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Research Article
Effect of Dietary Supplementation Based on an Ammoniated Palm Frond with Direct fed Microbials and Virgin Coconut Oil on the Growth Performance and Methane Production of Bali Cattle

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Abstract
Objective: The objectives of this study were to evaluate the effect of supplementation of an ammoniated palm frond-based diet with Direct-Fed Microbials (DFM) and Virgin Coconut Oil (VCO) on the *in vivo* methane production, Dry Matter Intake (DMI), Organic Matter Intake (OMI), Daily Gain (ADG) and nitrogen retention of Bali beef cattle. **Materials and Methods:** The DFMs used in this study were *Saccharomyces cerevisiae* (SC) and *Bacillus amyloliquefaciens* (BA) and the Virgin Coconut Oil (VCO) contained 51.95% C12:0. In a pilot study, 16 male Bali cattle were assigned treatments in a randomized complete block design. Cattle were fed a basal diet containing (dry matter basis) 40% ammoniated palm frond and 60% concentrate and the treatments were: a) control, b) SC 1% DM, c) SC 0.5% DM + BA 0.5% DM and d) SC 1% DM+VCO 2% DM. Data were analysed by analysis of variance (ANOVA) and differences among means were tested using Duncan's multiple range test (DMRT). **Results:** The results showed that supplementation with SC, SC+BA, SC+VCO significantly ($p<0.05$) reduced DMI and OMI but that the treatments were also able to increase ADG by 0.63, 0.63 and 0.71 kg day^{-1}, respectively. Supplementation with SC+VCO increased the feed efficiency and reduced methane gas production by up to 20.63% compared to the control and nitrogen retention tended to decrease with DFM and VCO supplementation. **Conclusion:** These results suggest that supplementation with SC+VCO generates the best results in Bali beef cattle growth performance, methane gas production and feed efficiency.

Key words: Direct-fed microbials, virgin coconut oil, methane, ammoniated palm frond, cattle

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The area under oil palm plantation in Indonesia is increasing annually[1] and palm frond is an agricultural by-product with the potential to be used as feed for ruminants[2]. The efficiency of rumen fermentation can be improved through a variety of approaches, including pre-treatment of the feed materials that will be given to cattle, the use of methanogenesis inhibitors and supplementation using microorganisms[3-5]. Direct-Fed Microbials (DFMs) are live microorganisms[6] and the direct fed microbial *Saccharomyces cerevisiae* can compete with bacterial starch to stimulate growth factors, such as organic acids or vitamins, thus stimulating the population of cellulolytic enzymes[7,8]. Thus the digestibilities of dry matter, organic matter and fibre can be improved and rumen fermentation can be increased when high-concentrate diets are supplemented with live Yeast Culture (YC)[9]. Therefore, supplementation with SC could improve nutrient digestibility and cattle growth performance[10]. Additionally, the DFM *Bacillus amyloliquefaciens* is cellulolytic and can be degraded crude fibre and produce the extracellular enzymes cellulase and hemicellulase[11]. Supplementation of an ammoniated palm frond-based diet with SC plus BA could increase dry matter and organic matter digestibility *in vitro*[12].

High fibre feed not only lowers the feed efficiency but also increases methane (CH_4) production in ruminants. The release of methane causes the loss of 6-13% of the energy in the feed[13]. However, Medium Chain Fatty Acids (MCFA) have the potential to suppress ruminal methanogenesis[14] and the reduction in archaeal numbers might be partially due to the defaunating effect of MCFA[15]. Virgin Coconut Oil (VCO) contains a high percentage of MCFA [7.19-8.81% C8:0, 5.65-6.59% C10:0, 46.9-48.0% C12:0 and 16.2-18.9% C14:0][16] but supplementation of the diets of lactating dairy cow with VCO at 29.8 g kg^{-1} DM do not affect the ruminal population of protozoa and methanogenic archaea[17]. Suryani *et al.*[12] found that supplementation with SC+VCO could decrease the proportion of acetate propionate and methane gas production by up to 45% compared to a control ammoniated palm frond-based diet *in vitro*.

We hypothesized that DFM and VCO supplementation would increase the growth performance of Bali cattle by reducing CH_4 production. Hence, the purpose of the current study was to evaluate the effect of DFM and VCO supplementation on feed intake, methane production and nitrogen retention in Bali cattle.

MATERIALS AND METHODS

The experiment was conducted from May-June, 2016, at the farm of the Animal Science Faculty of Andalas University, Indonesia.

Diet: Oil palm fronds without leaves and sticks that were previously treated with 6% urea were obtained from oil palm plantation of Andalas University, Indonesia. The DFMs used in the study were *Saccharomyces cerevisiae* and *Bacillus amyloliquefaciens*. The concentration of the live *Saccharomyces cerevisiae* of strain Meyen ex Hansen was 14×10^8colonies g^{-1}. The *Bacillus amyloliquefaciens* obtained commercially[11] at a concentration of 12×10^6 colonies g^{-1}. Virgin coconut oil was obtained from Andalas University, Indonesia and it consisted of 9.23% C8:0, 7.22% C10:0 and 51.95% C12:0. The basal diet contained (dry basis) 40% ammoniated palm frond and 60% concentrate. The compositions of the feeds including the concentrate are shown in Table 1.

Experimental design and procedure: Sixteen male Bali beef cattle of similar age (1.5-2 years) and initial body weight (110-174 kg) were placed in individual pens with free access to water and feed. The animals and treatments were randomly assigned in Randomized Complete Block Design (RCBD) with four replicate blocks and four treatment blocks, the treatments are shown in Table 1.

The feed was offered during the morning and afternoon. The experimental period was 26 days, twenty one of which were for adaptation and five for samplings. The offered and rejected feed was weighed to determine the total intake of DM and OM. During the collection period, the animals were fitted with bags with a harness to collect the faeces. The total faecal excretion was collected once daily and representative samples (10% of the total) were dried at 60°C overnight and kept in sealed bags until analysis. The feed and faecles were ground to pass through a 1 mm screen and formed into a composite sample. Dry matter, organic matter and nitrogen were analysed by standard methods[18].

Feed intake and feed efficiency: Dry Matter Intake (DMI) was calculated as the DM of the diet/100*the total diet intake and Organic Matter Intake (OMI) was calculated as the OM of the diet/100*the total diet intake. Feed intake was based on the metabolic body weight and calculated as the DMI or OMI/$BW^{0.75}$*1000 and feed efficiency was calculated as ADG/DM intake *100%.

Table 1: Ingredients and nutrient compositions of the experimental diets (DM basis)

	Diets			
Components	A	B	C	D
Ammoniated palm frond	40.00	40.00	40.00	40.00
Rice brand	20.00	20.00	20.00	20.00
Palm kernel	33.50	33.50	33.50	33.50
Corn	5.00	5.00	5.00	5.00
Mineral	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50
Total	100.0	100.00	100.00	100.00
Supplementation				
S. cerevisiae	-	1.00	0.50	1.00
B. amyloliquefaciens	-	-	0.50	-
VCO	-	-	-	2.00
Nutrient composition				
Crude protein	14.34	14.34	14.34	14.34
Fat	4.82	4.82	4.82	4.82
TDN	63.28	63.28	63.28	63.28
NDF	42.26	42.26	42.26	42.26
ADF	21.59	21.59	21.59	21.59

Table 2: Feed intake, daily weight gain, methane gas production and nitrogen retention of Bali cattle fed experimental diets

	Treatment				
Variables	Control	SC	SC+BA	SC+VCO	SEM
DMI (kg day^{-1})	3.16^{a}	3.01^{b}	2.99^{b}	2.57^{c}	0.143
DM/BW$^{0.75}$ intake (g kg^{-1} b.wt. $^{0.75}$ d^{-1})	79.94^{a}	75.68^{ab}	74.24^{ab}	67.36^{b}	1.790
OMI (kg day^{-1})	3.93^{a}	3.74^{b}	3.72^{b}	3.19^{c}	0.017
OM/BW$^{0.75}$ intake (g kg^{-1} b.wt. $^{0.75}$ d^{-1})	99.28^{a}	97.14^{a}	93.98^{a}	83.65^{b}	1.504
ADG (kg day^{-1})	0.53^{c}	0.63^{b}	0.63^{b}	0.71^{a}	0.007
Feed efficiency (%)	16.96^{c}	20.84^{b}	21.34^{b}	27.77^{a}	0.311
Methane production (L day^{-1})	109.01^{c}	103.27^{b}	102.61^{b}	86.52^{a}	0.501
Nitrogen retention (g day^{-1})	50.51^{a}	47.99^{a}	47.16^{a}	37.23^{b}	0.797

Description: Different letters (a,b,c) in the same row indicate significant differences between treatments ($p<0.05$)

Methane determination: Methane production was measured using the methane emission estimation method (Shibata's equation), as follows:

$$(\text{Methane production (L day}^{-1}) = -0.849\times\text{DMI2}+42.793\times\text{DMI}-17.766)$$

where, DMI = Dry Matter Intake (kg day^{-1})

Nitrogen retention: Nitrogen retention was calculated as the nitrogen intake minus the nitrogen contained in the faeces and urine[19]. This value can be used to determine whether the nitrogen balance is positive or negative.

Statistical analysis: Statistical analysis was performed using SAS program[20] and differences among the treatments were evaluated by one-way (ANOVA). Duncan's Multiple Range Test (DMRT) was used for comparison of means of treatments.

RESULTS AND DISCUSSION

The DMI, OMI, ADG, feed efficiency, methane production and nitrogen retention of the experimental rations with and without DFM and VCO supplementations are summarized in Table 2.

Table 2 shows that SC+VCO supplementation significantly ($p<0.05$) reduced DMI and OMI, while increasing ADG. Virgin coconut oil contains lauric acid, which has maximum digestibility, so this component can be digested more rapidly than other types of fat and quickly used as a source of energy[21,22]. Furthermore, the energy that it provided to the cattle decreased feed intake. Diet supplementation with DFM produced a beneficial microorganismal balance that reduced nutrient degradation in the rumen[23]. Therefore, DFM supplementation of beef cattle diets could improve growth performance, milk and meat production as has been shown in many studies[24,25].

Supplementation with SC+VCO could significantly ($p<0.05$) increase ADG. Increased ADG by a probiotic has been shown to increase DM and protein intake as well as nitrogen retention[26,27]. *Saccharomyces* sp., could stimulate the growth of rumen bacteria, especially cellulolytic bacteria that influence feed intake and digestibility and thus influence the ADG[28]. In this study, direct fed microbials, benefitted ruminants and influenced meat production[29] and daily gain (ADG) was higher in calves receiving a combination of *S. cerevisiae* and enzyme[30]. Supplementation with the DFMSC alone and combine with *B. amyloliquefaciens* (treatment B and C) had the same result on ADG. Qiao *et al.*[31] reported that *B. licheniformis* supplementation increased dry matter intake and milk protein percentages. Furthermore, the DFMSC could modify the rumen ecosystem to better degrade fibre[32].

Table 2 shows that supplementation with SC+VCO significantly ($p<0.05$) decreased methane gas production indicating that there was a synergistic effect when the DFM SC was combined with VCO. VCO has MCFA that decrease methane production but SC also inhibits methane production. This is supported by Ipharraguerre and Clark[33], who found that diet supplementation with coconut oil could decrease methanogens. Furthermore, coconut oil can reduce methane production by inhibiting *Archaea* methanogens in the rumen[14]. Supplementation with VCO as source of MCFA could also increase feed energy and thus increase ruminant productivity[34] and supplement MCFA could be directly absorbed by the rumen walls and potentially inhibit methanogenesis[35,36]. Furthermore, SC has the potential to reduce methane production because it can stimulate *acetogens* to compete with methanogen bacteria[37].

Table 2 shows that the highest mean feed efficiency value occurred under treatment D so SC+VCO supplementation could significantly ($p<0.05$) increase feed efficiency. When the feed efficiency value was higher, the feed were more efficient and higher quality, which reduced DM intake but increased the ADG under treatment D. Tillman *et al.*[38] stated that feed efficiency value depends on the total DM intake which could increase ADG. Furthermore, lactic acid production and DFM utilization in the rumen are related to feed efficiency and ruminant health[8,39]. Combining *S. cerevisiae* with enzymes could increase the feed efficiency of calves[30] and DFM supplementation of beef cattle feed could improve feed efficiency[40,41].

Supplementation with SC+VCO significantly ($p<0.05$) decreased nitrogen retention but although N retention was low under treatment D, there was a significant ($p<0.05$) increase in ADG compared with treatments A, B and C. Indicating an increase in microbial protein synthesis. The retention of N was low under treatment D because the feed intake was low and the protein intake indicated that little was being assimilated by the body. Chuzaemi[42] reported that the factors that affecting the nitrogen balance include nitrogen intake and nitrogen excretion through the faeces and urine. The protein in ruminants is derived from microbial proteins and the feed[43]. Sutardi[44] explained that not all the nitrogen that is consumed can be retained but is partially lost through faeces and urine. This experiment produced a positive N retention value indicating that the amount of N consumed was larger than the amount released[45].

CONCLUSION

Supplementation with the DFM SC alone, the combination of the DFMs SC+BA and the combination of SC+VCO could decrease nutrient intake but also increase ADG and feed efficiency as well as decrease methane production and nitrogen retention. Supplementation with SC+VCO resulted in the best improvement in Bali cattle growth performance and reduced methane gas production by up to 20.63%.

SIGNIFICANCE STATEMENT

This study demonstrates that supplementation with direct-fed microbials and virgin coconut oil can increase the growth performance and reduce the methane gas production and feed efficiency of Bali beef cattle. This study will enable researchers to further investigate the critical area of methane production by Bali cattle fed an ammoniated palm frond-based diet, which was previously unexplored. Thus, a new hypothesis regarding the effects of the supplement combination of DFM and VCO may be developed.

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