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Cellulolytic activities of the rumen ingesta of goats

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ABSTRACT A normal grass-fed goat has rumen pH non-inhibitory for the microbial population, highest ruminal concentration of sugar in the dorsal rumen and volatile fatty acids in the ventral rumen. Endoglucanase activity was 12.9 times higher than exoglucanase activity with maximum activity in the dorsal rumen. The digestion of POME fibre was better than crystalline cellulose and grass fibre with the latter the least digested. Apart from the swollen cellulose, the digestion of the other forms of cellulose was better in the ventral rumen than the other parts of the rumen.

Supplementing grass-fed goat with high carbohydrate and protein but low fibre-diet, increased the concentration of ruminal sugar and volatile fatty acids with maximum concentrations in the ventral rumen. The rumen microbial population increased and was highest in the dorsal rumen. The rumen pH decreased to a level inhibitory for cellulolytic activities. The relative digestibility of the different form of cellulose was the same as in the rumen of the grass fed goat but endoglucanase activity decreased by 1.8 times. However, apart from the swollen cellulose, the digestion of the other forms of cellulose was better in the dorsal part of the rumen.

However, diet supplementing of the grass-fed goat with low carbohydrate, low protein and low fibre diet with low cellulose content decreased the concentration of soluble sugar and volatile fatty acids. It also decreased the number of rumen microorganisms. The pattern was similar to that observed for the grass-fed goats. Swollen cellulose was again the best digestible form of cellulose but the activity was lower than that in the grass-fed goat. The digestion of crystalline cellulose was better than POME and grass fibre. The digestion of the different forms of cellulose was better in the ventral part of the rumen.

ABSTRAK Kambing biasa yang memakan rumput mempunyai pH rumen yang tidak merencat populasi mikroorganisma, kepekatan gula yang tertinggi di bahagian rumen dorsal dan asid lemak volatil di bahagian rumen ventral. Aktiviti endoglukanase adalah 12.9 kali lebih tinggi dari aktiviti eksoglukanase dengan aktiviti maksima di rumen dorsal. Gentian POME lebih mudah dihazamkan dari selulosa hablur dan gentian rumput dimana gentian rumput adalah tersukar dihazamkan. Selain dari selulosa kembang, selulosa dalam bentuk lain lebih baik dihazamkan di bahagian rumen ventral berbanding dengan bahagian rumen yang lain.

Makanan tambahan yang tinggi kandungan karbohidrat dan protein tetapi rendah kandungan gentian, meningkatkan kepekatan gula dan asid lemak volatil dalam rumen dengan kepekatan maksima di rumen ventral. Populasi mikroorganisma rumen meningkat dan tertinggi di rumen dorsal. pH rumen menurun ke paras yang merencat aktiviti selulolitik. Penghazaman relatif selulosa bentuk-bentuk berbeza serupa dengan rumen kambing yang diberikan makanan rumput tetapi aktiviti endoglukanase jatuh sehingga 1.8 kali. Selain dari selulosa kembang bentuk-bentuk selulosa lain dihazamkan dengan lebih baik di bahagian rumen dorsal.

Disebaliknya penambahan pemakanan kambing yang memakan rumput dengan makanan rendah karbohidrat, protein dan gentian yang rendah kandungan selulosa merendahkan kepekatan gula larut dan asid lemak volatil. Ia juga merendahkan bilangan mikroorganisma rumen. Cellulosa kembang sekali lagi merupakan bentuk selulosa yang terbaik dihazamkan tetapi aktiviti nya lebih rendah dari kambing yang memakan rumput sahaja. Selulosa hablur dihazamkan dengan lebih baik dari gentian POME dan gentian rumput. Semua bentuk selulosa dihazamkan dengan lebih baik di bahagian rumen ventral.

(rumen, feedstuff, cellulolytic activities, sugar, volatile fatty acids.)

INTRODUCTION

The presence of a specialized rumen involved in the pregastric digestion of feed materials enable the ruminant to consume various forage materials. The fermentation pattern of major feedstuff depends upon the relative solubility of feedstuff as well as the microbial fermentation pathways. The rumen microbial population can ferment highly soluble carbohydrate rapidly [1] but the digestion of the polysaccharides, predominantly cellulose and hemicellulose [2] requires the presence of the fibrolytic rumen bacteria [3].

The inclusion of low concentration of soluble carbohydrates in the diet causes the rapid increase in number of rumen bacteria and shortens the lag period for digestion of fibres [4] but high concentration of soluble carbohydrate increases the percentage of amylolytic and lactate-producing bacteria [5] and causes the pH of the rumen to decrease. This consequently decreases the protozoan population [6], the percentage of cellulolytic organisms and the digestibility of dry matter [7]. A high fibre diet causes the protozoa to reduce the total number of amylolytic bacteria leading to increased cellulolytic bacteria and enhanced cellulolytic activity in the rumen [8].

The presence of fat in the ruminant diet on the other hand decreases the digestibility of cellulose and crude fibre [9] whereas the inclusion of true protein in low quality roughage normally improves the rate of fibre digestion [10]. Malaysia produces a great deal of palm oil mill effluent (POME) but the role of POME in

rumen digestion has been poorly investigated. Feeding goats with POME-based concentrate has been found to reduce the number of total cellulolytic bacteria in the rumen that results in a population predominated by less active cellulolytic bacterial species [11].

The objective of the paper was to compare the pH, volatile fatty acid, sugar content of rumen in relation to the bacteria population and their cellulolytic activities in goats. The activities were compared in rumen of goats fed with normal grass (rumen A) to grass supplemented with beans from bean sprout production (rumen B) and grass supplemented with dried POME (rumen C).

MATERIALS AND METHODS

The rumens used in this study were from mature goats obtained from Perak. Once removed from the animals, the rumens were kept frozen in anaerobic condition and transported to the laboratory. The rumen was separated into the reticulum, the dorsal part of the rumen and the ventral part of the rumen and kept at -12°C until further use.

The pH of the rumen was measured using Chemcadet (Cole Palmer, USA) pH meter. The concentrations of total soluble sugar and volatile fatty acid (VFA) were determined in 3 replicates. Samples were suspended in equal volume of anaerobic dilution solution [12] mixed and centrifuged at 6000 g for 15 minutes. The concentration of total soluble sugar in the supernatant was determined by adding Anthrone reagent to rumen filtrate. The optical density at a wavelength of 620 nm was compared to glucose standard curve [13]. The volatile fatty acid (VFA) content was determined using a gas-liquid chromatography (Shimazu, Japan) according to Supelco Technical Bulletin 749 E (Supelco Inc.). Metaphosphoric acid (25%) was added in the ratio of 0.2 ml to 1.0 ml rumen filtrate, mixed, centrifuged and analyzed using Supelco SP1220 gas chromatography column.

The cellulolytic activities of the rumen were tested against micro-crystalline cellulose (Sigma Chemical Company, St. Louis, USA), acid swollen-cellulose, grass fibres and POME fibers. The acid swollen-cellulose was prepared by swelling the crystalline cellulose with 85% ortho-phosphoric acid [14].

The grass fibre was prepared from *P. purpureum* leaves. The leaves were dried at 100°C for 24 hours, ground and sieved through 1 mm pore size sieve. The sieved material was washed with hot water until sogluble carbohydrate was no longer detected in the filtrate. POME fibre was similarly prepared using freshly collected POME. The residual POME cake obtained after washing with hot water was dried and defated before use.

The cellulolytic activity was measured as the rate of glucose released by crude cellulase extract [15]. The rumen ingesta kept at 2-4°C, was centrifuged in oxygen-free atmosphere at 12,000 g for 20 minutes. The supernatant fluid was adsorbed to the cellulosic substrate under oxygen-free CO_2 atmosphere and then centrifuged at 5000 g for 10 minutes. The supernatant was discarded and replaced with chilled anaerobic dilution solution [12], mixed for 10 seconds and centrifuged at 5000 g for 10 minutes. The washing was repeated 3 times to ensure that the supernatant was free of soluble sugar. 1 ml of toluene was added to the tube, mixed and incubated for 24 hours at 38°C. After the incubation, the mixture was centrifuged at 5000 g for 10 minutes and the supernatant fluid was analyzed for the presence of sugar. Cellulolytic activity was expressed as net sugar production per 10 ml rumen fluid per 24 hours.

Enumeration of the total rumen bacteria was carried out using the VPI System (Bellco Glass Inc., USA) according to the Hungate roll-tubes technique for rumen bacteria [16]. All manipulation was carried out under CO_2 free of O_2 (Malaysian Oxygen) atmosphere. Traces of oxygen from the CO_2 (99.98% purity) was scrubbed with chromous acid before use. Samples were diluted using anaerobic dilution solution [12] and the total viable bacteria enumerated using Rumen fluid glucose, cellobiose starch agar [16].

RESULTS

pH and bacterial counts

The pH and the total viable bacteria counts of the different rumen are shown in Table 1. The pH of rumen was on the average lowest for rumen B. The average total viable rumen bacteria was highest in rumen B followed by rumen A and rumen C. The dorsal rumen supported the highest number of bacteria population whereas the least was in the reticulum.

Sugar and volatile fatty acid levels

The sugar concentration (Figure 1) was on the average highest for rumen B followed by rumen A and rumen C. However in rumen A sugar was highest in the dorsal rumen whereas in rumen B and C the highest sugar

concentration was in the ventral rumen and reticulum respectively.

The average VFA concentrations for the 3 rumen (Figure 2) was again highest for rumen B, followed by rumen A and rumen C. However in all cases the ventral rumen has the highest VFA concentration. The VFA concentrations in rumen A and rumen C were higher at lower ruminal sugar content, whereas in rumen B high VFA concentrations occurred at higher ruminal sugar content. Acetic acid was the major volatile fatty acids from the different parts of the rumens (Table 2). The molar percentage of acetate was comparable between the dorsal and ventral part of rumen A, but it was higher in the ventral than the dorsal part of rumen B and C. The concentration of propionate and butyrate generally decreased from dorsal rumen to ventral part of the rumen. The acetate to propionate ratio increased from dorsal rumen to the ventral part of rumen B and rumen C but decreased in rumen A.

Table 1. pH values and total viable bacterial count from different parts of the rumen.

Rumen: Parts of rumen	average pH	Total bacteria/mL	Average total bacterial/mL
A: Reticulum		3.3×10^7	
A: Dorsal-anterior	6.05	5.9×10^8	1.98 x 108
A: Dorsal-center		1.7×10^8	
A: Ventral		1.9×10^9	
B: Reticulum		1.1×10^9	
B: Dorsal-anterior	5.87	2.0×10^9	1.24 x 109
B: Dorsal-center		1.2×10^9	
B: Ventral		1.2×10^9	
C: Reticulum		2.6×10^7	
C: Dorsal-anterior	6.05	2.1×10^8	1.10 x 108
C: Dorsal-center		3.4×10^8	
C: Ventral		2.1×10^8	

Table 2. Molar percentage of major volatile fatty acids and ratio of acids (A) to propionic acids (P) from different parts of the rumen.

Rumen: Parts of rumen	Acetic	Propionic	n-Butyric	n-Valeric	A:P ratio
A: Recticulum	51.29	26.71	13.35	1.23	1.92: 1
A: Dorsal-rumen	58.87	22.21	11.66	1.24	2.65: 1
A: Ventral	58.68	23.03	11.52	1.10	2.55: 1
B: Recticulum	51.83	28.50	15.42	1.03	1.82: 1
B: Dorsal-rumen	51.86	28.58	15.59	1.12	1.81: 1
B: Ventral	56.02	26.04	14.18	1.20	2.13: 1
C: Recticulum	60.86	23.04	8.76	1.56	2.64: 1
C: Dorsal-rumen	53.44	25.06	13.43	1.31	2.13: 1
C: Ventral	58.25	21.37	12.56	1.25	2.73: 1

Digestion of cellulose

The activity of crude cellulase extract are shown in Figures 3-6. For all the substrates tested, rumen A showed better cellulase activities than that of rumen B or rumen C. Acid swollen-cellulose was the most readily digested cellulose with a digestion rate 1.8 times better in rumen A compared to rumen B and rumen C. The ratios of digestion of the acid swollen cellulose to the crystalline cellulose, POME fibres and grass fibres were 12.9, 10.9 and 22.2 for rumen A, 13.9, 10.1 and 16.2 for rumen B and 13.4, 15.7 and 24.0 for rumen C respectively.

Highest production of glucose from the acid swollen cellulose for rumen A was in the dorsal rumen whereas for the other forms of cellulose the digestion was higher in the ventral rumen. In rumen B, the swollen cellulose

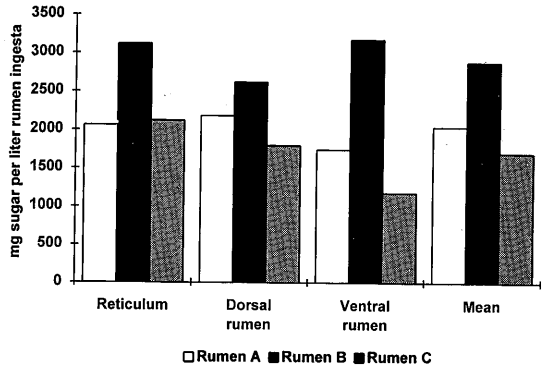

Figure 1. Concentrations of total soluble sugar from different rumens.

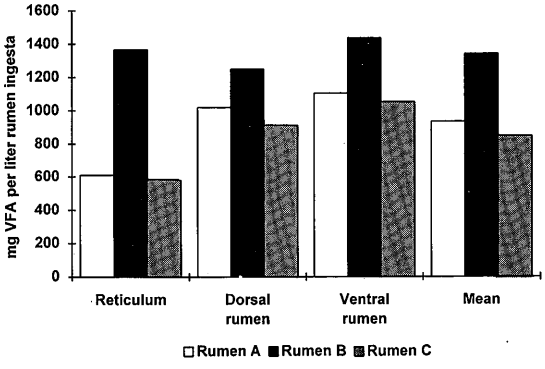

Figure 2. Concentrations of volatile fatty acids from different rumens.

was better digested in the ventral rumen and the other forms of cellulose were better digested in the dorsal part of the rumen. In rumen C, the digestion of swollen cellulose was comparable in the different parts of the reticulo-rumen and the other forms of cellulose were better digested in the ventral part of the rumen.

DISCUSSION

Ingested feedstuff is forced via the reticulum into the dorsal, and ventral part of the rumen, whereas the rumen digest is forced back via the reticulum into the omassum. During this process water is continuously added to the rumen. Thus the concentration of matter in the rumen depends on three main functions which includes the of rate of input of substances into the rumen, the rate of their enzymatic destruction, and the rate of their removal [17].

The rumen ingesta in the dorsal part of the rumen has characteristically been described to have higher total soluble sugar [18] and we noted similarly for rumen A. However the lower VFA content in the dorsal rumen compared to the ventral rumen suggested that readily fermentable carbohydrate was preferably utilized in the dorsal-rumen as evidenced from the higher total bacterial counts in that part of the rumen. This was supported by the decrease in the concentration of propionate and butyrate as the feedstuff moved from dorsal to the ventral part of the rumen.

In rumen A the digestion of swollen cellulose was higher in the dorsal part of rumen whereas the digestion of crystalline cellulose, grass and POME fibres were better in the ventral rumen. In general, large feed particles do not leave the rumen and would be retained for a longer period in the ventral part of the rumen, thus allowing more rapid microbial colonization and destruction of fibrous portion of the feed [19]. Since the products of cellulose fermentation were utilized as rapidly as they were formed [20], higher VFA in ventral rumen could be due to high cellulolytic activity. This contention is supported by the general trend of increasing acetate molar percentage from the dorsal to the

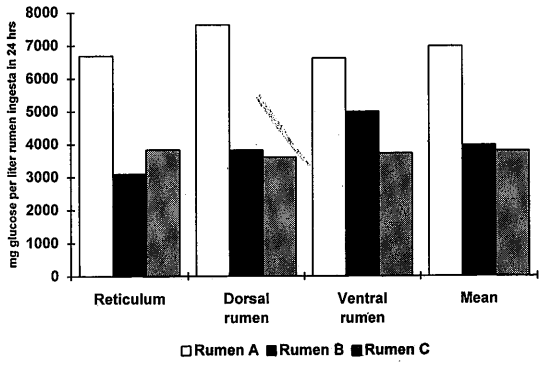

Figure 3. Digestion of swollen cellulose by rumen extracts.

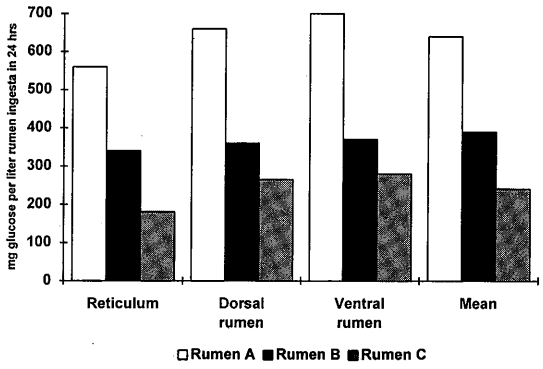

Figure 5. Digestion of POME fibres by rumen extracts.

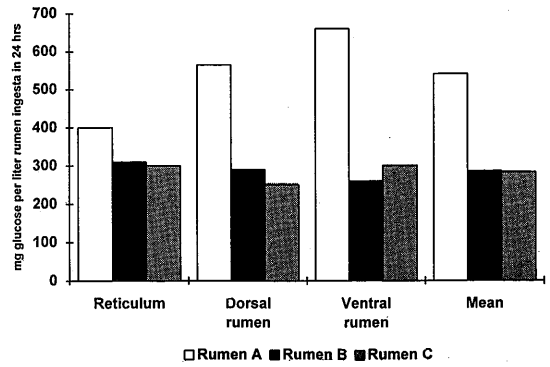

Figure 4. Digestion of crystalline cellulose by rumen extracts.

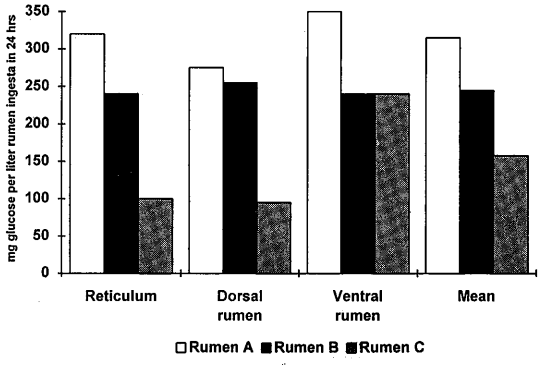

Figure 6. Digestion of grass fibres by rumen extracts.

ventral part of the rumen. Higher C2 to C3 VFA ratio in ventral rumen was generally caused by active cellulolytic activities [21] and could be further enhanced by the low soluble sugar concentration in the ventral rumen [15].

Supplementation of feedstuff with readily digestible carbohydrate and high protein feed in rumen B resulted in higher population of rumen bacteria, the production of more VFA but lower rumen pH. This could have changed the activities of rumen microorganisms [6]. High cellulase production could be attributed to the high bacterial count [4] but digestibility was not merely the function of the high number and proportion of fibrolytic rumen bacteria since cellulase activity could be inhibited by the presence of high soluble sugar and starch [22]. The addition of sugars or starch to the ruminant diet caused rapid fermentation of the carbohydrates with excessive production of acids. The acidic environment inhibited the growth of the cellulolytic bacterial population and has been suggested to suppress the cellulase activity in the rumen [6]. Washout of culture of *Bacteroides succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens* occurred at pH 6.0, 5.9, and 6.15 respectively [23] and lowering the pH to 6.0 almost completely inhibited attack of cotton by mixed ruminal bacteria [24].

The rumen pH was not the sole factor for the decrease in ruminal cellulolytic activity. The concentration of soluble sugar and the pH of rumen C were comparable to that of rumen A but showed lower cellulolytic activity than rumen A. Besides preferential substrate utilization or other catabolite regulatory mechanisms, concentrate feeds could be a major determinant of microbial competition in the rumen since it could also affect requirement for maintenance energy and consequently microbial growth rate [25, 26]. The fibre content of POME was only 60% of that of the grass of which cellulose of the former was only 40% of the total fibre. The lower fibre content could have lowered the proportion of cellulolytic bacteria and consequently the decline in numbers of cellulolytic bacteria as observed in rumen C. The presence of fat in the ruminant diet could also decrease the digestibility of cellulose and crude fibre and could cause a shift in the microbial composition which is responsible for the crude fibre digestion [9]. In addition, the low protein content of ruminant diet could further decrease the digestion of cellulose in the rumen [10]. Similar observations has been made for ruminants fed with ground or pelleted fibrous lignocellulose feed [27].

Effect of the feedstuff on the rumen system was further extended to the differences observed in the cellulolytic activities in rumen A, B, and C. The degree of order of crystallinity is critical in determing the biodegradability of cellulosic plant materials. Efficient and complete hydrolysis of cellulose is thought to be the result of the combined synergistic action of both the endoglucanase and the exoglucanase [28]. Higher digestion of swollen cellulose as compared to crystalline cellulose was common for mixed ruminal bacteria and pure cultures of cellulolytic bacteria [14]. Both exoglucanase activities and endoglucanase activities were higher in rumen A as compared to rumen B and rumen C. However, the endoglucanase activity of rumen B was comparable with rumen C but the exoglucanase activity was higher in the latter. This difference could be due to the change in the distribution of cellulolytic bacteria species using the different diets. This could be true since *Ruminococcus* was shown to be dominant in normal grass diet where efficient digestion of the cellulosic fibres was attributed to higher production of exoglucanase [29]. *Ruminococcus albus* and *Ruminococcus flavefaciens* produced endoglucanase or exoglucanase and hydrolyzed ball-milled filter paper, alfalfa fibre, and microcrystalline cellulose [15]. On the other hand, *Fibrobacter succinogenes* has lower exoglucanase activity but produced higher endoglucanase which suited for fibre such as swollen cellulose [30].

The enhanced degradation rate of swollen cellulose over crystalline cellulose could be due to the decrease in crystallinity [14] and increase in the surface area [30]. However the low digestibility of grass fibre compared to crystalline cellulose, apart from crystallinity, could be attributed to lignification of cell walls and the presence of protective plant tissues layer [20, 29]. POME fibre although lignocellulosic in nature, was subjected to high temperature at low pH during the extraction stage in the factory which could have caused its higher digestibility compared to the grass fibre.

Hence it could be concluded that supplementing the grass-fed goats with bean wastes did not alter the relative digestibility of the different forms of cellulose but altered the distribution of the rumen cellulolytic activities. Grass-fed goats with dried POME in the diet caused the decrease in the number of rumen microorganisms and also the rumen cellulolytic activities.

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