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# Effect of Various Feed Additives on the Methane Emissions from Beef Cattle Based on an Ammoniated Palm Frond Feeds

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## Abstract

Methane gas has a very significant contribution to the increase in greenhouse gases (GHG) globally. The livestock sector, especially ruminants, causes the issue of increasing GHG concentrations. The chapter presents the issue of reducing methane gas production from cattle. Various experiments to reduce methane gas production from ruminants have been carried out and have shown varying results. This series of results of the author's research on reducing methane gas production in livestock in beef cattle based on agriculture by-product to animal feed is addressed with this background. Agriculture by-products such as oil palm fronds and rice straw can be used to feed beef cattle in Indonesia. However, agriculture by-product as animal feed can reduce feed efficiency and increase methane gas production due to the high lignin content. Therefore, various alternatives are carried out to optimize the utilization of this plantation waste. One of them is the use of feed additives and methanogenesis inhibitors. The author's series of research using feed additives (direct-fed microbial) and various methanogenesis inhibitors (plant bioactive compounds and dietary lipids) were tested to determine their effect on nutrient digestibility and methane gas production in feed based on plantation waste. Experiments were carried out *in vitro* and *in vivo* on various types of ruminants. Plant bioactive compounds such as tannins are proven to reduce methane production through their ability to defaunate in the rumen. Tannins may also have direct effect on methanogens and indirectly by reducing fiber digestion. In addition, direct-fed microbial (DFM) feed additives such as *Saccharomyces cerevisiae*, *Bacillus amyloliquifaciens*, and *Aspergillus oryzae* can be used in ruminants to increase livestock productivity. Furthermore, virgin coconut oil as a dietary lipid contains medium-chain fatty acids, mainly lauric acid, which can inhibit the development of ciliates of protozoa and methanogenic bacteria that produce methane in the rumen.

**Keywords:** feed additive, direct fed microbials, virgin coconut oils, tannins and saponin, methane emissions, beef cattle, ammoniated palm frond

## 1. Introduction

The main problem in the development of ruminant livestock production in Indonesia, such as beef cattle, is the difficulty of meeting the availability of forage sustainably, both in quality and quantity. Therefore, the use of plantation waste such as palm fronds, rice straw as animal feed is an alternative that can be done to overcome the problem of feed availability. The utilization of plantation waste as ruminant feed is still minimal due to the high content of lignin [1] which causes low digestibility [1–3]. To optimize plantation waste as animal feed, it is necessary to combine processing techniques and optimize bioprocesses in the rumen [3], which aims to increase the microbial population and streamline the fermentation process in the rumen.

Supplementation of direct-fed microbial (DFM) and methanogenesis inhibitors is a way that can be done to increase the efficiency of rumen fermentation [3–5]. DFM is a feed additive product that contains a source of live microorganisms [6], can modify the rumen ecosystem [7], synthesize nutrients so that their availability can increase livestock growth [8]. *S. cerevisiae* is one of the DFM microbes that can be added together with other bacteria and fungi such as *Aspergillus* sp. and *Bacillus* sp. [3]. The administration of *S. cerevisiae* as an additive to live microbes into the body will affect the host by improving the balance of rumen microorganisms [9]. *S. cerevisiae* can compete with starch bacteria [10].

High-fiber feeds such as plantation waste reduce not only the efficiency of feed use [11] but also increase the production of methane gas (CH<sub>4</sub>) [12]. In the livestock sector, methane is one of the gaseous products of fermented feed ingredients by rumen microbes. Ruminants account for more than 75% of methane emissions from total greenhouse emissions [13]. The release of methane causes an increase in the concentration of CH<sub>4</sub> in the air and causes energy loss of 6–13% from the feed [14]. Many livestock nutritionists try to reduce methane production because they feel responsible for the contribution of the livestock sector to atmospheric pollution by methane, as one of the pollutants that is always associated with global warming [15]. Decreased methane production in the rumen is closely related to the metabolic activity of protozoa [16]. Ciliated protozoa in the rumen are in symbiosis with methane bacteria, so that by reducing the population of ciliated protozoa, it will reduce the availability of hydrogen for the formation of methane [17].

Tannins are plant bioactive compounds that can reduce methane production because they act as protozoal defaunation agents [18]. The results of the meta-analysis of *in vivo* experiments with tannins reported by Jayanegara et al., [19] revealed that the concentration of tannins is closely related to the production of CH<sub>4</sub> produced. Different sources of tannins have been shown to have different impacts on CH<sub>4</sub> production. This is probably because the composition and types of tannins [12] are different from different sources. In addition to tannins, Virgin coconut oil (VCO) contains many medium-chain fatty acids (MCFA). Medium-chain fatty acids (MCFA) are known to have a high potential to suppress rumen methanogenic bacteria [20]. The most abundant MCFA in VCO was lauric acid (C12: 0) 51.95% [21]. Soliva *et al.*, [22] stated that lauric acid (C12: 0) is more effective in suppressing methanogenesis than myristic acid (C14: 0). The ability of VCO to modify the rumen ecosystem depends on the level of its addition in the feed [23]. The high lauric acid content in VCO will allow VCO to have the ability as a defaunation agent against ciliated protozoa and inhibit archaea methanogens in the rumen.

Based on the description above, this chapter book presents several reviews of the results of the author’s research, which combines a combination of processing techniques and optimization of bioprocesses in the rumen to increase the value of benefits from plantation waste that can be packaged into complete quality rations, able to increase livestock productivity and reduce beef cattle methane production.

2. Direct fed microbial and virgin coconut oils on methane gas production

2.1 Effect direct-fed microbes on rumen microbial population

Direct-fed microbes (DFM) have comparable results to probiotics. DFM is a feed product that contains a source of live microorganisms [6]. DFM is commonly used as a supplement to increase livestock production. DFM commonly used in ruminants is yeast. DFM works to modify the rumen ecosystem to create an optimal environment for the development of rumen microbes. The provision of DFM as an additive to live microbes in the feed will affect the host by improving the balance of rumen microor-ganisms [9].

The three-stage series of research has been conducted by Suryani *et al.*, [3]. Phase I is a research aimed at optimizing the bioprocess in the rumen through DFM to increase the rumen microbial population. Three types of DFM were used, namely *Saccharomyces cerevisiae*, *Aspergillus oryzae*, and *Bacillus amyloliquefacien*. The substrate used was based on palm frond, which had previously been ammoniated using 6% urea. The evaluation was carried out *in vitro* [24] to determine nutrient degradation and rumen fermentability. The effect of DFM supplementation on rumen fermentability [3] is shown in **Table 1**.

The results showed that DFM supplementation in feed based on plantation waste in the form of ammoniated palm frond could increase rumen fermentability. The

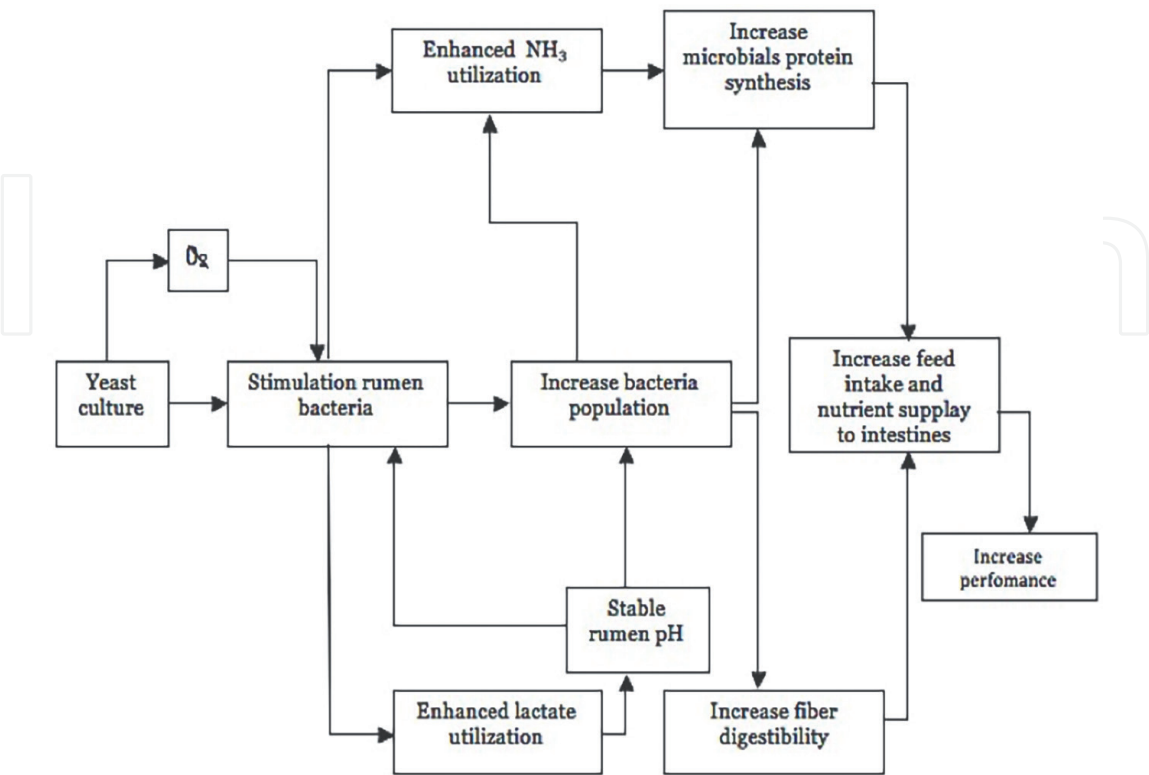
Treatments	Parameters		
	VFA (mM)	NH <sub>3</sub> (mM)	Bacteria population (cell mL <sup>-1</sup> )
P0	108.35 <sup>c</sup>	12.28 <sup>d</sup>	1.61 x 10 <sup>9e</sup>
P1	130.69 <sup>ab</sup>	14.97 <sup>ab</sup>	2.49 x 10 <sup>9ab</sup>
P2	125.10 <sup>cd</sup>	14.47 <sup>ab</sup>	2.37 x 10 <sup>9bc</sup>
P3	123.24 <sup>cd</sup>	13.73 <sup>bc</sup>	2.40 x 10 <sup>9bc</sup>
P4	126.97 <sup>bc</sup>	15.25 <sup>a</sup>	2.41 x 10 <sup>9bc</sup>
P5	132.55 <sup>a</sup>	15.75 <sup>a</sup>	2.55 x 10 <sup>9a</sup>
P6	121.38 <sup>d</sup>	13.06 <sup>cd</sup>	2.35 x 10 <sup>9c</sup>
P7	121.38 <sup>d</sup>	12.78 <sup>cd</sup>	1.93 x 10 <sup>9c</sup>
SE	1.806	0.425	3.33

Source: Suryani *et al.*, 2016, DOI: 10.3923/pjn.2017.599.604  
Numbers followed by different lowercase letters in the same column (a, b, c, d, and e) were significantly different (P < 0.05), SC: *Saccharomyces cerevisiae*, AO: *Aspergillus oryzae*, BA: *Bacillus amilolyquifaciens*, P0: Ammoniated palm fronds, P1: P0 + SC (1%), P2: P0 + AO (1%), P3: P0 + BA (1%), P4: P0 + SC (0.5%) + AO (0.5%), P5: P0 + SC (0.5%) + BA (0.5%), P6: P0 + AO (0.5%) + BA (0.5%), P7: P0 + SC (0.3%) + AO (0.3%) + BA (0.3%), supplementation of DFM % on dry matter basis;

**Table 1.**  
Supplementation of DFM in ammoniated palm fronds on fermentability and bacteria population *in vitro*.

bacterial population increased from  $1.61 \times 10^9$  to  $2.35 \times 10^9$  cell mL<sup>-1</sup>. These results are following the results of research [1, 9] where the addition of probiotics in the ration can stimulate the development of microbes in the rumen and increase the digestibility of food in livestock. The way yeast works in the rumen can utilize oxygen to ensure anaerobic conditions for rumen bacteria and stimulate specific rumen bacterial populations [25] (**Figure 1**). However, there was a tendency for the bacterial population to decrease in the combination supplementation of three types of DFM (P7). It was suspected that there was an accumulation of rumen microbial growth so that bacteria in the rumen competed in digesting feed. The total NH<sub>3</sub> and VFA concentrations increased from 12.28 mM to 14.28 mM and 108.35 mM to 125.90 mM. Desnoyers *et al.*, [26] stated that yeast supplementation could increase the concentration of VFA (2.1 mmol L<sup>-1</sup>) and decrease the concentration of lactate.

Furthermore, DFM fungal *A. oryzae* can reduce oxygen in the rumen [27]. This situation was followed by increased ammonia and lactic acid utilization so that the rumen pH was stable. Anaerobic conditions and stable rumen pH allow more optimal microbial protein synthesis so that the total population of rumen bacteria increases and the digestibility of crude fiber increases. Increased digestibility of crude fiber will increase the consumption and supply of nutrients to the intestines, so that it is expected to increase the overall response of livestock production. Meanwhile, *B. amylolyquifaciens* DFM can produce cellulase enzymes [28], so when yeast is combined with fungal or bacterial DFM, it can increase rumen fermentation with high VFA results. The increase in rumen fermentability was also followed by dry matter and organic matter digestibility which increased from 47.5% (without DFM) to 51.55% (with DFM) and 48.89% to 52.41% [3]. DFM *S. cerevisiae* can be used individually or in combination with *A. oryzae* or *B. amylolyquifaciens*. However, when viewed from the average value produced, the *S. cerevisiae* + *B. amylolyquifaciens* combination gave the best rumen digestibility and



**Figure 1.**  
Mode of action DFM in the rumen.



fermentability results. This is because *S. cerevisiae* can produce growth factors for microbial growth from organic acids, B vitamins, and amino acids to stimulate rumen microbial activity and development [29]. A brief diagram illustrating the working principle of DFM in the rumen. It can be seen in **Figure 1** [25] modified.

Yeast culture uses oxygen to metabolize feed particles into sugars and oligosaccharides to produce peptides and amino acids as end products used by bacteria. Most rumen microorganisms are anaerobic, so the utilization of oxygen by yeast culture will increase the optimum conditions in the rumen. These conditions will protect the anaerobic rumen bacteria from damage by O<sub>2</sub>. They created better conditions for the growth of cellulolytic bacteria so that the number of cellulolytic bacteria increases and improves digestion in the rumen [30].

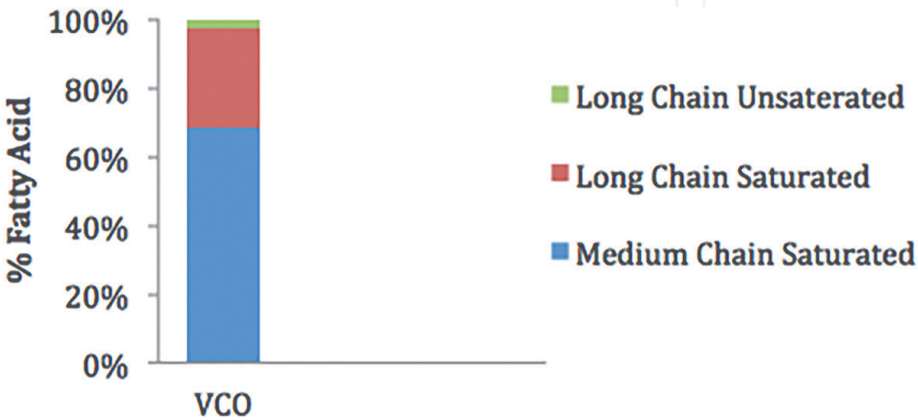
Yeast activity as DFM can regulate rumen biological activity by stimulating lactic acid utilization and reducing ammonia production, so that rumen pH is stable and increases nutrient absorption and VFA profile [31]. Supplementation can support livestock productivity by increasing intestinal development, mucosal immunity, nutrient absorption, and inhibiting pathogenic bacteria. This will have an impact on improving livestock health and performance [32].

## 2.2 Effect virgin coconut oils on methane gas concentration

In another study, to streamline the digestive process in the rumen, Suryani *et al.*, [3] continued the best DFM results from the 1st stage of the experiment to be combined with methane emission reducers. Virgin coconut oil (VCO), rich in MCFA, is used to reduce methane emissions. VCO is oil produced from fresh coconuts. VCO contains lauric acid (C12:0), which effectively suppresses methanogenic bacteria and rumen protozoa [5]. The VCO used in this study contained lauric acid (C12: 0) 51.95% [21] (**Figure 2**).

The purpose of this experiment is to get the best VCO level combined with the best type of DFM stage 1 on ammoniated palm fronds. The three VCO levels tested were 2, 3, and 4% DM. The two best types of DFM from stage 1 used as controls were *S. cerevisiae* and *S. cerevisiae* + *B. amyloliquifaciens* 1% DM. Experiments were carried out *in vitro* according to the method [24].

The effect of combined VCO and DFM supplementation on methane gas concentration and rumen protozoa population *in vitro* on ammoniated palm frond-based feed can be seen in **Table 2**, **Figures 3** and **4**.

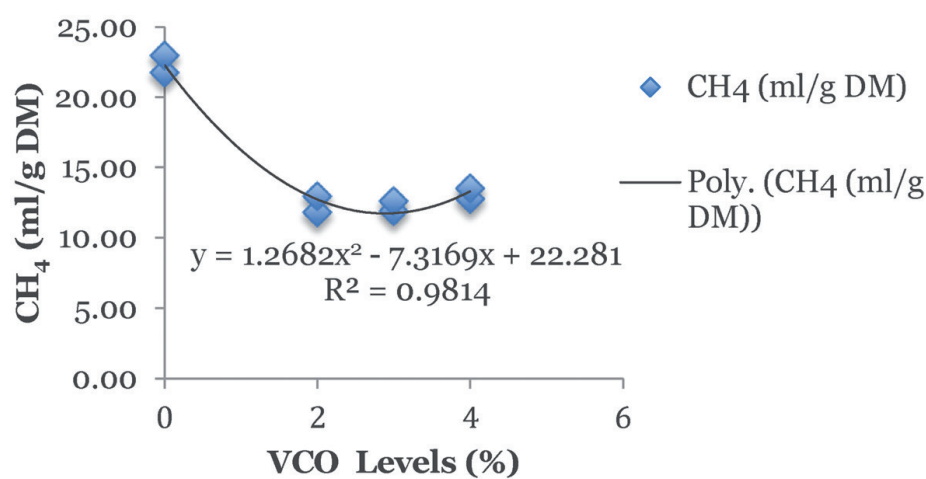


**Figure 2.**  
Fatty acid composition of VCO.

Treatments	CH <sub>4</sub> (ml/g DM)	Protozoa (cell/mL-1)
P1: SC 1% + 0% VCO	21.74 ± 1.16	7.08 x 10 <sup>4</sup> ± 0.23
P2: SC 0.5% + BA 0.5% + 0% VCO	22.94 ± 0.84	7.23 x 10 <sup>4</sup> ± 0.36
P3: SC 1% + 2% VCO	11.78 ± 0.62	1.92 x 10 <sup>4</sup> ± 0.09
P4: SC 0.5% + BA 0.5% + 2% VCO	12.92 ± 0.22	2.23 x 10 <sup>4</sup> ± 0.09
P5: SC 1% + 3% VCO	11.87 ± 0.79	1.97 x 10 <sup>4</sup> ± 0.09
P6: SC 0.5% + BA 0.5% + 3% VCO	12.58 ± 0.15	2.65 x 10 <sup>4</sup> ± 0.15
P7: SC 1% + 4% VCO	12.75 ± 0.93	3.38 x 10 <sup>4</sup> ± 0.09
P8: SC 0.5% + BA 0.5% + 4% VCO	13.49 ± 0.09	3.28 x 10 <sup>4</sup> ± 0.15

Note. Substrate based on Ammoniated palm frond treated with 6% urea, DFM supplementation and VCO level on dry matter basis, SC: *Saccharomyces cerevisiae*, AO: *Aspergillus oryzae* BA: *Bacillus amylolyquifaciens*, VCO: Virgin coconut oils;

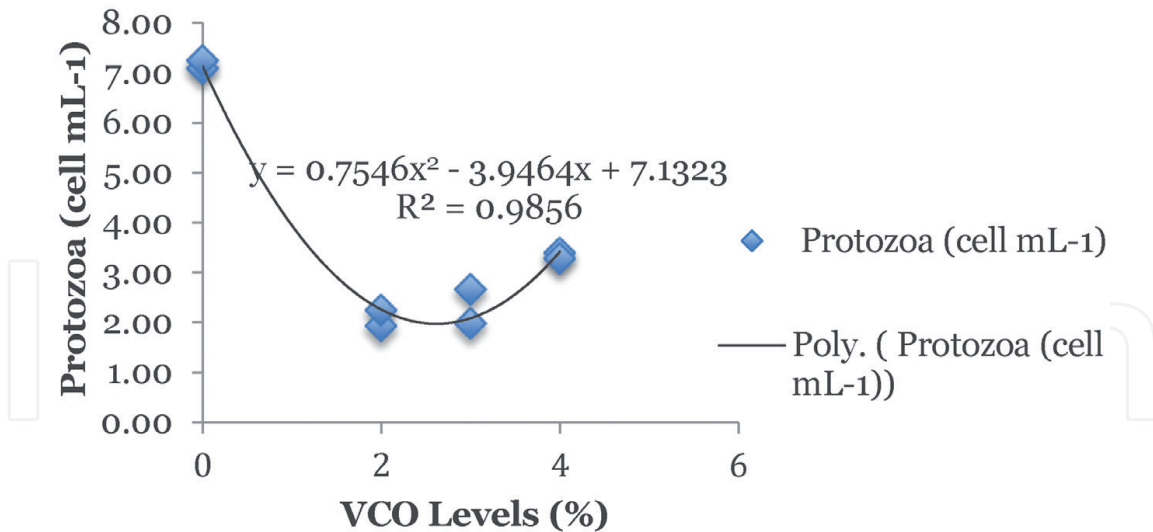
**Table 2.**  
Production of methane (CH<sub>4</sub>) and protozoa population from the fermentation of ammoniated palm fronds in vitro in the rumen for each DFM type level and VCO levels.



**Figure 3.**  
The relationship between DFM + VCO levels and methane production from rumen fermentation of ammoniated palm frond during 48 hours incubation.

The results of the orthogonal polynomial test show a quadratic relationship ( $P < 0.05$ ) between the level of VCO ( $X$ , %) and the concentration of methane gas in the rumen ( $Y$ , mM) with the equation  $y = 1.2682x^2 - 7.3169x + 22.281$  and the coefficient of determination  $R^2 = 0.98137$  (**Figure 3**).

Based on the orthogonal polynomial test, methane gas concentration at the level of 2% VCO addition with DFM *S. cerevisiae* and *S. cerevisiae* + *B. amylolyquifaciens* decreased by 48.11% and 43.67%, respectively. The addition of a 3% VCO level also decreased methane gas concentration compared to without supplementation and resulted in an average of 11.87 mM and 12.58 mM. The decrease in methane gas concentration occurs because VCO is rich in MCFA, mainly lauric acid (C12:0) (**Figure 2**), which is effective in suppressing methanogenic bacteria and rumen protozoa [5]. Lauric acid is the most toxic to protozoa [33] and is the most potent antiprotozoal that inhibits ciliated protozoa's growth and activity (mainly *Entodinium spp.*) [22]. The decrease in ciliate protozoa population due to defaunation causes a decrease in the symbiosis between ciliate protozoa and methanogens, thereby reducing the availability of hydrogen for methane formation [17].



**Figure 4.**  
The relationship between DFM + VCO levels and the population of protozoa produced by fermenting the rumen of ammoniated palm frond during 48 hours of incubation.

Furthermore, Dohmet *et al.*, [33] reported that lauric acid (C12:0) and myristic acid (C14:0) could reduce methanogenesis in the rumen and significantly reduce total methanogenic bacteria. This result is also supported by Machmuller *et al.*, [20]. The effect of coconut oil supplementation is to reduce methane by inhibiting the metabolic activity of archaea methanogens directly in the rumen.

Supplementation of *S. cerevisiae* and VCO DFM at all levels (P3, P5, and P7) can reduce methane concentration better than the combination of DFM *S. cerevisiae* + *B. amyloliquifaciens* and VCO at all levels (P4, P6, and P8). This indicates that when *S. cerevisiae* type DFM combined with VCO can support a decrease in methane concentration in rumen fermentation activity, this is also suspected because *S. cerevisiae* as DFM also can reduce methane. Yeast supplementation can also stimulate acetogenins to compete for hydrogen with methanogens, thereby reducing methane emissions [34].

The results of the orthogonal polynomial test give a quadratic relationship between the VCO level (X, %) and the protozoa population (Y, cell/mL-1), the Eq.  $Y = 0.7546x^2 - 3.9464x + 7.1323$  and the coefficient of determination ( $R^2$ ) = 0.98564 is shown on **Figure 4**. The average population of protozoa with the addition of VCO in the rumen can be seen in **Table 3**.

Based on the orthogonal polynomial test, the protozoa population decreased with VCO supplementation. Supplementation of 2% and 3% VCO (P3,P4,P5,P6) on palm fronds with the addition of DFM *S. cerevisiae* and *S. cerevisiae* + *B. amyloliquifaciens* reduced the protozoa population by 72.88%, 69.15%, 72, 17 and 63.32%, respectively. This result was also followed by a decrease in methane gas concentration in this treatment. Protozoa populations are closely related to rumen methane production [35]. 7 to 37% of methanogens live in symbiosis with protozoa in the rumen [5]. The results of this combination of DFM and VCO supplementation resulted in a decrease in the percentage of protozoa population, which was the same as that obtained by Kongmun *et al.* [36] that the protozoa decreased 68–75% by supplementing with 7% coconut oil. Furthermore, this result is greater than that obtained [37] that coconut oil and lauric acid supplementation reduced the protozoan population by up to 40%.

Meanwhile, total protozoa (especially *Entodinium spp*) decreased by 96% due to lauric acid supplementation compared to myristic acid on a concentrate rich substrate [38]. This indicates that DFM supplementation in high-fiber feeds such as palm



Variables	Treatments				SE
	A	B	C	D	
DM Intake (kg day <sup>-1</sup> )	3.16 <sup>a</sup>	3.01 <sup>b</sup>	2.99 <sup>b</sup>	2.57 <sup>c</sup>	0.143
DM/BW <sup>0.75</sup> (g kg <sup>-1</sup> b.wt. <sup>0.75</sup> d <sup>-1</sup> )	79.94 <sup>a</sup>	75.68 <sup>ab</sup>	74.24 <sup>ab</sup>	67.36 <sup>b</sup>	1.790
OM Intake (kg/h/d)	3.93 <sup>a</sup>	3.74 <sup>b</sup>	3.72 <sup>b</sup>	3.19 <sup>c</sup>	0.017
OM /BW <sup>0.75</sup> (g kg <sup>-1</sup> b.wt. <sup>0.75</sup> d <sup>-1</sup> )	99.28 <sup>a</sup>	97.14 <sup>a</sup>	93.98 <sup>a</sup>	83.65 <sup>b</sup>	1.504
ADG (kg day <sup>-1</sup> )	0.53 <sup>c</sup>	0.63 <sup>b</sup>	0.63 <sup>b</sup>	0.71 <sup>a</sup>	0.007
Feed Efficiency (%)	16.96 <sup>c</sup>	20.84 <sup>b</sup>	21.34 <sup>b</sup>	27.77 <sup>a</sup>	0.311
Methane production (L day <sup>-1</sup> )	109.01 <sup>c</sup>	103.27 <sup>b</sup>	102.61 <sup>b</sup>	86.52 <sup>a</sup>	0.501
Nitrogen intake (g day <sup>-1</sup> )	59.20 <sup>a</sup>	56.34 <sup>b</sup>	55.96 <sup>b</sup>	47.84 <sup>c</sup>	0.815
Nitrogen retention (g day <sup>-1</sup> )	50.51 <sup>a</sup>	47.99 <sup>a</sup>	47.16 <sup>a</sup>	37.23 <sup>b</sup>	0.797

Source: Suryani *et al.*, 2017,<http://dx.doi.org/10.3923/pjn.2017.599.604>.  
Numbers followed by different lowercase letters (a, b, c) in the same row are significantly different ( $P < 0.05$ ), A: 100% Complete feed, B: A + 1% SC, C: A + 0.5% SC + 0.5% BA, D: A + 2% VCO + 1% SC. DM: Dry matter, OM: Organic matter, BW: Body weight, ADG: Average daily gain, SC: *Saccharomyces cerevisiae*, BA: *Bacillus amyloliquifaciens*, VCO: Virgin coconut oils;

**Table 3.**  
Effect of DFM and VCO supplementation on consumption, ADG, efficiency, and methane production of Bali cattle.

oil plantation waste plays an important role in modifying the rumen ecosystem so that the addition of VCO at the right level can reduce the concentration of methane and protozoa without reducing nutrient degradation. From the results of this study, it is recommended that 2% VCO be used for cattle *in vivo* because levels 3 and 4% give almost the same average results.

In other studies, Suryani *et al* [24] continued the experimental *in vitro* studies of stages I and II into a complete ration formulation based on ammoniated palm fronds prepared with a TDN content of 63.28%. *In vivo* tests were carried out using 16 Bali cattle to determine the effect of adding DFM *S. cerevisiae*, *S. cerevisiae* + *B. amyloliquifaciens*, and *S. cerevisiae* + 2% VCO on livestock productivity. Blood samples were collected to determine the effect of DFM and VCO supplementation on the blood profile. Blood samples were taken once before the cattle were fed in the morning (fasting). Blood samples were taken through the jugular vein using a 10 ml capacity syringe and placed in a vacutainer. Blood serum was separated using centrifugation at 3000 rpm for 10 minutes. Analysis of glucose levels, total protein, urea, BUN, albumin, triglycerides, total cholesterol, HDL, and LDL was carried out using the HumaStar 80® Auto Analyzer. A statistical test was carried out to determine the effect of treatment on the observed parameters, using a variance according to the design used. If there was a significant effect, it was continued with Duncan's test [39].

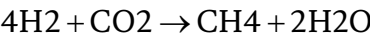
The effect of DFM and VCO supplementation on Bali cattle on performance and methane gas production [21] is shown in **Table 3**.

DFM and VCO supplementation decreased methane production by 5.26, 5.87, and 20.63% respectively. The highest ration efficiency was in DFM *S. cerevisiae* + 2% VCO supplementation, followed by ADG at 0.70 (kg/h/d) and decreased methane production by 20.63% [21]. DFM yeast was reported to have the ability to reduce methane production by 28% [40]. Yeast supplementation could also stimulate acetogens to compete for hydrogen with methanogens, thereby reducing methane emissions [41]. With reduced methane production in the rumen, it can increase feed energy, which

positively affects livestock performance. This can be seen from the decrease in DM and OM consumption but can increase Efficiency and ADG. The digestibility of DM, OM, NDF, ADF, Cellulose, and TDN also increased with DFM supplementation and the combination of DFM *S. cerevisiae* + VCO [21]. The mechanism of DFM can reduce methane production, presumably because DFM microorganisms can stimulate the development of rumen microbes in digesting feed so that fermentation of carbohydrates in the rumen results in high production of propionate. In the rumen, propionate production requires H<sub>2</sub> bound to glucose which is described in the following equation.



Therefore, to reduce hydrogen production to methane, hydrogen must be switched to propionate production via lactate or fumarate [42]. H<sub>2</sub> and CO<sub>2</sub> are substrates used to form methane. According to Wilkie [43] the role of hydrogen in the methane production process is as a source of electrons, so the low level of H<sub>2</sub> in the rumen is an indication of activity using H<sub>2</sub> to reduce CO<sub>2</sub> to CH<sub>4</sub>. In addition, to form one mole of CH<sub>4</sub> requires four moles of H<sub>2</sub>. The rate of H<sub>2</sub> utilization is four times the rate of methane production so that H<sub>2</sub> in the rumen never accumulates. The following is the stoichiometry of the carbohydrate fermentation reaction in producing methane gas in the rumen:



The effect of DFM and VCO supplementation on Bali cattle on blood profile can be seen in **Table 4**.

The results showed that DFM and VCO supplementation had a very significant effect (*p* < 0.05) in reducing cholesterol, LDL and increasing HDL blood levels of Bali

Variables	Treatments				SE
	A	B	C	D	
Cholesterol (mg/dl)	137.18 <sup>a</sup>	124.25 <sup>b</sup>	122.00 <sup>b</sup>	108.69 <sup>c</sup>	1.508
Triglycerides (mg/dl)	87.29	89.08	95.70	108.49	3.305
LDL (mg/dl)	76.14 <sup>a</sup>	72.49 <sup>b</sup>	70.43 <sup>b</sup>	67.18 <sup>c</sup>	0.501
HDL (mg/dl)	184.00 <sup>a</sup>	170.07 <sup>b</sup>	168.05 <sup>b</sup>	147.00 <sup>c</sup>	1.814
Urea (mg/dl)	29.42	24.53	24.06	19.53	0.954
Protein (g/dl)	6.17	6.99	7.18	7.66	0.226
Albumin (g/dl)	3.13	3.31	3.45	3.56	0.046
Glucose (mg/dl)	70.09	73.75	76.69	80.08	0.679

Numbers followed by different lowercase letters (a, b, c) in the same row are significantly different (*P* < 0.05), A: 100% Complete feed, B: A + 1% SC, C: A + 0.5% SC + 0.5% BA, D: A + 2% VCO + 1% SC. DFM: Direct fed microbials, SC: *Saccharomyces cerevisiae*, BA: *Bacillus amyloliquifaciens*, VCO: Virgin coconut oils, LDL: low density lipoprotein, HDL: high density lipoprotein.

**Table 4.**  
Blood profile of complete diet based on ammoniated palm fronds supplemented with DFM and VCO.

cattle. DFM and VCO supplementation had no significant effect ( $P > 0.05$ ) on triglycerides, urea, protein, albumin, and glucose. VCO contains MCFA, which is a saturated fatty acid (**Figure 1**), its addition in the ration if consumed by livestock can help lower cholesterol because of the nature of this fatty acid, which can be absorbed directly by the animal's body so that it does not cause fat accumulation that causes cholesterol. This is supported by Fernando *et al.* [44], which states that MCFA is directly converted into energy in the liver and increases metabolic rate, and reduces fat deposits in the body. MCFA has a very high solubility in water and requires fewer digestive enzymes, making it burnt into energy. MCFA is burned to produce energy and encourages the combustion of LCFA [45]. So there is a significant decrease in the amount of LDL and is followed by an increase in HDL in the blood. The calories contained are also lower than long-chain fatty acids [46]. Reducing fat deposits in the body can lower LDL cholesterol and increase HDL cholesterol [47].

This study can conclude that individual *S. cerevisiae* DFM supplementation and *S. cerevisiae* + *B. amyloliquifaciens* combination can optimize bioprocesses in the rumen. VCO supplementation level of 2% can be used to suppress methane production. Supplementation of *S. cerevisiae* type DFM and 2% VCO level can be considered to optimize bioprocesses in the rumen, increasing performance and reducing methane production in Bali cattle fed complete rations based on ammoniated palm fronds.

### 3. Effect of different source tannins on methane gas production

Bioactive compounds, including polyphenols, carotenoids, omega-3 fatty acids, vitamins, organic acids, nucleotides, and nucleosides, have attracted significant attention for their role in preventing several chronic diseases in humans. In animal husbandry, especially ruminant nutrition, bioactive plant polyphenolic compounds such as tannins and saponins have been studied extensively for optimizing bioprocesses in the rumen through feed manipulation. Manipulation of feed using tannins as an agent of rumen defaunation is one way to overcome global climate change due to the effects of greenhouse gases, one of which is caused by methane gas from ruminants [18]. Feeds containing tannins will be anti-nutrients that limit livestock production when the crude protein concentration in the feed is high because it can reduce the absorption of amino acids [48]. Tannins can also cause poisoning if consumed by livestock in excess, and there are many *in vitro* and *in vivo* studies that describe the methane inhibitory effect of tannins [19]. The study results Staerfl *et al.* [49] proved that the use of tannins could reduce CH<sub>4</sub> emissions by up to 36% in bulls fed grass, corn silage, and concentrate rations. Not many studies have explored the use of tannins in feed based on plantation waste. Therefore, the authors are interested in conducting a series of experiments using tannins from different sources. Plant bioactive compounds used are tannins derived from *gambir leaves waste* (GLW) and obtained from two different sources or areas, namely GLW Payahkumbuh and Painan. GLW was added at different levels (10, 15, 20%) to the ammonium palm midrib substrate with the addition of 4% urea [50]. Experiments were carried out *in vitro* and *in vivo*.

In another *in vitro* study in the same group, the authors also tried to compare *Gliricidia sepium* in animal feed based on rice straw plantation waste [51]. *Gliricidia sepium* is a bioactive plant compound containing thick tannins and saponins capable of modifying the number of rumen microbes such as archaea, protozoa, and fibrolytic bacteria that affect the fermentative process and production of methane gas [52]. The study was conducted *in vitro*. Complete feed is prepared based on ammoniated

rice straw. Three levels of *Gliricidia sepium* tested were 10, 20, and 30% DM basis. The study results, the effect of different sources and levels of tannins on dry matter digestibility (DM), organic matter (OM), methane gas concentration, protozoa, and bacteria can be seen in **Table 5**.

The results showed that different sources and doses of tannins proved to have different effects on decreasing methane production [50]. The *in vitro* study results showed that supplementation of 15% GLW and 10% GLW, which had a total tannin concentration of 12.5 and 15.6% dry matter, respectively, could reduce methane gas concentration by 53% and 45% compared to control. The decrease in methane gas was followed by a decrease in the protozoa population by 53.89% compared to control. Different levels and sources of GLW had no significant effect ( $P > 0.05$ ) on the total bacterial population. However, there is a tendency for the bacterial population to increase as the population of protozoa and methane decreases. Tannins decrease methane production by reducing methanogenic bacteria and protozoa [53]. Furthermore, it was reported that condensed tannins extracted from different plants had different effects on rumen fermentation characteristics. This is because it is associated with different chemical structures and molecular weights [54, 55]. Condensed tannins extracted from different plants have varied activities in binding carbohydrates and proteins [56].

Furthermore, the *in vitro* results of the addition of GLW as a source of tannins were tested *in vivo* on three Simmental cattle [12] with a weight ranging between 179 and 190 kg using the BSL design. The results showed that two sources of tannin levels could increase nutrient digestibility but had no effect on protein digestibility,

Treatments	DM (%)	OM (%)	Protozoa population (cell mL <sup>-1</sup> )	CH <sub>4</sub> (mL/g DM)	VFA Total (mM)	A: P Ratio
T0	48.45 <sup>b</sup>	51.34 <sup>b</sup>	11.43 x 10 <sup>4a</sup>	27.22 <sup>a</sup>	71.00 <sup>b</sup>	3.98 <sup>a</sup>
B1	51.59 <sup>ab</sup>	54.17 <sup>ab</sup>	2.3 x 10 <sup>4c</sup>	23.64 <sup>ab</sup>	83.70 <sup>ab</sup>	2.70 <sup>c</sup>
B2	52.09 <sup>a</sup>	57.30 <sup>a</sup>	1.4 x 10 <sup>4c</sup>	12.67 <sup>c</sup>	95.78 <sup>a</sup>	3.52 <sup>ab</sup>
B3	50.93 <sup>ab</sup>	53.15 <sup>ab</sup>	4.8 x 10 <sup>4b</sup>	13.14 <sup>c</sup>	65.94 <sup>b</sup>	3.38 <sup>ab</sup>
C1	51.08 <sup>ab</sup>	54.16 <sup>ab</sup>	4.7 x 10 <sup>4a</sup>	15.13 <sup>c</sup>	75.49 <sup>ab</sup>	2.58 <sup>b</sup>
C2	50.69 <sup>ab</sup>	52.83 <sup>ab</sup>	9.3 x 10 <sup>4b</sup>	17.12 <sup>c</sup>	79.40 <sup>ab</sup>	3.65 <sup>a</sup>
C3	48.65 <sup>b</sup>	51.04 <sup>b</sup>	8.8 x 10 <sup>4a</sup>	21.90 <sup>b</sup>	62.44 <sup>b</sup>	3.40 <sup>b</sup>
A	58.83 <sup>c</sup>	59.50 <sup>c</sup>	6.3 x 10 <sup>5a</sup>	22.72 <sup>a</sup>	72.00	2.14 <sup>b</sup>
B	62.5 <sup>b</sup>	63.72 <sup>b</sup>	5.8 x 10 <sup>5b</sup>	21.46 <sup>b</sup>	74.25	1.50 <sup>a</sup>
C	66.33 <sup>a</sup>	68.66 <sup>a</sup>	4.9 x 10 <sup>5c</sup>	16.27 <sup>c</sup>	75.45	1.70 <sup>a</sup>
D	68.54 <sup>a</sup>	69.50 <sup>a</sup>	4.7 x 10 <sup>5c</sup>	14.14 <sup>c</sup>	76.8	1.33 <sup>a</sup>

Sources: Ningrat et al., 2017; DOI: 10.3923/ajas.2017.47.53; Zain et al., 2020; DOI:10.18517/ijaseit.10.2.11242. Different superscripts in the same column highly significant effect ( $p < 0.05$ ), T0: Oil palm frond ammoniated previously treated by 4% urea as control, B1: A + 10% GLW Payakumbuh, B2: A + 15% GLW Payakumbuh, B3: A + 20% GLW Payakumbuh, C1: A + 10% GLW Painan, C2: A + 15% GLW Painan, C3: A + 20% GLW Painan. A: 40% ammoniated rice straw +60% concentrate, B: 40% ammoniated rice straw +50% concentrate + 10% *Gliricidia sepium*, C: 40% ammoniated rice straw +40% concentrate +20% *Gliricidia sepium*, D: 40% ammoniated rice straw +30%, DM: Dry matter, OM: Organic matter, VFA: Volatile fatty acid, GLW: gambir leaves waste;

**Table 5.**  
Effect different sources and doses of tannin on dry matter (DM), organic matter (OM), protozoa population, methane (CH<sub>4</sub>) production, VFA total, and acetate: Propionate ratio based on agriculture by-product as feed in the rumen.



urinary allantoin, and nutrient consumption. The addition of 15% GLW tannins and 10% GLW Painan in the ration significantly increased ADG and decreased methane production compared to controls, namely 0.65, 0.90, 0.92 kg/day, and 2.48, 1.28, 1.26 MJ/day [12]. Saponins contained in GLW can increase the efficiency of rumen fermentation through the mechanism of reducing the population of protozoa [57]. The decrease in the protozoa population will cause the availability of H<sub>2</sub> for methanogens to decrease [58]. The reduction in protozoa population supports stabilization of rumen pH and an increase in the population of cellulolytic microorganisms. Thus, decreased methanogenesis will increase the efficiency of digestibility in high fiber rations and livestock performance.

The addition of *G. sepium* in the diet resulted in a decrease in methane production and the highest protozoa population at the levels of 20 and 30%, namely 12.67, 13.16 mM, and  $4.9 \times 10^5$ ,  $4.7 \times 10^5$  cell/ml *in vitro*. However, there was no significant difference ( $P > 0.05$ ) between the two levels. The treatment had no significant effect ( $p > 0.05$ ) on total VFA, acetate, butyrate, valerate + isovalerate + isobutyrate. Acetate propionate ratio decreased respectively to 2.14, 1.50, 1.70, 1.33. The propionate concentration increased by 43.87% compared to the control, and there was no significant difference ( $P > 0.05$ ) between levels of *gliricidia* addition [51]. Plant bioactive compound *Gliricidia sepium* contains tannins and saponins, which effectively reduce the population of protozoa and methane production. The feed used in this study was based on agricultural waste with high fiber content. In addition to saponin's structure, which can affect protozoa's activity, the type of feed given can also affect the fermentation process in the rumen [59]. In the study Zain *et al.*, [51] the types of protozoa that survived the addition of *Gliricidia sepium* were not identified. However, the results obtained showed that the saponins and tannins in *Gliricidia sepium* could inhibit certain types of protozoa that cause a decrease in protozoa population in the rumen. The decrease in methane production and the protozoa population with 20 and 30% *Gliricidia sepium* can increase the digestibility of dry matter and organic matter produced [51].

The potential of plant bioactive compounds such as tannins and saponins as defaunation agents and reducing methane emissions can be combined with direct-fed microbes. There is not much literature on decreasing methane production that combines the two in *in vivo* studies. *In vitro* studies Arowolo *et al.* [60] stated that there is a synergistic effect between probiotics and plant bioactive compounds simultaneously to stabilize the rumen fermentation process and reduce methane production. However, it still requires further studies at the *in vivo* level. Based on these results, Ningrat *et al.* [61] conducted a test of *Gliricidia sepium* and DFM *S. cerevisiae* supplementation to improve the performance of Simmental cattle while reducing methane gas production. It was found that the combined supplementation of 1% SC and 15% *Gliricidia sepium* significantly increased the digestibility of DM, and OM, ADG, and methane gas production compared to *S. cerevisiae* and *Gliricidia sepium* supplementation individually. The decrease in methane production with the addition of SC, GLW, and the combination of *S. cerevisiae* + *Gliricidia sepium* respectively 1.42, 1.35, and 1.02 MJ.day<sup>-1</sup> [61]. These results prove that yeast culture can work synergistically when combined with reducing agents. Emissions of methane plant bioactive compounds such as tannins and saponins. Tannin compounds inhibit the activity of methanogens [62] and can defaunate [63]. Pineiro-Vazquez *et al.* [64] reported the results of an *in vivo* evaluation showing the effect of 80% Leucaena sp. (21% condensed tannins) in the diet composition was able to reduce methane emissions by 61.3% without affecting nutrient intake and VFA production in the *Bos taurus* × *Bos indicus* cross.

## 4. Conclusion

In conclusion, the overall reduction in methane production in agriculture by-products as feed-based beef cattle can be made by improving feed quality through a combination of processing techniques and efforts to optimize bioprocesses in the rumen, which include supplementation of feed additives such as direct-fed microbials, methanogenesis inhibitors and plant bioactive compounds. Supplementation of DFM type *S. cerevisiae* 1% combined with 2% VCO can reduce methane production by 20.36% and increase ADG by 0.70 kg/day in Bali cattle. Plant bioactive compounds, especially tannins from *Gliricidia sepium*, can be used up to 15% in amniotic palm frond-based rations. *Gliricidia sepium*, which contains tannins and saponins at levels of 20 and 30% dry matter in complete rations, can also reduce methane, protozoa population and increase livestock performance. The combination of DFM *S. cerevisiae* and *Gliricidia sepium* can also be used to reduce methane gas production in Simmental cattle fed complete feed based on 46.61% amniotic palm fronds compared to controls.

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