DIGESTION OF CELLULOSIC MATERIALS FROM LOCAL FORAGE (*PENNISETUM PURPUREUM*) AND PALM OIL MILL EFFLUENT (POME) BY THE RUMEN BACTERIAL POPULATION FROM GOAT

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TO

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ABSTRACT

Rumen samples were obtained from goats fed on *Pennisetum purpureum* (Rumen A), *P. purpureum* supplemented with *Vigna* bean sprout waste (Rumen B) and *P. purpureum* supplemented with palm oil mill effluent [POME](Rumen C). The average total viable bacteria per gram of rumen content were 1.98×10^8, 1.24×10^9 and 1.10×10^8 for rumen A, B and C respectively. Bacterial number was lowest in the reticulum of all types of rumen, but was highest in the dorsal-anterior part of rumen A and rumen B and in the dorsal-center part of rumen C. The occurrence of cellulolytic bacteria was highest in rumen B and lowest in rumen C. Addition of bean waste caused an increase in percentage of cellulolytic bacteria whereas the usage of POME caused eight-fold decrease in cellulolytic bacteria population.

Total soluble sugar and total volatile fatty acids concentrations were highest in rumen B followed by rumen A and rumen C. However, the concentration of total soluble sugar decreased from reticulum to the ventral part of rumen C. In rumen A and B, although the soluble sugar concentration was lowest in the ventral part of the rumen, the highest sugar concentration was in the dorsal-anterior part of the rumen A and in the ventral part of the rumen B. However, the distribution of VFA in the different part of

rumen A and C was the reverse of the pattern for soluble sugar. In rumen B, VFA concentration decreased from reticulum to the dorsal-anterior part of rumen and then increased to the ventral part of rumen.

Between the three rumens samples, rumen A showed highest cellulolytic activity against grass fibre, POME fibre, microcrystalline cellulose and acid-swollen cellulose followed by rumen B, and rumen C. In rumen A and rumen C digestion of grass fibre and microcrystalline cellulose was more active in the ventral part of rumen whereas the digestion of acid-swollen cellulose and POME fibre was more active in the dorsal part of rumen. On the contrary, the digestion of grass fibre, microcrystalline cellulose and POME fibre was more active in the dorsal part of rumen whereas digestion of acid-swollen cellulose was more active in the ventral part of rumen B.

Eleven percent of the cellulolytic bacteria isolated from the rumen of goat fed with *P. purpureum* showed positive cellulolytic activity against acid-swollen cellulose and microcrystalline cellulose. *Ruminococcus flavefaciens* was the most dominant (34.62%) cellulolytic bacteria isolated followed by *Fibrobacter succinogenes* (30.77%), *Ruminococcus albus* (11.54%), *Butyrivibrio fibrisolvens* (11.52%), *Eubacterium cellulosolvens* (7.69%) and *Clostridium cellobioparum* (3.85%). This was in agreement with general trend for rumen cellulolytic bacterial population. However, the presence of only 6.8% of

cellulolytic bacteria in rumen fed with POME as compared to grass could be attributed to the different in the nutrient status of the two substrates. Although the concentration of nitrogen-free extract was approximately equal in both rumens, *P. purpureum* was characterised by higher fibre content (29.40%) compared to POME (16.80%) but POME contained higher fat, protein and soluble sugar.

The suitability of POME as medium for growth and cellulolytic activity of pure cultures of ruminal cellulolytic bacteria was studied using whole POME, POME extracts, and POME extracts added with acid-swollen and microcrystalline cellulose. *R. albus, F. succinogenes, R. flavefaciens, C. cellobioparum and B fibrisolvens* grew in all the POME or POME-based media. However, *B. fibrisolvens* and *C. cellobioparum* showed better growth in all media compared to the other cellulolytic bacteria but the former had much lower cellulolytic activity than *R. albus* and *R. flavefaciens*. *R. flavefaciens* produced extracellular cellulase in POME extract in the absence of cellulose whereas *R. albus* require the presence of cellulose for higher production of extracellular cellulase. *F. succinogenes* showed poor growth and low extracellular cellulase production in POME or POME-based media.

Fermentation of POME and *P. purpureum* by rumen bacterial population from grass-fed goats was studied in a semi-batch fermentation system simulating the rumen system. Soluble sugar in both substrates was rapidly utilised

followed by rapid increase in total rumen bacteria, total cellulolytic bacteria and volatile fatty acid concentration. However, molar ratio of fatty acids and fatty acid utilisation pattern were different in both systems. The acetic acid to propionic acid ratio indicated the presence of active cellulolytic bacterial population in both systems. However, the population adapted to grow on *P. purpureum* were better in cellulolytic activity against grass fibre, POME fibre and microcrystalline cellulose, whereas the population adapted to grow on POME were better in cellulolytic activity against acid-swollen cellulose. This study suggested that there was a change of bacterial population grown on POME.

ABSTRAK

Sampel-sampel rumen telah diperolehi dari kambing yang makan *Pennisetum pupureum* (Rumen A), *P. pupureum* yang ditambah dengan sisa kacang *Vigna* bertunas (Rumen B), dan *P. purpureum* yang ditambah dengan efluen kilang kelapa sawit [POME] (Rumen C). Purata jumlah bakteria hidup setiap gram kandungan rumen ialah 1.98×10^{5} bagi rumen A, 1.24×10^{5} bagi rumen B, dan 1.10×10^{5} bagi rumen C. Retikulum kesemua jenis rumen mengandungi bilangan bakteria terendah, tetapi tertinggi di bahagian anterior dorsal rumen A dan rumen B dan di bahagian tengah dorsal rumen C. Rumen B mengandungi bilangan bakteria selulolitik tertinggi dan rumen C mengandungi bilangan bakteria selulolitik terendah. Sisa kacang yang ditambah dalam makanan kambing mengakibatkan peningkatan peratusan bilangan bakteria selulolitik manakala penambahan POME menyebabkan penurunan bilangan bakteria selulolitik sebanyak 6 kali ganda.

Jumlah gula larut dan kepekatan asid lemak meruwap adalah tertinggi dalam kandungan rumen B, diikuti oleh rumen A dan rumen C. Kepekatan gula larut menurun dari retikulum ke bahagian ventral rumen C. Walaupun kepekatan gula juga terendah di bahagian ventral rumen A dan rumen B tetapi kepekatan gula paling tinggi di bahagian anterior dorsal rumen A dan di bahagian ventral rumen B. Walau bagaimanapun, corak taburan VFA dalam berbagai bahagian rumen A dan rumen

C adalah berlawanan dari corak taburan kepekatan gula larut di dalam rumen-rumen tersebut. Dalam rumen B, kepekatan VFA berkurangan dari retikulum ke bahagian anterior dorsal dan kemudiannya meningkat di bahagian ventral rumen.

Diantara ketiga-tiga rumen, rumen A menunjukkan aktiviti selulolitik tertinggi terhadap serabut rumput *P. purpureum*, serabut POME, selulosa mikrokristal dan selulosa kembang-asid. Aktiviti selulotik kandungan rumen B lebih tinggi dari rumen C. Dalam rumen A dan rumen C, penghazaman serabut rumput dan selulosa mikrokristal adalah lebih aktif di bahagian ventral rumen manakala penghazaman serabut POME dan selulosa kembang-asid lebih aktif di bahagian dorsal rumen. Sebaliknya, penghazaman serabut rumput, selulosa mikrokristal dan serabut POME adalah lebih aktif di bahagian dorsal rumen B manakala penghazaman selulosa kembang-asid di dalam rumen B paling aktif di bahagian ventral.

Hanya sebelas peratus dari bakteria yang diasingkan dari rumen kambing yang makan *P.purpureum* sahaja menunjukkan aktiviti selulolitik terhadap selulosa mikrokristal atau selulosa kembang-asid. *Ruminococcus flavefaciens* adalah bakteria selulolitik paling banyak diasingkan dari rumen ini (34.62%) diikuti oleh *Fibrobacter succinogenes* (30.77%), *Ruminococcus albus* (11.54%), *Butyrivibrio fibrisolvens* (11.52%), *Eubacterium cellulosolvens* (7.69%) dan *Clostridium cellobioparum*

(3.85%). Keputusan ini bersamaan dengan corak normal populasi bakteria selulolitik dalam rumen. Walau bagaimanapun, peratusan bakteria selulolitik di dalam rumen kambing yang makan POME lebih rendah iaitu sebanyak 6.8% dan ini adalah disebabkan perbezaan status nutrien diantara *P. purpureum* dengan POME. Walaupun kepekatan ektrak bebas-nitrogen hampir sama dalam kedua-dua *P. purpureum* dan POME, *P. purpureum* mengandungai selulosa lebih tinggi kepekatannya (29.40%) berbanding dengan POME (16.80%). POME pula lebih tinggi kandungan lemak, protein, dan gula larut berbanding dengan *P. purpureum*.

Kesesuaian POME sebagai medium pertumbuhan dan penghasilan aktiviti selulolitik beberapa kultur tulin bakteria selulolitik rumen telah dikaji menggunakan POME, ektrak POME, dan ektrak POME yang ditambah dengan selulosa mikrokristal dan selulosa kembang-asid. *R. albus, R. flavefaciens, C. cellobioparum* dan *B. fibrisolvens* boleh tumbuh dalam POME atau medium berasaskan POME. *Walaupun B. fibrisolvens* dan *C. cellobioparum* menunjukkan pertumbuhan lebih baik di dalam kesemua media pertumbuhan berbanding dengan bakteria selulolitik yang lain tetapi aktiviti selulolitik kedua-duanya adalah jauh lebih rendah *dari R. albus* dan *R. flavefaciens*. *R. flavefaciens* menghasilkan selulase luar-sel di dalam ektrak POME walaupun dalam ketiadaan selulosa manakala *R. albus* memerlukan kehadiran selulosa bagi menghasilkan selulase luar-sel yang lebih

banyak. *F. succinogenes* menunjukkan kadar pertumbuhan dan penghasilan selulase luar-sel paling rendah dalam kesemua media pertumbuhan.

Fermentasi POME dan *P. purpureum* oleh populasi bakteria rumen dari rumen kambing yang makan *P. purpureum* telah dikaji dalam sistem fermentasi separa-kelompok yang meniru sistem rumen asli. Didapati bahawa gula larut dalam kedua-dua substrat digunakan secara pantas dan diikuti dengan pertambahan pantas bilangan jumlah bakteria, bilangan bakteria selulolitik, dan kepekatan asid lemak meruwap. Walau bagaimanapun, nisbah molar asid-asid lemak dan corak penggunaan semula asid-asid lemak berbeza di dalam sistem fermentasi POME dengan sistem fermentasi *P. purpureum*. Nisbah asid asetik dengan asid propionik menunjukkan terdapatnya populasi bakteria selulolitik yang aktif di dalam kedua-dua sistem fermentasi. Walau bagaimanapun, populasi bakteria yang telah menyesuaikan diri hidup menggunakan substrat *P. purpureum* menunjukkan aktiviti selulolitik lebih tinggi terhadap serabut *P.purpureum*, selulosa mikrokristal dan serabut POME berbanding dengan populasi bakteria yang telah menyesuaikan diri hidup mengunakan POME. Walau bagaimanapun, populasi bakteria yang telah menyesuaikan diri hidup menggunakan POME menunjukkan aktiviti selulolitik yang baik terhadap selulosa kembang-asid berbanding dengan populasi bakteria yang telah menyesuaikan diri hidup menggunakan substrat *P. purpureum*. Kajian ini menunjukkan terdapatnya perubahan populasi bakteria setelah tumbuh menggunakan substrat POME.

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