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Effects of microbial fermented liquid (MFL) supplementation on gas production kinetics and digestibility using *in vitro* gas production technique

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Abstract The effects of microbial fermented liquid (MFL) supplementation on rumen fermentation and digestibility of dairy steer using *in vitro* gas production technique was studied. The two rumen fistulated dairy steers as a rumen fluid donor was used. The levels of source of microbes (yeast (Y) and microbial fermented liquid (MFL) were incorporated with the levels of supplementation (0, 10, 20 and 30 % of concentrate) was recorded. It was found that the intercept value (a) and IVDMD and IVOMD were interacted ($p<0.01$) between microbial source and supplement levels, while, supplement levels affected on a and the insoluble fraction (b), microbial source affected on b, potential extent of gas production (a+b) and cumulative gas production at 72 h and IVOMD. Moreover, supplementation of MFL with 20 % of concentrate were the highest ($p<0.05$) of b, c, a+b, cumulative gas production at 72 h, IVDMD and IVOMD. In conclusion, supplementation of MFL could improve nutritional digestibility and a possible productivity in ruminants.

Keywords: microbial fermented liquid (MFL), yeast, rumen fermentation, digestibility, *in vitro* gas production

Introduction

The rumen ecosystem which have fermentation process by rumen microorganism (bacteria, protozoa and fungi) to produce volatile fatty acids (VFAs) such as C_2, C_3, C_4 and microbial protein which beneficial for ruminants

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under anaerobic condition, pH 6.5-7 and temperature 39℃ for optimal experiment. Therefore, manipulation of rumen ecosystem is very important for improved ruminant's productivity. There are variety methods used to improved rumen fermentation such as supplementation of essential oil, tannin, saponin, and microorganism. Present, many researcher try to use microorganism in ruminants feed in term of direct feed or supplementation of feed. Microorganisms that are commonly used in direct feed microorganism (DFM) for ruminants could be classified mainly as lactic acid producing bacteria (LAB), second is lactic acid utilizing bacteria (LUB) and other microorganisms (Elghandour *et al*., 2015) or other microorganism's species like Lactobacillus, Bifidobacterium, Enterococcus, Streptococcus, Bacillus, Propionibacterium, *Megasphaera elsdenii* and Prevotellabryantii, included some fungal species of yeast such as Saccharomyces and Aspergillus (Seo *et al*., 2010).

Yeast can stimulate the growth of various microorganisms in the rumen. Many researchers reported that yeast culture improved feed intake, feed conversion efficiency, growth rate and nutrient digestibility in cost effective (Ghazanfar *et al*., 2015), rumen fiber fermentation, rumen microbial protein (MCP) synthesis, rumen pH (Leicester *et al*., 2016). The addition of yeast could improved rumen fermentation and ruminals productivity (Dolezal *et al*., 2012).

The use of Microbial Fermented Liquid (MFL) is one of mixed microbial which contain lactic acid and microorganisms as the main components. When used as additive to fermentation in rumen, thereby increasing the microbial, lactic acid and quality of the smell better (Khonyoung and Wonnakom, 2010). MFL also helps for rumen manipulation and efficient in ruminants. MFL be able to balance the microflora within the intestines of animals, which increases the uptake of nutrients and helps to reduce methane production. In addition, lactic acid bacteria in MFL help in fermentation and the degradation cellulose and lignin (Worku *et al*., 2016). Furthermore, microbial inoculation not only affects on rumen fermentation but also animal improved performance as indicated by increased milk yield and weight gain (Kung *et al*., 2003).

However, the used of MFL in ruminant still limited of data. There for the objective of this study was to study effects of microbial fermented liquid (MFL) supplementation on gas production kinetics and digestibility using *in vitro* gas production technique.

Materials and methods

Dietary substrate treatments and design treatments

The experiment consisted of 18% CP and 80% TDN (Table 1.) used as experiments substrates. The experimental diets were milled to pass through a 1-

mm screen sieve (Cyclotec Mill, Tecator, Sweden) and used for chemical analysis using AOAC (1995) and in the *in vitro* gas test. Samples were prepared and weighed (total dietary feed mixture 0.5 g of dry matter; DM) into 50 mL bottles for various time incubations. Feed ingredients and chemical compositions of concentrate are shown in Table 1. The experiment was performed using a 2×4 factorial arrangement in a completely randomized design (CRD). Factor A was 2 levels of microbial source (yeast (Y) and microbial fermented liquid (MFL)) and factor B was 4 levels of supplementation (0, 10, 20 and 30 % of concentrate).

Microbial fermented liquid (MFL) and yeast preparation

Preparation of MFL and yeast were adapted form the method of Polyorach *et al*. (2013). Activated (A) MFL and yeast using 20 g put into a flask and added 20 g cane sugar and 100 ml distilled water, mixed well and keep at room temperature (27-33°C) for 1 h. Liquid culture medium (B) was prepared using 48 g molasses was weighed and dissolved in 100 ml distilled water, followed by addition of 48 g urea (B). Mixed (A) and (B) at 1:1 ratio then incubated at room temperature 48 h. then can used in this experiment.

Experimental animal and preparation of rumen inoculum

Two males, rumen-fistulated dairy steer with body weight of 350±30 kg were used as rumen fluid donors. Dairy steer rumen fluid was collected from animals fed with concentrate (14% CP and 80% TDN) at 2.5% of BW in two equal portions, at 08:00 h and 16:00 h and this experimental used rice straw as a roughage source was fed on *ad libitum* basis. The animals were adaptation for feed and environment for 14 d before sampling for rumen fluid where they were fed twice a day with water freely available. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks and then bring to the laboratory.

In vitro fermentation of substrates

Samples 0.5 g of concentrate were weighed into 50 ml. serum bottles and supplementation with the respective levels source of microbes (Y and MFL) for each treatment, three replications were prepared. Ruminal fluid from each animal was mixed with the artificial saliva solution of Menke and Steingass (1988) at 39°C under continuous flushing with CO_2 for *in vitro* gas test.

Sample collection and analysis

Gas production kinetics: dthe occurrence of gas between the incubation times the gas production kinetics were recorded at 0, 2, 4, 6, 8, 12, 24, 48 and 72h. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as follows:

$$y = a+b\ (1-e^{(-ct)})$$

Where a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction, t = incubation time, (a+b) = the potential extent of gas production y = gas produced at time ‘‘t’’

Then a and b were estimated the amount of gas produced by the degradation of the insoluble components but can be degraded or potential value of gas production d = a+b

Digestibility: In vitro degradability was determined after termination of incubation at 24 h, when the contents were filtered through pre-weighed Gooch crucibles (40 mm of porosity) and residual dry matter was estimated. The percent loss in weight was determined and presented as *in vitro* dry matter degradability (IVDMD). The dried feed sample and residue left above was ashed at 550℃ for determination of *in vitro* organic matter degradability (IVOMD) (Tilley and Terry, 1963).

Statistical analysis

Data were analyzed as 2×4 factorial arrangement in a completely randomized design (CRD) using Proc ANOVA (SAS, 1985) and analysed using the model:

$$Y_{ij} = \mu+A_i+B_j+AB_{ij}+\varepsilon_{ij}$$

Where: Y = observations; μ = overall mean; A_i = effect of factor A (Microbial Sources, i = 1 to 2); B_j = effect of factor B (Supplement levels, j = 1 to 4), AB_{ij} = interaction between factor A and B, and ε_{ij} = the residual effect. Multiple comparisons among treatment means were performed by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980). Supplement level’s trends were analyzed by using orthogonal polynomials. Differences

between means with $p<0.05$ were accepted as representing statistically significant differences.

Results

Feed ingredients and chemical compositions

This experiment used cassava chip and rice bran as a main carbohydrate sources and used soybean meal as a main protein source. Chemical compositions of experimental diet there were dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and TDN the value were 95.3, 93.6, 18.2, 20.4, 14.5 and 82.2, respectively (Table 1).

Gas Kinetic Sand Cumulative Gas Production

Effects of microbial fermentation (MFL) supplementation on gas production kinetic and degradability using *in vitro* gas production technique are shown in Table 2 and Figur 1. Under this experiment found that the gas production from the immediately soluble fraction (a) there were interactions between microbial sources and supplement levels by MFL supplemented groups at 30% of concentrate was the highest ($p<0.01$) and yeast supplemented groups at 10% of concentrate was the lowest. When considering factor of microbial sources found that have affected on gas production from the insoluble fraction (b), gas potential extent of gas production (a+b) and cumulative gas production at 72 h. By MFL supplemented groups higher than yeast supplemented groups ($p<0.01$). However, factor of microbial sources did not affect (a) and (c) ($p<0.05$).

In addition, when considering supplement levels found that affected on a and b by MFL supplementation groups was higher than yeast supplemented groups ($p<0.01$), while did not affected on c, a+b and cumulative gas production at 72 h ($p<0.05$).

In vitro digestibility

The *in vitro* degradabilities are shown in Table 2. This results were found that *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) there were interactions ($p<0.01$) between microbial sources and supplement levels. Found that digestibility of IVDMD in yeast supplemented at 20% of concentrate (79.52%) was the highest ($p<0.01$) and

level at 0% of concentrate (68.78%) lowest. Furthermore, IVOMD in MFL supplemented at 20% of concentrate (96.75%) was the highest ($p<0.01$) and yeast supplemented groups lowest at 0% of concentrate (92.96%).

Table 1. Feed ingredient composition of dietary treatments used in the experiment

Item	Concentrate
Ingredient, % of DM	
Cassava chip	49.4
Rice bran	30.0
Soybean meal	15.0
Molasses	2.0
Urea	2.1
Sulfur	0.5
Mineral mixture	0.5
Salt	0.5
Chemical composition, % of DM	
Dry matter (DM)	95.3
Organic Matter (OM)	93.6
Crude Protein (CP)	18.2
Neutral detergent fiber (NDF)	20.4
Acid detergent fiber (ADF)	14.5
TDN*	82.2

*TDN by calculation

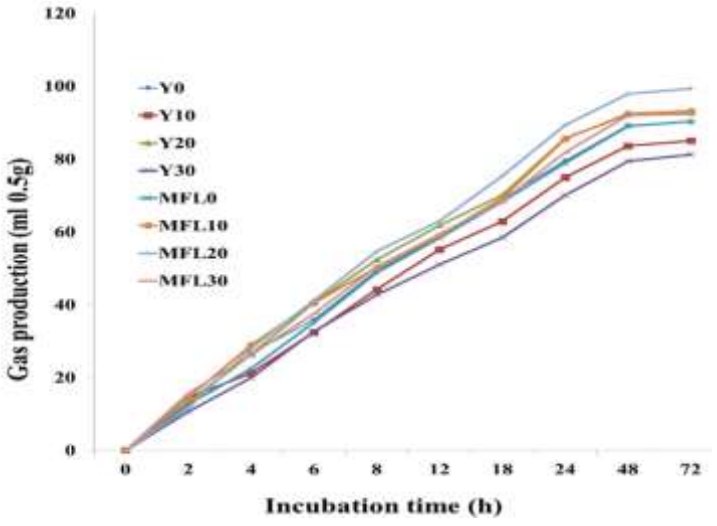

Figure 1. Cumulative gas production affected by Microbial fermented liquid (MFL) supplementation

Table 2. Effect of microbial fermentation (MFL) supplementation on gas production kinetic and degradability using in *vitro gas* production technique

	Supplement levels	Gas kenetics[2]				Gas (72 h) ml/0.5 g DM substrate	*In vitro Degradability* (%)	
		a	b	c	a+b		IVDMD	IVOMD
Yeast	0	-0.24^{b}	90.32^{cd}	0.089^{ba}	90.08^{bc}	90.17^{bc}	68.78^{c}	92.96^{c}
	10	-0.04^{b}	85.24^{cd}	0.083^{b}	85.20^{dc}	85.00^{dc}	77.53^{ba}	95.11^{b}
	20	-0.98^{cd}	93.94^{b}	0.093^{a}	92.70^{ba}	92.70^{ba}	79.52^{a}	95.51^{ba}
	30	-0.53^{cb}	81.30^{d}	0.83^{b}	80.77^{d}	81.20^{d}	72.70^{bc}	93.74^{c}
MFL[1]	0	-1.16^{cd}	91.47^{b}	0.086^{ba}	90.30^{bc}	90.27^{bc}	76.80^{ba}	96.27^{ba}
	10	0.61^{a}	92.81^{b}	0.088^{ba}	93.42^{ba}	93.10^{ba}	74.44^{bac}	95.46^{ba}
	20	-1.45^{d}	100.77^{a}	0.089^{ba}	99.32^{a}	99.37^{a}	79.33^{a}	96.75^{a}
	30	0.70^{a}	91.87^{b}	0.086^{ba}	92.57^{b}	92.37^{ba}	74.78^{ba}	95.49^{ba}
SEM		0.208	1.962	0.003	2.005	2.229	1.682	0.405
Comparison								
Microbial Sources		ns	**	ns	**	**	ns	**
Supplement levels		**	**	ns	ns	ns	ns	ns
Interaction		**	ns	ns	ns	ns	**	**
Orthogonal polynomial								
Supplement levels (linear)		ns	ns	ns	ns	ns	ns	ns
Supplement levels (quadratic)		ns	**	ns	ns	*	ns	ns
Supplement levels (cubic)		**	**	ns	**	**	ns	ns

a,b,c Value on the same row with different superscripts differ(P<0.05), *P<0.05, **P<0.01, ns= non-significant different, SEM=Standard error of the mean, IVDMD= *In vitro* Dry matter digestibility, IVOMD = *In vitro* organic matter digestibility. 1MFL=Microbial fermented liquid, 2a= The gas production from the immediately soluble fraction, b= The gas production from the insoluble fraction, c= The gas production rate constant for constant for the insoluble fraction (b); a+b = The gas potential extent of gas production.

Discussion

This study demonstrated that the result under this experiment by MFL supplemented groups have favourably altered in vitro ruminal fermentation of concentrate feed which similar to reported by Contreras-Govea *et al*. (2011) who supplemented LAB compare with control group found that LAB supplemented groups were able to cumulative gas production highest than control group (non-supplement). Moreover, Ridwan *et al.* (2018) reported that gas production of 2 ml of LAB supplementation group was higher than control group. This might be due to MFL affected on improving ruminal fermentation resulted on improved ruminal microbial productions (Weinberg *et al*., 2004). Ramaswami *et al*. (2005) reported that LAB are the major component of MFL therefore when supplement YFL in animal feed was affected on microbial ecosystems to assist in increasing the microbial populations within the rumen. It can also reduced the concentration of NH_3-N, reducing methane production (Quinn *et al*., 2009). LAB also has an affected on the reduction As reported by Seo *et al*. (2010) microorganisms can be improved in lactic acid and MFL fermentation with LAB that reduce the concentration of lactic acid that effects the acidity. However, from the results from this experiment (Table2.) showed that cumulative gas production of yeast supplement group decrease due to that yeast supplementation did not modify ciliate protozoa this affects the digestibility lower and effect to gas production (Lila *et al*., 2004).

In this experiment of digestibility of IVDMD and IVOMD found that in yeast supplemented at 20% of concentrate was the highest which similar to Tang *et al*. (2008) who reported that supplementation of yeast indicate that effects of increase of rumen microbial that led to higher IVDMD as a result of yeast supplementation might be attributed it ability of yeast to remove oxygen from the rumen environment. Yeasts enhances the ruminal fermentative process and the action of microorganisms and able to stabilize ruminal pH affects digestibility (Rossi *et al*., 2004).

In addition might be due to in MFL contain LAB which is the main component that improves microorganisms with in the rumen fermentation process. According to Ellis *et al*. (2016) reported that LAB supplementation can increased microbial population are altered to higher counts of fibrolytic bacteria, suggesting an improvement of fibre digestion. Similar Carvalho *et al*. (2013) reported that using 1.5% urea and 1% Lactobacillus with *L. plantarum* the inclusion of microbial inoculants strongly improved the effective ruminal degradability. In addition, Ridwin *et al*. (2018) reported that supplementation of LAB at 2 ml found the relationship LAB addition and substrates degradation which affected to dry matter digestibility (DMD) and organic matter

digestibility (OMD) by value higher at 74.44% and 73.67% respectively could be LAB increases digestibility when added directly to the rumen fluid (Weinberg *et al*., 2007).

Conclusion

In conclusions, supplementation of MFL at 20% of concentrate gas production and digestibility. Howere, these findings should be applied further in *in vivo* experiment in order to increase ruminants production efficiency.

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