

APPLICATION OF CELLULOLYTIC MIXED CULTURES FOR RUMINANT PRODUCTIVITY: 3. THE EFFECT OF CELLULOLYTIC MIXED CULTURES INOCULATION ON CELLULASE ACTIVITY AND VOLATILE FATTY ACID PRODUCTION OF CATTLE RUMEN FLUID BY IN VITRO ANALYSIS

Zaenal Bachrudin, Retno Kurniawati, Lies Mira Yusiati,
Ristiano Utomo and Widyantoro

ABSTRACT

The objective of this experiment was determined the effects of cellulolytic mixed cultures (CMC) inoculation on cellulase activity and Volatile fatty acid (VFA) production of cattle rumen by in vitro analysis. CMC as starter having high activity of endo-glucanase and β -glucosidase were obtained by isolation and selection of rumen microbes from buffalo. Rumen fluid (RF) as microbial donor was taken from rumen cannulated cattle that were fed 60% rice straw and 40% concentrate for 15 days. Series of different kind ratio between CMC: RF as starter for in vitro rice straw fermentation (treatments) were designed using one way completely randomized design as follow: 1) CMC₁₀₀ (100% of CMC: 0% of RF); 2) CMC₈₀ (80% of CMC: 20% of RF); 3) CMC₆₀ (60% of CMC: 40% of RF); CMC₄₀ (40% of CMC: 60% of RF); 5) CMC₂₀ (20% of CMC: 80% of RF); 6) CMC₀ (0% of CMC: 100% of RF). All treatments have 3 replications and were incubated at 39 C for 48 hours. Different variable was determined by Duncan's new multiple range test. The result of this study showed that CMC inoculation in rice straw fermentation by RF gave effect ($P < .05\%$) on cellulase activity and VFA production. The highest activity of endo-glucanase and β -glucosidase activity namely were in CMC₈₀ (80% of CMC: 20% of RF) and CMC₁₀₀ (100% of CMC: 0% of RF) respectively. While the highest concentration acetate, propionate and butyrate produced was CMC₈₀ (80% of CMC: 20% of RF). Finally the starter having ratio of 80% of CMC: 20% of RF (CMC₈₀) showed the best treatments.

Key words: Cellulolytic mixed cultures, Fermentation, Endo-glucanase, β -glucosidase

INTRODUCTION

The potency of ruminants is able to utilize renewable carbohydrates that are abundant in the nature due to the activities of cellulolytic microorganisms. The variety of the microorganisms is in the form of bacteria, fungi and protozoa (Bryant, 1973). The high capability of ruminants in utilization of components crude fiber like cellulose, hemicellulose, and pectin to convert protein microbes would be more useful for human being as animal protein.

The buffaloes have capability to digest the crude fiber higher than other ruminants. Therefore the ecological of microbes in the rumen of those ruminants may different other ruminants. One of the efforts to improve the capability of

saccharification in the rumen is a manipulation of rumen fermentation in the rumen by the inoculation of microbes as probiotics of microbial sources. Dawson *et al.*, (1990) stated that yeast inoculation into cattle would improve 5 to 40 time of cellulolytic activity in the rumen. Teh *et al.*, (1987); Wiedmeier *et al.*, (1987) showed that yeast cultures was able to develop VFA production, pH in the rumen as well as the population of anaerobic bacteria include cellulolytic bacteria.

In addition of nutritional factors there is an other possibility factors that determine the microbial growth in the rumen of young animal is the microbial inoculation coming from adult animals (Dehority and Orpin, 1988). Fuller (1989) said that addition of a life microbe as probiotic improved the animal

performance. The reason of this improvement is because there was a balance condition in the intestine.

Kennedy *et al* (1992) stated that buffaloes have received rice straw as basic feed could maintain the ammonia content in the rumen by increasing urea recycle from blood circulation into the rumen. While the buffaloes was able to synthesize microbial nitrogen more efficiently those cattle. The addition of urea in the ration increases the fibriolytic higher in the buffaloes compared to in the cattle. Finally the rumen microorganisms in the buffaloes could digest crude fiber better significantly than other (Kennedy *et al.*, 1992).

Based on the situation above, it is important to have study of isolation and selection of microbial cellulolytic from the rumen of buffaloes and their application for improvement of crude fiber degradation in the rumen of animals.

The objective of the present study is to utilization of cellulolytic mix-cultures as starter that have been isolated and selected, to improve the cellulose fermentation in the rumen of cattle by in vitro analysis.

MATERIALS AND METHODS

Production of cellulolytic mix-cultures as starter

Cellulolytic mix-cultures that was used for this study is compose of pure isolates called KD-1, KD-4; KD-8; KD-11; KB-1; KC-6; KH-5 and KH-6. First those isolates was cultured separately by anaerobic method for 12-hour incubation. Then those mix-cultures were incubated using molasses as carbon sources.

In vitro analysis of cellulolytic mix-cultures (CMC) as starter

The in vitro analysis for the activity of cellulolytic mix-cultures as starter used anaerobic fermented. Such fermenter is simulated for rumen conditions. Anaerobic conditions were done by O₂ free CO₂ gas. The pH of 6 – 7 and the temperature of 39 °C in the fermenter was kept constantly during the study. The various inoculum for the in

vitro analysis is the mixture between of cellulolytic mix-cultures and rumen fluid of cattle as microbial donor which have different ratio as follow: i) 0% of (RF) : 100 % of CMC; ii) 20 % of RF: 80 % of CMC; iii) 40 % of RF: 60 % of CMC; iv) 60 % of RF: 40 % of CMC; v) 80 % of RF: 20 % of CMC; vi) 100 % of RF : 0 % of CMC. Variables used to evaluate of the activity of various inoculums by in vitro determination are endo-glucanase, β -glucosidase, and VFA production.

Analysis of enzymes activity

β -glucosidase determination used O-nitrophenil-beta-D-glukopiranosida as substrate. While endo-glucanase activity used the reducing sugar determination that was produced by hydrolysis of carboxyl methyl cellulose by addition of ferricyanida as oxidation (Halliwell *et al.*, 1985)

RESULTS AND DISCUSSION

The effect of various inoculate on VFA Production

The effect of various ratio Cmix-cultures and rumen fluid of cattle as starter on VFA production of cellulose fermentation by in vitro analysis could be found at table 1.

According to the table 1 that, acetate production increased significantly on the ratio of 80 % of CMC and 20% of RF as starter. While the lowest production of those acid could be found at inoculum, which compose of 100% of RF or without CMC addition. As type of acetate production, the propionate and butyrate also showed highest content significantly as response of CMC80 inoculation. The highest amount of acetate, propionate and butyrate is 121.50, 67.48, and 11.74 respectively.

The effect of various inoculate on the activity of cellulase

The effect of various starter inoculation of cellulose fermentation by in vitro analysis on the activity of cellulase can be seen at table 2.

The table 2 showed that the starter containing 80% of CMC and 20% of RF

Table 1. The effect of various ratio Cmix-cultures and rumen fluid of cattle as starter on VFA production (mM) of cellulose fermentation by in vitro analysis

No	Sample	VFA content (mM)		
		Acetate	Propionate	Butyrate
1	CMC100	46.17	23.42 ^c	5.11 ^c
2	CMC80	121.50 ^a	67.48 ^a	11.74 ^a
3	CMC60	50.66 ^b	38.33 ^b	9.32 ^b
4	CMC40	49.11 ^b	30.95 ^b	5.68 ^c
5	CMC20	42.78 ^b	27.52 ^c	6.25 ^c
6	CMC0	38.50 ^b	29.50 ^c	6.18 ^c

CMC100 (Ratio: 100% of CMC:0% of RF); CMC80 (Ratio: 80% of CMC:20% of RF); CMC60 (Ratio: 60% of CMC:40% of RF); CMC40 (Ratio: 40% of CMC:60% of RF); CMC20 (Ratio: 20% of CMC:80% of RF); CMC0 (Ratio: 0% of CMC:100% of RF).

Means with the same small letter superscripts within a column are not significantly different at $P < .01$ and $P < .05$.

effected on the activity of endo-glucanase significantly different. While the highest activity of β -glucosidase was found at starter containing 100% of CMC. The highest activity of endo-glucanase was found at starter of CMC80. It seems that there is synergism interaction between CMC and rumen fluid as microbial donor. It mean that cellulolytic mix-culture improve significantly on cellulose fermentation by in vitro analysis. The result was supported with Baldwin and Allison (1983). They stated that mix-culture in the ecosystem of the rumen would work

synergism well for cellulose digestion. Bachrudin *et al.* (1996) stated that cellulose digestion would improve more effectively by using mix-cultures rather than monocultures. This thought was supported to the Van Soest (1982). He said that rumen microbes were conducted the cellulose digestion in a cluster. Acetate, propionate and butyrate was produced significantly as responses of starter containing 80% of CMC and 20% of cattle RF as microbe donor. This amount of production fermentation is corresponding to the highest activity of endo-glucanase (table

Table 2. The effect of various starter inoculation of cellulose fermentation by in vitro analysis on the activity of cellulase

No	Sample	CMC-ase(mg D-glucose/ mg protein/minute	β -glucosidase (mg m-Nitto/mg protein/minute
1	CMC100	0.025 ^b	0.012 ^a
2	CMC80	0.032 ^a	0.008 ^c
3	CMC60	0.012 ^c	0.009 ^{bc}
4	CMC40	0.010 ^d	0.010 ^{ab}
5	CMC20	0.011 ^c	0.004 ^d
6	CMC0	0.010 ^d	0.007 ^c

CMC100 (Ratio: 100% of CMC: 0% of RF); CMC80 (Ratio: 80% of CMC: 20% of RF); CMC60 (Ratio: 60% of CMC: 40% of RF); CMC40 (Ratio: 40% of CMC: 60% of RF); CMC20 (Ratio: 20% of CMC: 80% of RF); CMC0 (Ratio: 0% of CMC: 100% of RF).

Means with the same small letter superscripts within a column are not significantly different at $P < .01$ and $P < .05$.

2). It means that by higher endo-glucanase activities will provide more glucose in the medium and consequently by the glucose fermentation produce more VFA.

CONCLUSION

Inoculation of starter containing 80% of CMC and 20% of RF from cattle as microbial donor enhanced the VFA production by in vitro analysis.

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