CHAPTER 6

GENERAL DISCUSSIONS & CONCLUSIONS

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Cellulose is the chief component of ruminal feedstuff. The ability of the ruminant to adapt to various cellulosic diets depends largely on the types and composition of the cellulolytic bacteria in the rumen (Grub and Dehority, 1975). The composition and activity of the microbial flora of the rumen varies according to the composition of the diet (Mackie, Gilchrist, Robert, Hannah, and Schwartz, 1978) and its physical form (Thorley, Sharpe, and Bryant, 1968). In the current study, effect of POME on the rumen bacterial population was studied and compared with *P. purpureum*.

Rumen ingesta of goat fed only on *P. purpureum* contained all important cellulolytic bacteria species (Chapter 3) and was shown to have high cellulolytic activity against recalcitrant fibre such as crystalline cellulose and grass fibre (Chapter 5). However, when this bacterial population were grown in POME, cellulolytic activity was decreased (Chapter 5) and it has been reported that *R. albus* and *F. succinogenes* were eliminated from the rumen of goat fed with POME (Abdul Manan, 1987). Chemical analysis of POME (Chapter 4) and *P. purpureum* (Chapter 5) showed differences

in their nutrients content and this could attribute to the difference in bacterial activity and their composition in POME and grass. POME contains lower crude fibre, cellulose, and protein but relatively high in fat when compared to grass. Soluble sugar content was higher than in grass and this soluble sugar is more readily fermentable as shown in Chapter 5. The amount of cellulose was low in POME and exists primarily in colloidal form.

The presence of high amount of readily fermentable sugar as in POME or *Vigna* bean sprout caused high increase in total rumen bacteria population (Chapter 2, Chapter 4 and Chapter 5). However, high concentration of readily fermentable sugar decreases the rumen bacterial cellulolytic activity. The analyses on the rumen digesta in the present studies showed that in Rumen A (*P. purpureum*) and Rumen C (POME) where pH at most part of the rumens were 6.0 or higher, cellulolytic activity was relatively higher in the ventral that was low in soluble sugar concentration. In rumen B where the pH of dorsal and ventral was below 6.0, the cellulolytic activity was comparable in both locations although the ventral part of the rumen has lower soluble carbohydrate concentration. Rumen B had higher soluble carbohydrates at every rumen part compared to rumen A and although the ventral part of rumen B had the same pH as the ventral part of rumen A, rumen A had higher cellulolytic

activity. These results suggested that there was depression on cellulase activity in the presence of soluble carbohydrates.

In the semi-batch system, fermentation was carried out at constant pH of 6.8 and the initial soluble sugar present in culture fluid was 6.6 g/l and 8.5 g/l in grass and POME system respectively. From 3-hour sample, grass showed higher cellulolytic activity probably due to lower sugar concentration compared to POME after which cellulolytic activities decreased when the sugar concentration decreased. Dropped in cellulolytic activity during this period could be due to drop in bacterial count (Henning *et al*, 1980). The cellulolytic activity dropped until the soluble sugar concentration was about 0.3 g/l to 0.1 g/l. During this period acetic to propionate increased corresponded to the increased in total cellulolytic count in both systems suggesting that cellulolytic population was more active when the soluble sugar concentration was lower. At sugar concentration less than 0.1 g/l cellulolytic activities increased in both systems indicates the absence of suppression of cellulolytic activity by soluble sugars. During this period, acetate to propionate ratio and VFA pattern remained constant in both systems. This could be due to selection of specific bacterial population growing under

low sugar concentration dependent on the presence of cellulolytic bacterial population.

Although grass has higher nitrogen-free extract than POME, nitrogen-free extract in POME are readily utilizable (Shaiful *et al*, 1987) whereas in grass, due to its cell wall structure the nitrogen-free extract has to be released by the action of cell wall degraders (Cheng, Fay, Howart, and Costerton, 1980). Extractable carbohydrates and cellulose were the main carbohydrate sources for rumen microorganisms. In grass the NFE was 1.9 times higher than cellulose whereas in POME the NFE was 9.0 times higher than cellulose. Thus, NFE would be more important in POME as carbohydrates source and this would have caused the lower cellulolytic activity in POME since the rate of fermentation was controlled by the extractable carbohydrates (Leedle *et al*, 1982 .

Several theories have been suggested to explain depressing effect of readily fermentable carbohydrate on fibre digestion: a preference for readily fermentable carbohydrate rather than fibre component, competition for essential nutrients by the microbial populations using the fermentable carbohydrates, and decreased in pH caused by rapid fermentation of carbohydrate resulted in preferential proliferation of the non-fiber digesting populations (El-

shazly, Dehority, and Johnson, 1961; Burrough, Gerlaugh, and Bethke, 1949). However, Simpson (1984) and Simpson *et al* (1977) demonstrated that a variety of carbon sources added to *in vitro* culture of rumen microbes growing on cellulose depressed the cellulose digestion with no drop of pH. High level of readily fermentable carbohydrate was reported to decrease cellulose digestion *in vivo* (Head, 1983; Henning, Van der Linden, Matheyse, Nauhaus, Schwartz, and Gilchrist, 1980; Joanning, Johnson, and Barry, 1981) and *in vitro* (Belasco, 1956; Simpson, March, and Merola, 1977).

Feeding POME-based concentrate (0.247 g glucose per gram PBC) to goat with a daily intake of more than 600 g PBC would cause the addition of more than 150 g glucose to the rumen per day. It has been shown that subculturing *Fibrobacter succinogenes* up to 10 times on 4% glucose reduced the capability of the bacteria for adhering to cellulose by 35% (Roger *et al*, 1990) and it was reported that glucose and cellobiose repressed certain cellulase genes (Taylor, Crosby, McGavin, Forsberg, and Thomas (1987). Thus, maintaining goat to a high intake of PBC may cause the lowering or eliminating important cellulolytic species such as *Fibrobacter succinogenes* and may cause lower cellulolytic activity in the rumen content of goat fed PBC.

High initial rate of VFA production when using POME (Chapter 5) would result in low pH if the frequency of feeding is not control. Reducing the pH from 6.8 to approximately 6.0 were shown to cause moderate depression of fibre digestion whereas a decreased in pH below 6.0 caused severe inhibition in batch and continuous culture (Mould, Orskov, and Mann, 1984; Crawford, Hoover, and Knowlton, 1980). Groleau and Forsberg (1983) showed that the activity of isolated fibrolytic enzymes remained high in pH range of 6.8 to 6.0. Decreased in number of cellulolytic microbes have not been associated with small decreased in pH (Leedle *et al*, 1982). Precipitous loss of fibrolytic activity at pH below 6.0 was shown to be related to the reduction in growth of cellulolytic bacteria (Russell, Sharpe, and Baldwin, 1979) and washout of cellulolytic bacteria from continuous culture (Russell and Dombrowski, 1980).

pH lower than 6.0 has been shown by Roger *et al* 1990 to lower the number of adhering cells of *Fibrobacter succinogenes* and Shriver, Hoover, Sargent, Crawford, and Thayne (1986) found that the quantity of mixed ruminal bacteria attached to fibre was reduced by 43% as compared with the average value for pH 6.2 to 7.0. This pH effect on the bacterial attachment could be the probable cause for low cellulolytic activity in the rumen of goat fed with grass supplemented with *Vigna* bean processing wastes.

Cellulolytic organisms require primarily ammonia as nitrogen source (Bryant, 1973). However, amino acids or peptides are required by cellulolytic organisms for maintenance of fibre digestion (Van Gylswyck, 1970). Ammonia concentration required by adherent, fibre-digesting organisms are greater than the free-floating organisms in rumen fluid. However, high requirements for amino acids or peptides have been found for particle-free organisms in rumen fluid (Russell, Sharp, and Baldwin, 1979), amylolytic organisms (Maeng and Baldwin, 1976), and other sugar-using organisms in rumen (Hungate, 1966). Thus, it is suggested that lower cellulolytic activity observed in POME was associated with the rapid growth of amylolytic and other sugar utilizers that simultaneously utilized amino acids and peptides which in turn limited the availability of amino acids and ammonium for fibrolytic microbes.

Analysis on the volatile fatty acid pattern(Chapter 5) supported this contention. Fatty acids such as iso-butyric, iso-valeric, and methylbutyric were required for the growth of cellulolytic microbes (Bryant, 1973) and cellulose digestions were improved by of addition of these acids (Gorosito, Russell, and Van Soest, 1985; Varga, Hoover, Junkins, and Shriver, 1982). Requirement for the fatty acids was associated with requirement for amino acids since fatty acids could be produced from the

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deamination of valine, leucine, and iso-leucine respectively. In the POME fermentation system it was possible that high uptake of amino acids occurred and could have caused faster depletion of iso-acids (iso-acids exhausted from 0.5 day fermentation). The unavailability of the iso-acids for the cellulolytic bacteria could be another factor for the low cellulolytic activity in POME system.

High protein content in POME and immediate availability of high concentration of sugars would cause a different fermentation pattern as observed in the semi-batch fermentation studies. Total VFA increased and as expected butyrate concentration increased (Annison and Lewis, 1966). High initial soluble sugar present resulted in high proportion of valerate and together with the decreased in iso-acids proportion with POME as substrate indicated that an altered microbial population had occurred (Erfle, Boila, Teather, Mahadevan, and Sauer, 1982). The shift in population using POME could have involved the cellulolytic population as shown by the changes in relative rate of digestion pattern in semi-batch POME fermentation.

A unique feature of palm oil mill effluent is its relatively high proportion of the dry matter is made up of colloidal carbohydrates and proteins (Stanton, 1974; Huang, Ong, Seow, and Tan, 1978). The colloidal form of cellulose may influence the type and proportion of different

cellulolytic bacteria when POME is introduced into rumen. Enzymatic hydrolysis of cellulose was affected by the structural properties of cellulose (Lee, Kim, Ryu, and Taguchi, 1983; Ryu, Lee, Tassinari, and Macy, 1982). Low cellulolytic activity showed by *Fibrobacter succinogenes* grown on POME compared to crystalline and swollen cellulose as the cellulose sources (Chapter 4) indicated that POME was lacking in the requited form of cellulose for the bacteria. This bacteria was found to be the numerically predominant cellulolytic bacteria when the proportion of crystalline cellulose in the animal diet was increased (review by Groleau et al, 1981).

Due to method of extraction, POME was high in residual oil (Hua, Kean, Ming, and Ying, 1975). Lipid were shown to have no effect on rumen or total-tract digestibility of starch or nitrogen-free extract fraction of diet (Van Nevel and Demeyer, 1988) but exert negative effect on crude fibre digestibility (White, 1958). The saturated fatty acids with C_{12} chain length have the most widespread inhibitory effect on pure cultures cellulolytic bacteria (Nieman, 1954). Based on the report by Chin (1981) it was calculated that the concentration of individual fatty acid in POME were lauric, 0.014; myristic, 0.068; palmitic, 2.93; stearic, 0.036; oleic, 2.72; and linoleic, 0.694 g/liter of POME. All of these acids inhibited growth of *Ruminococcus* at concentration as low as 0.005 g/l (Handerson, 1973) but the

inhibition varied with the strain used (Van Nevel and Demeyer, 1988). Negative effect of fatty acid on fibre digestion may contribute to the lower cellulolytic activity in POME or elimination of *Ruminococcus* in rumen of goat fed with POME.

POME contains significant amount of phenolics compound (about 6 g per litre POME) (Ho *et al*, 1984). Amongst the phenolics compound found in POME are vanilic, p-hydroxybenzaldehyde, vanilin and syringaldehyde (Shaiful - personnal communication). Vanilic acid has been shown to inhibit growth of *R. albus*, *R. flavefaciens and F. succinogenes* (Chesson *et al*, 1982) and this may contribute to elimination of Ruminococci and *F. succinogenes* in rumen of goat fed with POME and consequently lower cellulolytic activity in POME.

The lower growth rate of *F. succinogenes* and *R. flavefaciens* compared to *Butyrivibrio* and *Clostridium* Chapter 4) could be the cause for it to be less dominant in mixed population with POME as substrate. Although *R. albus* showed fairly high growth in POME, they were more sensitive to low pH (Hiltner *et al*, 1983), a situation which most probably had occurred in POME fermentation.

Unlike POME, *P. purpureum* seemed to be a better substrate for bacterial growth and attachment. From the semi-batch experiment and the analysis on the rumen ingesta of *P. purpureum* fed goat it was shown that soluble sugar

concentration was low. Nutrient-rich tissue of forage was mostly internal (Cheng *et al*, 1989). Consequently, the overall rate of digestion of this tissue depends upon microbial access to the inside tissue. The released of nitrogen-free extract would be gradual (Cheng, *et al*, 1980) and this would regulate the fermentation rate and maintained the optimum pH for the cellulolytic bacteria to proliferate on the cellulosic materials. This could be true in the semi-batch experiment where the proportion of volatile fatty acids was indicative of a normal rumen and the system was able to maintain high count of bacteria (>10^8 cell/ml) after 6 turnovers of 12 hours.

Its fibre crystallinity was higher than swollen cellulose and would favour the attachment and colonization of more diverse cellulolytic species. This would include the most active fibre digesting *F. succinogenes*. The quality of forages influenced the major type of bacteria attached to it (Akin, 1980). *R. albus* and *R. flavefaciens* were more prevalent on *Medigo sativa* (Kistner, 1965) while *F. succinogenes* or *B.fibrisolvens* predominant on *Eragrostis tef* (Akin 1979) and *Festuca arundinace* (Akin, 1980). High proportion of both Ruminococci and *Fibrobacter* found in the rumen of *P. purpureum* fed goat in this study indicated that *P. purpureum* was suitable to support the growth and activity of normal rumen microorganisms.

CONCLUSIONS

1. The nutritional status of feedstuff given to goats affects the number, distribution and activities of the rumen bacteria.
2. *P. purpureum* is a better substrate for rumen bacterial population than POME since cellulolytic activity against grass fibre, crystalline cellulose, and acid swollen cellulose of the culture filtrate of mixed rumen population grown on grass (in vitro study in Chapter 5 and in green rumen in Chapter 2)are higher than the population grown on POME.
3. Rumen of goat fed with *P. purpureum* contains all important cellulolytic bacteria species indicating that *P. purpureum* is a good substrate for maintenance of a normal and active cellulolytic rumen bacterial population.
4. All important cellulolytic bacterial species could grow in POME and produce cellulolytic enzymes. However, growth of *R. flavefaciens* and *F. succinogenes* are poor than the less important cellulolytic species such as *C. cellobioparum*, and *B. fibrisolvens*.
5. Bacterial activities (such as cellulolytic activity, VFA production and utilization, and sugar utilization) were different in POME as compared to *P. purpureum* indicating that change in bacterial population had occurred.