CHAPTER 2.

CHARACTERISATION OF RUMEN INGESTA FROM GOATS FED WITH *P. PURPUREUM*; *P. PURPUREUM* WITH *VIGNA* BEAN SPROUT WASTES; AND *P. PURPUREUM* AND POME-BASED CONCENTRATE.

2.0. INTRODUCTION

The shortage of goats as the preferred source of meat and the destructive nature of grazing goats have resulted in the increase in the usage of semi-intensive stall feeding system by the local goat breeders in Malaysia. However the shortage of proper forage ground in favour of other economically commercial crops has hindered the intensification of the livestock production. Potential cheap local feed materials introduced by the small holders were based on the acceptability by the goats rather than any scientific studies on the nutrient contents and its digestibility.

Crop residues such as paddy straw and cocoa pods are normally rejected by the goats but the usage of dried POME and rejected beans from *Vigna* bean sprouts cottage industry have gained popularity amongst the small holders in the rural areas of Malaysia. This chapter investigates the bacterial concentration, soluble sugar concentration, fatty acids content and cellulolytic activity of rumen ingesta from goats fed with: (i) *Pennisetum purpureum*, (ii) *P. purpureum* supplemented with *Vigna* bean sprout wastes, and (iii) *P. purpureum* supplemented with POME-based concentrate.

2.1. BACTERIA COUNT

2.1.1. Materials and Methods

2.1.1a. Rumen samples

Two rumen samples per category were obtained from goats fed on Napier grass (*Pennisetum purpureum*) (Rumen A), *P. purpureum* supplemented with *Vigna* bean sprout wastes (Rumen B), and *P. purpureum* supplemented with palm oil mill effluent (POME)-based concentrate (comprising of POME, 60.67%; poultry dung, 28.40%; molasses, 5.47%; and brewer's yeast, 5.47%) (Rumen C). The digestive tracts of slaughtered goat were tightly tied at the oesophageal and abomasal ends, placed in double plastic bags and immediately frozen with dry ice contained in polystyrene box. The frozen rumen was transported to the laboratory and kept at -12^{o}C until further use. Care was taken to ensure that the rumens were not punctured so as to maintain the anaerobiosis of the rumen content.

2.1.1b. Preparation of oxygen-free carbon dioxide

The use of commercial carbon dioxide gas (Malayan Oxygen) for culturing rumen bacteria was found to be unsuitable as it contained traces of oxygen sufficient to prevent reduced condition. The trace oxygen was removed by passing the CO_2 gas through chromous acid solution and Supelco gas purifier (Supelco Inc., Belfonte, USA).

Chromous acid solution was prepared as described by McBee (1977). 75 g of twenty-mesh zinc granules were treated with 50 ml 3N hydrochloric acid (HCl) and stirred vigorously for 30 second. 50 ml of water that contained 5 ml of saturated $HgCl_2$ solution was added and stirred for 3 min. The washing solution was decanted and the zinc was well washed with distilled water. The water was decanted and the zinc was placed in a gas-washing bottle. 60 g of chromium potassium sulphate dissolved in 250 ml distilled water was added to the zinc followed by introduction of 0.5 ml of concentrated H_2SO_4. CO_2 gas was passed through the bottle for a few minutes to displace all air. The material was allowed to stand until the solution turned to a blue color. The solution was transferred via a tube to a new pre-gassed washing bottle and used as the chromous acid oxygen scrubber. The outlet from the scrubber was connected to Supelco gas purifier and channeled to three glass tubing each filled with a sterile swinex filter set with a 0.22 µm membrane filter and a gassing laboratory cannula.

2.1.1c. Preparation of pre-reduced dilution solution

Dilution solution was prepared according to Bryant and Burkey, (1953). It contained: K_2HPO_4, 0.45g; NaCl, 0.9g; $(NH_4)_2SO_4$, 0.9g; KH_2PO_4, 0.45g; $CaCl_2$, 0.9g; $MgSO_47H_2O$, 0.19g; cycteine.$HCl.H_2O$, 0.25g; $NaCO_3$, 4g; and Resazurin, 0.0001g, and distilled water, 1000 ml.

The ingredients except Cysteine.HCl.H_2O and Na_2CO_3 were mixed in conical flask and brought to boiling for 3 minutes. After the solution has cooled down to 55°C, a stream of oxygen-free CO_2 was passed into the conical flask to displace the atmosphere above the dilution solution. The dilution solution was then dispensed into bottle pre-gassed with oxygen-free CO_2, gassed out, capped, and pressed using a Bellco press (Bellco Glass Inc., Vineland, New Jersey). The bottles were sterilised at 121°C for 30 minutes. After sterilisation, the dilution solution was cooled to 50°C in a water bath. The required filter-sterilised solution of cysteine.HCl.H_2O and $NaCO_3$ solution were then added aseptically under continuous gassing with oxygen-free CO_2 until the resazurin indicator turned from pink to colourless. The bottles were tightly capped and kept for further use.

2.1.1d. Media for roll tube

Medium 10, a medium described by Caldwell and Bryant (1966) and Thorley, Sharpe, and Latham (1968) as to be adequate in replacing the rumen fluid for culturing rumen bacteria was used in this study. It was modified with the inclusion of 1% (dry wt/v) acid-swollen cellulose (see section 2.2.3 a1), and yeast extract at 0.1% concentration and was designated as complete medium (CM medium).

The composition of the CM medium per litre is as follows: K_2HPO_4, 0.45g; NaCl, 0.9g; $(NH_4)_2SO_4$, 0.9g; KH_2PO_4, 0.45g; $CaCl_2$, 0.09g; $MgSO_47H_2O$, 0.19g; Cysteine.HCl.H_2O, 0.5g; sodium sulphide, 0.25g; Na_2CO_3, 4g; Resazurin, 0.0001g; 0.5 each of glucose, cellobiose, and soluble starch; Trypticase, 5g; yeast extract, 1g; hemin, 0.0001g; Agar, 5g; and volatile fatty acid mixture solution, 3.1 ml. The volatile fatty acids mixture is made up of 17 ml acetic acid, 6 ml propionic acid, 4 ml butyric acid, and 1 ml each of iso-butyric, n-valeric, i-valeric, and methylbutyric acid.

The ingredients were mixed in conical flask except cysteine.HCl, sodium sulphide, sugars, sodium carbonate, and hemin and pH was adjusted to 6.8. The mixture was boiled for 3 minute and cooled to 50°C in a water bath and was bubbled with oxygen-free CO_2. The medium was then dispensed into pre-gassed roll tube, capped with butyl rubber stopper, pressed and autoclaved. After sterilisation, the tubes were cooled to 50°C in water bath The required quantity of filter sterilised sugars solution, hemin, and Na_2CO_3 were added aseptically and the medium was gassed with CO_2. Cysteine.HCl.H_2O and Sodium sulphide were added and the bottle was tightly capped and maintained molten in water bath for further use.

2.1.1e. Rumen content sampling and inoculum preparation

The whole rumen, dilution solution, and media were placed in an anaerobic cabinet (Forma Scientific, Model 1029, Ohio) with a CO_2, 5.5%; H_2, 5.5%; N_2, 89% gas phase. Samples of rumen ingesta were taken from four parts of the rumen: (i) the reticulum, (ii) the dorsal-anterior part of the rumen, (iii) the dorsal-centre part of the rumen and (iv) the ventral part of the rumen.

Three replicates of 10 ml rumen content were taken at each sampling sites with the aid of cut off 20 ml sterile disposable plastic syringes. Each of the rumen samples was diluted with 20 ml anaerobic dilution medium in 50 ml-roll streak tube (Bellco Glass Inc.) and vortex mixed. 1 ml of the diluted rumen ingesta was transferred to a bottle containing 9 ml anaerobic dilution medium and 5.0 g. sterile sands and vortex mixed for 3 minutes. The samples were then serially diluted to 10^{-10} dilution using 1 ml-hypodermic syringes (Terumo Inc. Ltd., Japan). Duplicates of 0.1 ml from 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} dilution were inoculated into roll tube containing 7.0 ml of molten pre-reduced complete medium (CM medium). The inoculated tubes were taken out from the anaerobic cabinet and roll tubes were prepared by rapidly rotating the tubes using a tube spinner (Bellco Glass Inc.). The solidified tubes were incubated in an upright position at 38°C up to a period of 3 weeks.

2.1.1f. Total viable and total cellulolytic bacteria counts.

The incubated roll tubes were checked for growth at every two days interval, and colonies from the roll tubes that contained 60-100 colonies were counted using a binocular dissecting microscope. The colonies were categorised for good and poor clearing and no clearing of the cellulose in the tubes. Fungal colonies are morphologically distinct (with hair-like periphery called thallus) and were excluded. The present growth medium is not suitable to support protozoal growth and protozoa do not form colony as bacteria do on a growth medium containing agar.

2.1.2. Results

The average of the total bacteria counts for the different part of rumens is shown in figure 2.1. The count was highest in rumen B, followed by rumen A and rumen C. In all rumens, bacterial count was lowest in the reticulum. Bacterial count was highest in dorsal-anterior part of rumen A and rumen B whereas in rumen C highest count of bacteria was at the dorsal-center part of the rumen.

The means of the total counts of viable bacteria and the counts of the cellulolytic bacteria for different

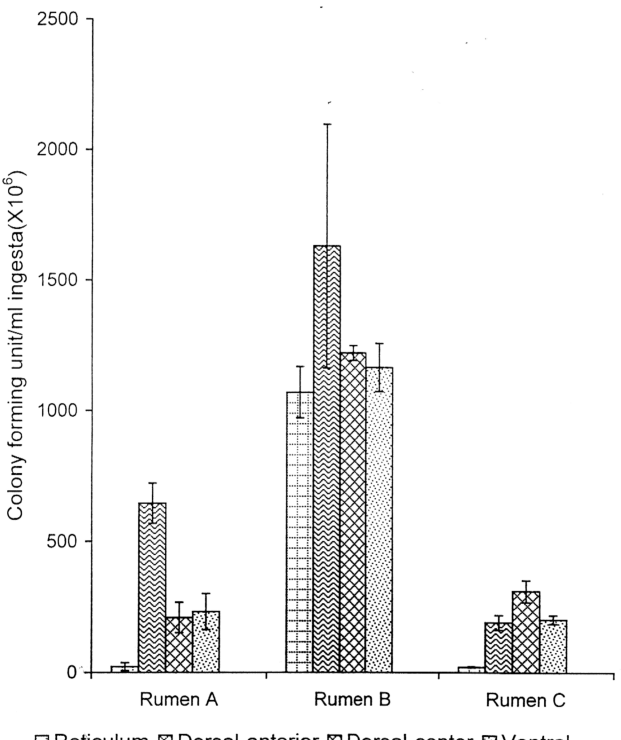
part of the rumen were calculated and the percentage of the cellulolytic bacteria for each rumen is shown in table 2.1. The supplementation of grass with bean waste increased the total count as well as the percentage of cellulolytic bacteria whereas feeding goats with pelleted concentrate (rumen C) reduced the percentage of the cellulolytic bacteria as well as total number of bacteria.

Table 2.1:

Rumen ingesta at the different parts of rumen were cultured in roll tube. After incubation, bacterial colonies were counted. Total bacteria count is the average bacteria number in the reticulum, the dorsal-anterior, the dorsal-center and the ventral part of rumen. Colonies that digested cellulose (shown with clearing of cellulose around the colonies) were taken as cellulolytic colonies.

Rumen	Total bacteria/ml ingesta	Total cellulolytic bacteria/ml ingesta	Percentage cellulolytic bacteria
Rumen A	$2.12 \pm 0.25 \times 10^{8}$	$2.23 \pm 0.30 \times 10^{7}$	10.57
Rumen B	$1.24 \pm 0.23 \times 10^{9}$	$2.87 \pm 0.22 \times 10^{8}$	23.14
Rumen C	$6.24 \pm 0.44 \times 10^{7}$	$8.53 \pm 0.25 \times 10^{6}$	1.36

Figure 2.1: Total viable bacteria number at different parts of rumen. Rumen A was from goat fed on *P.purpureum*, Rumen B was from goat fed on *P. purpureum* aand *Vigna* bean wastes, and Rumen C was from goat fed on *P. purpureum* and POME concentrate.



2.2. ANALYSES ON RUMEN INGESTA

On the completion of the sampling for the enumeration of rumen bacteria, the whole reticulo-rumen was transferred out from the anaerobic cabinet and kept in a -12°C freezer. Rumen contents from the different part of the frozen rumen were collected, thawed to room temperature and were strained by squeezing the content in two layers of cheesecloth. The filtrate was kept in a storage bottle at -12°C until further use.

2.2.1 Total soluble sugar.

2.2.1a. Materials and methods

The rumen filtrate was centrifuged at 6000*g* for 15 minutes to remove any particulate matter. Sugar concentration was determined by the Anthrone method described by Trevelyan and Harrison, (1952). 0.1 ml of the supernatant was placed in a 18 mm x 150 mm-screw-cap culture tube and diluted with 2.4 ml of distilled water. The tubes were placed in ice bath to cool. 5 ml. of Anthrone reagent (0.2g Anthrone dissolved in 100 ml concentrated sulphuric acid and chilled for 2 hours) was added and mixed well with vortex mixer. The tubes were placed in an ice bath until all the tubes were prepared. The tubes were then placed in a boiling bath for 15 minutes, cooled in ice bath for 2-3 minutes and then were let to stand at room temperature for 5-10 minutes. Optical density was determined by Spectronic

20 (Bausch and Lomb) at a wavelength of 620 µm against a reagent blank. Standard curve was determined using glucose standard at 50, 100, and 150 µg glucose per ml.

2.2.1b. Results

Total soluble sugar at the different part of rumen is shown in figure 2.2. Sugar concentration was on the average highest for rumen B followed by rumen A and rumen C. In rumen A, sugar concentration was highest at the dorsal-anterior part of rumen followed by reticulum, dorsal-center and ventral part of rumen. In rumen B, highest sugar concentration was at the ventral part of rumen, followed by reticulum, dorsal-anterior, and dorsal-center part of rumen. In rumen C, sugar concentration decrease from reticulum to the ventral part of rumen.

2.2.2. Volatile fatty acid content.

2.2.2a. Materials and methods.

The volatile fatty acids (VFA) content was determined according to Supelco Technical Bulletin No. 749E (Supelco Inc.). 1 ml. of rumen content filtrate was added to and thoroughly mixed with 0.2 ml. 25% metaphosphoric acid and allowed to stand for 30 min. The mixture was centrifuged at 4000 *g* for 10 min. The supernatant was analysed by gas-liquid chromatography (Shimadzu Model GC-14A) using a glass column purchased from Supelco Inc. packed with material code

number SP 1220. Column temperature was 145°C and nitrogen flow rate was 80 ml/min. The peaks resolved were compared to standard VFA that comprises of formic acid, acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid and n-valeric acid at 120, 10.5, 9.9, 9.5, 9.5, 9.9 and 9.8 µg/µl respectively.

2.2.2b. Results

Total VFA at the different part of the rumen is shown in figure 2.3. The concentration of VFA was on the average highest for rumen B, followed by rumen A and rumen C. In rumen A and rumen C, VFA concentration increase from reticulum to the ventral part of rumen. In rumen B, VFA concentration increase from the dorsal-anterior part of the rumen to the ventral part of rumen but VFA concentration in reticulum was higher than the dorsal-anterior part of the rumen.

Table 2.2 showed the major individual VFA at the different parts of the rumen. Acetic acid was the major acid found in the rumens followed by propionic, butyric and valeric acid. Rumen B contained higher amount of butyric and valeric acid. There was a general trend of increase in the acetate to propionate ratio from dorsal-anterior towards the ventral part of rumen B and rumen C. In rumen A, the acetate to propionate ratio was comparable at the dorsal-anterior, dorsal-central and the ventral part of the rumen but the concentration was higher than in the reticulum.

Figure 2.2: Concentration of soluble sugar expressed as glucose at the different parts of rumen of goat fed on *P. purpureum* (Rumen A), *P.purpureum* and *Vigna* bean waste (Rumen B), and *P.purpureum* and POME concentrate (Rumen C)

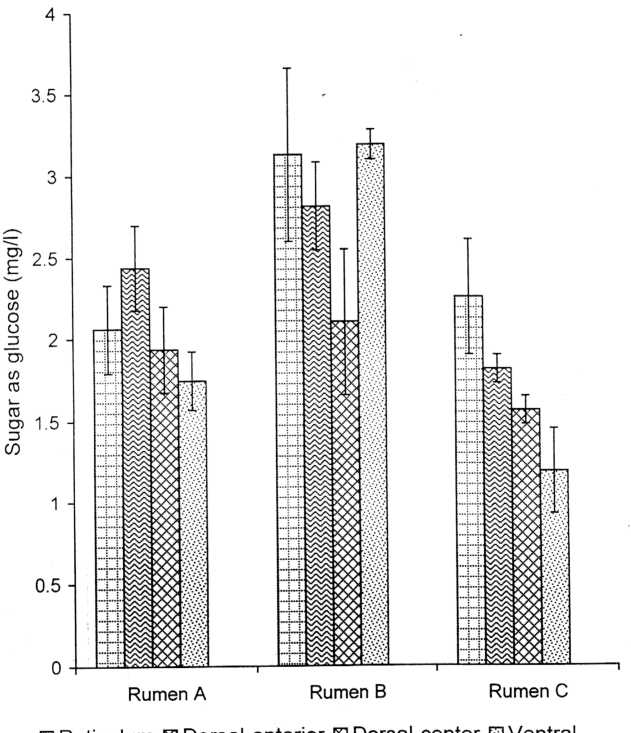


Figure 2.3: Total volatile fatty acid concentration at the different parts of rumen A (goat fed on *P. purpureum*), rumen B (goat fed on *P. purpureum* and *Vigna* bean waste), and rumen C (goat fed on *P.purpureum* and POME concentrate).

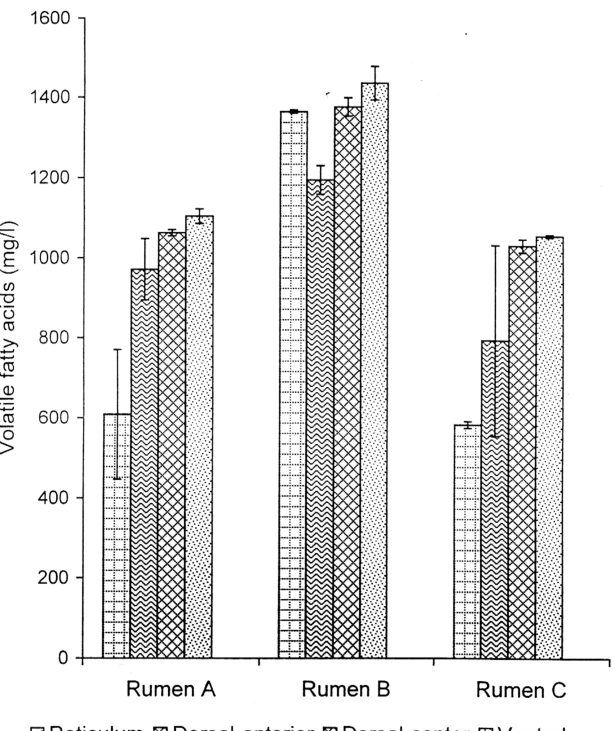


Table 2.2: Rumen ingesta at the different parts of rumen was analysed for formic, acetic, propionic, iso-butyric, n-butyric, iso-valeric, and n-valeric acid. The acid concentration was expressed in mole. The figures in the table below are molar percentage of the various fatty acids. Molar percentage of acetic acid (as for the other acids) is calculated as follows:

$$\text{Molar percentage of acetic acid} = \frac{\text{Acetic acid (mole)}}{\text{Formic acid (mole) + Acetic acid (mole) + Iso-butyric acid (mole) + n-butyric acid (mole) + Iso-valeric (mole) + n-valeric (mole)}} \times 100$$

Rumen	Part of rumen	Acetic	Propionic	n-butyric	n-valeric
A	Reticulum	51.29	26.71	13.35	1.23
	Dorsal-anterior	59.16	21.66	11.65	1.28
	Dorsal-centre	58.59	22.76	11.67	1.20
	Ventral	58.68	23.03	11.52	1.10
B	Reticulum	51.83	28.50	15.42	1.03
	Dorsal-anterior	51.64	29.17	1.11	15.38
	Dorsal-centre	52.07	27.99	15.79	1.12
	Ventral	56.20	26.40	14.18	1.20
C	Reticulum	60.86	23.04	8.76	1.56
	Dorsal-anterior	48.56	27.19	14.86	1.48
	Dorsal-centre	58.33	22.94	1.13	12.00
	Ventral	58.25	21.37	12.56	1.25

2.2.3. Cellulolytic activity

2.2.3a. Materials and methods

2.2.3a.1. Cellulosic substrates

Four substrates were used in the determination of cellulolytic activity.

1. Microcrystalline cellulose
2. Acid-swollen cellulose
3. Grass fibres
4. POME fibres

The microcrystalline cellulose was purchased form Sigma Chemical Company, St. Louis, USA. The acid-swollen cellulose was prepared according to the method described by Taya, Honma, Ohmiya, Kobayashi and Shimizu (1981). Three grams of crystalline cellulose was mixed with 100 ml. of 85% orthophosphoric acid prechilled at 3°C. The slurry was kept at 2-3°C for 30 min. It was then mixed with 20 volumes of tap water. The precipitate was filtered under reduced pressure and thoroughly washed with distilled water. Part of the cake was dried at 70°C for one day to determine the water content. The residual cake was used without drying as the source of acid-swollen cellulose.

The grass fibre was prepared from *Pennisetum purpureum* leaves. The leaves were cut into 2-4 cm. length and blended with tap water in the mixer blender. The leaves were filtered and squeezed through double layer of cheesecloth. The residue was boiled and filtered. Washing with hot water was repeated for five times until the soluble carbohydrate was no longer detectable. The grass fibre was dried at 100°C overnight, ground and sieved through 1 mm. pore-size sieve (Townson and Merser Ltd., England).

In order to obtain POME fibre, POME sludge collected from local palm oil processing mill was first boiled for 10 min. The hot sludge was filtered through double layer of absorbent cotton and washed with equal volume of hot boiling water for 5 times. The residual cake was removed, dried and resuspended in dietyl-ether. The mixture was filtered using Whatman filter paper (fast) and further washed for 3 times to remove any residual fats. The residue was then dried in stainless steel tray at 100°C overnight and then ground and sieves using 1 mm. pore-size sieve.

2.2.3a.2. Measurement of cellulolytic activity

Determination of cellulolytic activity was done according to the method of Smith, Yu and Hungate (1973). The rumen ingesta filtrate was first kept at 2-4°C and then

centrifuged at 1000 *g* for 20 min. 10 ml of the supernatant fluid was introduced into the Venoject tube (Terumo Corp., Japan) containing 100 mg of cellulosic substrate. The tube was gassed with oxygen-free carbon dioxide and kept at 3°C for 2 hours to allow adsorption of cellulase to the cellulosic material. The tube was vortex-mixed every 15 minute to ensure all the enzymes could adsorb to the cellulosic material. The tube was then centrifuged at 5000 *g* for 10 min. The supernatant fluid was discarded and replaced with 10 ml of chilled anaerobic dilution solution (section 2.1.1b), mixed gently for 10 second and centrifuged at 5000 *g* for 10 min. The washing was repeated for 3 times (as traces of soluble sugar were detected from single washing). Two ml of the supernatant fluid from the third washing was sampled out and kept frozen. This was done to check for the presence of background sugar. One ml of toluene was then added to the remaining fluid in the tube, mixed and incubated for 24 hours at 38°C. After incubation, the mixture was then centrifuged at 5000 *g* for 10 min. Both the supernatant fluids of the incubated and the unincubated samples were analysed for the presence of sugar as described in section 2.2.1a.

2.2.3b. Results.

The activities of crude cellulase extracts are shown in figure 2.4a, 2.4b, 2.4c and 2.4d. Acid-swollen cellulose was the most readily digestible form of cellulose whereas the lowest production of glucose was observed in the digestion of grass fibre. POME fibre was better digested than crystalline cellulose in rumen A and rumen B. In all cases, rumen A showed better cellulase activity than that of rumen B and rumen C. Rumen B showed better cellulase activity for POME and grass fibre than rumen C which was better in digesting crytalline cellulose than rumen B. Highest production of glucose from the swollen cellulose and POME fibre in rumen A and rumen C was observed in the anterior part of the dorsal rumen but the ventral part of rumen showed better cellulase activity for crystalline cellulose and grass fibre. Highest activity for swollen cellulose in rumen B was observed in the ventral part of the rumen whereas for all of the other forms of cellulose, digestion was better in the anterior part of the dorsal rumen.

Figure 2.4a: Cellulolytic activity of rumen ingesta for acid swollen cellulose at the different parts of rumen A (goat fed on *P.purpureum*), Rumen B (goat fed on *P. purpureum* and *Vigna* bean waste, and Rumen C (goat fed on *P.purpureum* and POME concentrate)

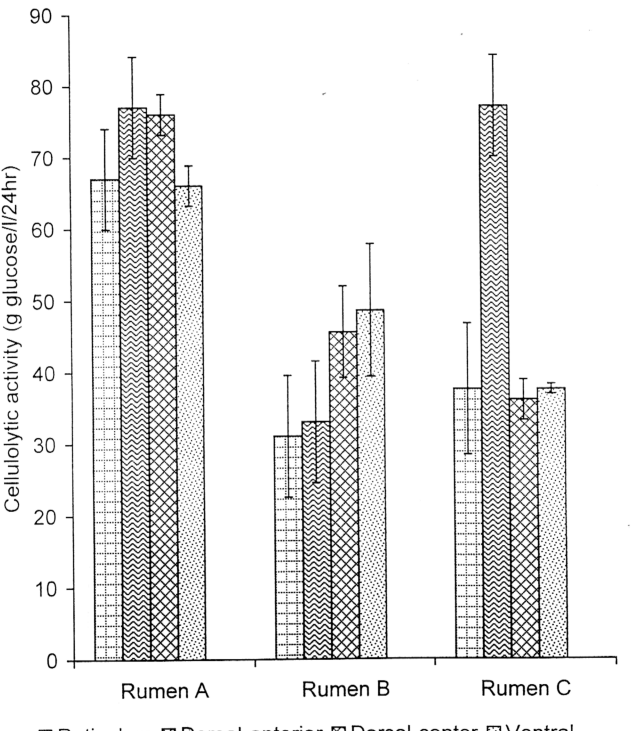


Figure 2.4b: Cellulolytic activity of rumen ingesta for crystalline cellulose at the different parts of rumen A (goat fed on *P.purpureum*), Rumen B (goat fed on *P. purpureum* and *Vigna* bean waste) and Rumen C (goat fed on *P.purpureum* and POME concentrate).

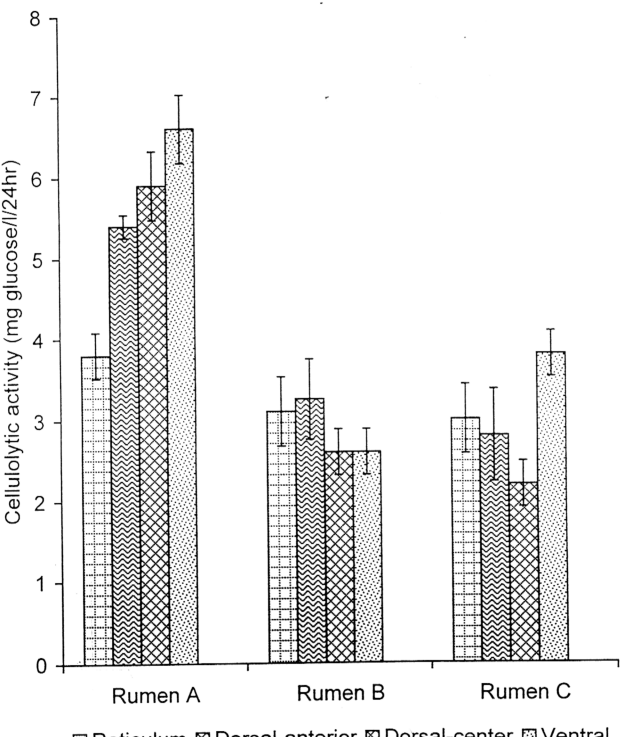


Figure 2.4c: Cellulolytic activity of rumen ingesta for POME fibre at the different parts of rumen A (goat fed on *P. purpureum*, rumen B (goat fed on *P.purpureum* with *Vigna* bean waste) and rumen C (goat fed on *P.purpureum* and POME concentrate).

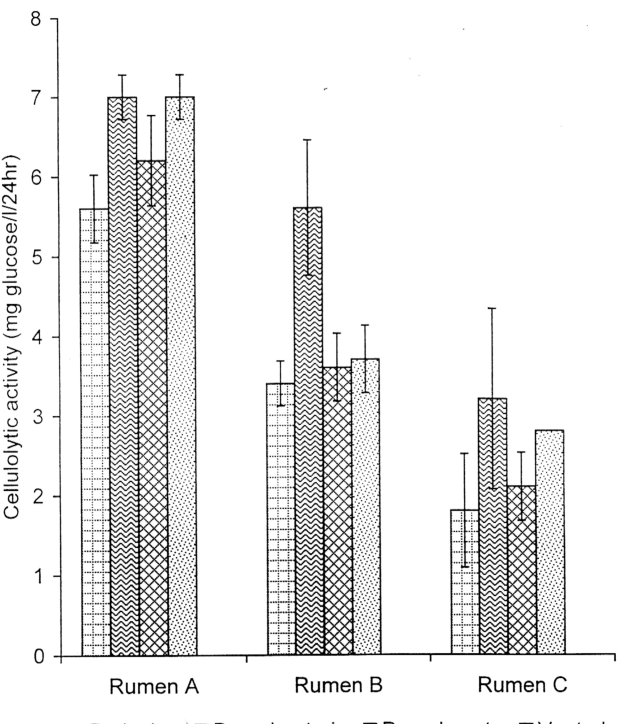
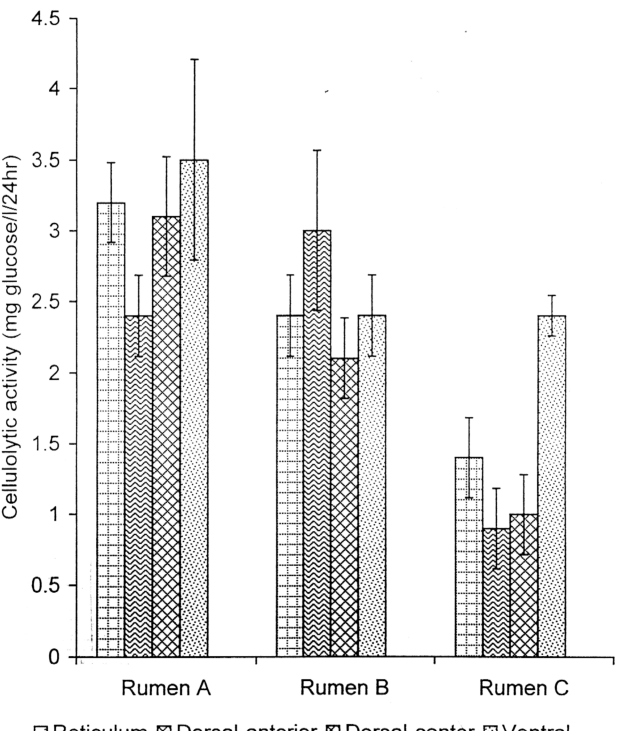


Figure 2.4d: Cellulolytic activity of rumen ingesta for grass fibres at the different parts of rumen A (goat fed on *P.purpureum*), rumen B (goat fed on *P.purpureum* with *Vigna* bean waste), and rumen C (goat fed on *P.purpureum* and POME concentrate).



2.2.4. pH

2.2.4a. Materials and methods

The pH of the filtrate from rumen ingesta was measured using Chemcadet (Cole-Palmer) pH meter (digital). The pH meter was first calibrated with pH 4 and pH 7 standards before pH measurements were taken. The pH of the different parts of the rumen was carried out from 3 sub-samples.

2.2.4b. Results.

The pH of the different parts of the rumen is shown in table 2.2. The pH of the rumen was on the average lowest for rumen B. However, except for rumen B, the other rumens have lower pH in the ventral region compared to the reticulum and the dorsal-anterior part. The pH in the dorsal-centre was lower than dorsal-anterior in the case of rumen A and rumen C but no difference was observed in rumen B.

Table 2.3

pH at the different parts of rumen of goat fed on *P. purpureum* (A), *P.purpureum* and *Vigna* bean waste (B), and *P. purpureum* and POME-based concentrate

Rumen	Part of rumen	pH
A	Reticulum	6.25 ± 0.07
	Dorsal-anterior	6.05 ± 0.07
	Dorsal-centre	6.00 ± 0.00
	Ventral	5.90 ± 0.00
B	Reticulum	5.90 ± 0.00
	Dorsal-anterior	5.85 ± 0.07
	Dorsal-centre	5.85 ± 0.07
	Ventral	5.85 ± 0.07
C	Reticulum	6.10 ± 0.00
	Dorsal-anterior	6.15 ± 0.07
	Dorsal-centre	6.05 ± 0.07
	Ventral	6.05 ± 0.07

2.3. DISCUSSION

The rumen system can best be described as a semi-continuous system where feedstuff is forced down to the reticulum, the dorsal and the ventral part of the rumen. Water is continuously added through input of saliva and products are removed through outward movement of digesta together with exchange of soluble materials through the wall of the rumen. Mixing of rumen is aided by the rumen contraction. For a reasonably mixed rumen content, the concentration of substances in the rumen is the function of rate of input of substances into the rumen, the rate of their destruction, and the rate of their removal (Czerkawski, 1986).

The decreasing concentration of soluble sugar from the reticulum to the ventral part of the rumen suggested that the readily fermentable carbohydrate be preferably utilised in the reticulo-rumen. This was supported by the decrease in the propionate and butyrate concentration as the feedstuff moved from the dorsal-anterior to the ventral. The presence of higher energy available for microbial growth in the dorsal has similarly observed by Smith, Sweeny and Rooney (1956). The dorsal ingesta were characteristically higher in total sugar, nitrogen, ammonia and crude fibre. This could possibly explained the presence of higher bacteria population in the dorsal part of rumen A, B and C. The highest bacteria population in the dorsal part and

relatively high count in the ventral as compared to the reticulum appeared to be in agreement with the work of Bryant and Robinson (1968) who showed that the means of logorithms of count of dorsal samples were always significantly higher than those of the corresponding ventral samples. In addition, when silage was fed, the bacterial number was higher in the ventral rumen than the reticulum.

Despite the high content of readily digestible substrate in the reticulum the bacterial population was still low. This could be related to rapidly changing ruminal condition at feeding (Leedle, Bryant and Hespell, 1982) where water intake at feeding could lead to osmotic shock effect (Rogers, Marks, Davis and Clark, 1979) and oxygen entering via water and feed might have restricted those fermentative bacteria innately more susceptible to it. The intake of water may also have diluted the reticular content.

The effect of available energy for growth also explained the presence of higher bacterial population in rumen B. The latter was supplemented with readily digestible carbohydrate and high protein present in the rejected non-sprouting *Vigna* beans. Fermentation of high digestible carbohydrate and protein changed the rumen fermentation pattern in rumen B since it has higher propionate and butyrate concentration. This could have resulted from an adaptive balance between amylolytic and lactate-utilising bacteria (Mackie and Gilchrist, 1979).

Although soluble sugar was lower in the ventral rumen for all rumens studied, total VFA appeared to be higher and hence lower pH. This result was rather contrary from those reported by other workers (Smith *et al*, 1956) who have correlated the presence of high energy to the higher VFA in the dorsal rumen. The difference could be due to difference in the efficiency of reticulo-rumen movements in mixing the digesta which varies with the physical state of the content where the speed of mixing decrease with increase particle size (Hungate 1966). In General, large feed particles do not leave the rumen (Czerkawski, 1986). Thus, fibrous material especially in rumen A would be retained for a longer period in the ventral part of the rumen, thus allowing more rapidly microbial colonisation and destruction of fibrous portion of the feed. Balch and Johnson (1956) reported that rate of breakdown of cotton thread anchored in the ventral rumen was higher as compared to the dorsal rumen. Similarly, The ventral rumen content digested more cellulose *in vitro* than the digestion by the dorsal rumen content (Smith *et al*, 1956). Since the products of cellulose fermentation were utilized as rapidly as they were formed (Pavlostathis, Miller and Wolin, 1988), higher VFA in the ventral rumen could be due to high cellulolytic activity. This contention is supported by the general trend of increasing of acetate molar percentage from the dorsal-anterior to the ventral part of the rumen. Murphy, Baldwin and Koong (1982), reported that higher C2 to C3 VFA ratio in ventral rumen was generally caused by active cellulolytic activities and could be further enhanced by the low soluble

sugar concentration in the ventral rumen (Smith, Yu and Hungate, 1973).

High cellulase production in the dorsal rumen could be attribute to the high bacterial count (Hiltner and Dehority, 1983) but digestibility was not merely the function of the high number and proportion of fibrolytic bacteria (Jung and Varel, 1988) since the cellulase activity in the dorsal and reticular content might have been inhibited by the presence of high soluble sugar (Russell and Baldwin, 1978; Moulds, Orskov and Gould, 1983).

Supplementation of feedstuff with readily digestible high protein feed as in rumen B and the usage of concentrates in rumen C, would have changed the activities of rumen micro-organisms (Mackie and Gilchrist, 1979; Wedekind, Muntefering and Barker, 1986). The presence of highly digestible substrate would have caused lower rumen pH and hence would have lowered the cellulolytic activity as observed for rumen B. This has been shown in pure cultures of rumen bacteria (Russell and Dombrowski, 1980) as well as for mixed culture of rumen bacteria (Stewart, 1977; Terry, Tilley and Outen, 1969). Russell *et al* (1980) showed that wash out of culture of *F. succinogenes, R. albus* and *R. flavefaciens* occurred at pH 6.0, 5.9 and 6.15 respectively and lowering the pH to 6.0 almost completely inhibited the attack of cotton by mixed ruminal bacteria (Stewart, 1977).

The rumen pH was not the sole factor for the decrease in ruminal cellulolytic activity. The concentration of soluble sugar and the pH of the rumen C were comparable to that of rumen A but showed lower cellulolytic activity than rumen A. Besides preferential substrate utilisation or other catabolite regulatory mechanisms (Russell *et al*, 1978) concentrate feed can be a major determinant of microbial competition in the rumen. This includes requirement for maintenance energy (Russell *et al*, 1979a), substrate affinities (Russell and Baldwin, 1979b) and growth rate (Russell, Delfino and Baldwin, 1979). The fibre content of PBC 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 would have lowered the proportion of cellulolytic bacteria and consequently the decline in the number of cellulolytic bacteria as observed in rumen C. This has similarly been observed by Thorley, Sharpe and Bryant (1968) for ruminants fed with ground pelleted feed and by Henning *et al* (1980) and Grubb and Dehority (1975) with concentrates.

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. Both exoglucanase activities (digestion of crystalline cellulose) and endoglucanase activity (digestion of swollen cellulose) was higher in rumen A as compared to rumen B and rumen C. Although the endoglucanase activity of rumen B was

comparable with rumen C, the exoglucanase activity was higher in the latter.

The feedstuff also affected the distribution of cellulolytic activities in the rumen. The exoglucanase activity was higher in the ventral part of rumen A and rumen C but both rumen showed better endoglucanase activity in the dorsal part of rumen. The reverse was observed for rumen B. In rumen B, the cellulolytic activity was most active in the dorsal part of rumen whereas in rumen A and C the digestion of more resistant cellulose (crystalline cellulose and grass fibre) was more active in the ventral part of rumen but for swollen cellulose and POME fibre, it was more active in the dorsal part of rumen. This difference could be due to change in the distribution of cellulolytic bacterial species using the different diets (Thorley, Sharpe and Bryant, 1968). This could be true since *Ruminococcus* was shown by Gardner, Doerner and White (1987) to be dominant in normal grass diet where efficient digestion of cellulosic fibre was attributed to high production of exoglucanase. On the other hand, *F. succinogenes* has lower exoglucanase activity but produced higher endoglucanase which is better suited for fibre such as swollen cellulose (Groleau and Forsberg, 1981).

Relative amount of different forms of cellulase produced in all rumens followed the same order: swollen cellulose > POME fibre > crystalline cellulose > grass fibre. High digestion of swollen cellulose as compared to crystalline cellulose was also observed *in vitro* digestion employing mixed ruminal bacteria (Weimer, Lopez-guisa and French, 1990) and pure cultures of cellulolytic bacteria

(Weimer, French and Calamari, 1991; Taya, Honma, Kobayashi and Shimizu, 1981). The enhanced degradation rate of swollen cellulose over crystalline cellulose could be due to the decrease in the crystallinity (Taya *et al*, 198,Weimer *et al*, 1990). The lowest rate of degradation of grass fibre could be attributed to the crystallinity, lignification of the cell walls and the presence of protective plant tissue layers (Chesson and Forsberg, 1988).

POME fibre is lignocellulosic in nature. The pre-treatment of POME fibre during the oil extraction stage has subjected it to high temperature at low pH. This could cause its higher digestibility. However, fibre digestion was found to be better in forage-fed goat and as such, feeding the goat with concentrate might have altered the distribution of the bacteria and consequently, lowered the cellulolytic activity.

In conclusion, diet composition affects the bacteria content, volatile fatty acid content, concentration of soluble sugar, and cellulolytic activity of the rumen content. Feeding goat with *P.purpureum* supplemented with POME decrease the number of cellulolytic bacteria and cellulolytic activity compared to rumen of goat fed mainly on *P.purpureum*, whereas, supplementation of *Vigna* bean waste increase the total bacterial number as well as the cellulolytic number, volatile fatty acid content and soluble sugar concentration, but decrease the cellulolytic activity.