., 11(6): 224-231.

Umucalilar HD, Glen N, Hayirli A, Alatas MS (2010). Potential role of inulin in rumen fermentation. Rev. Med. Vet., 161(1):3e9.

Wang L, Zhang G, Li Y, Zhang Y (2020). Effect of high forage/concentrate diet on volatile fatty acid production and the microorganism involved in vfa production in cow rumen. Animals, 10(2): 223. https://doi.org/10.3390/ani10020223

Wang, Y., X. Nan, Y. Zhao,I. Jiang, H. Wang, D. Hua, F. Zhang, Y. Wang, J. Liu, J. Yao and B. Xiong. 2021. Dietary supplementation with inulin improves lactation performance and serum lipids by regulating the rumen microbiome and metabolome in dairy cows. Animal Nutrition, 7(1), 294–302. https://doi.org/10.1016/j.aninu.2021.01.006

Zhang R, Wang X, Sun X (2021). Role of inulin in modifying rumen fermentation and reducing methane emissions in ruminants. J. Dairy Sci., *104*(3): 2167-2179. https://doi.org/10.3168/jds.2021-20362

Zhao XH, Gong JM, Zhou S, Liu CJ, Qu MR (2016). The effect of starch. inulin. and degradable protein on ruminal fermentation and microbial growth in rumen simulation technique. Ital. J. Anim. Sci., 13(1): 189–95. https://doi.org/10.4081/ijas.2014.3121

Zhao Y, Wang Y, Nan X, Jiang L, Wang,Y, Liu J, Yao J, Rahman MT, Xiong B (2022). Responses of lactation Rumen Fermentation and Blood Biochemical Parameters with Increasing Dietary Inulin Supplementation in Mid-Lactation Dairy Cows. Agriculture, 12(4): 521. https://doi.org/103390/agriculture12040521