MICROBIAL COLONIZATION AND DIGESTION OF FEED MATERIALS IN CATTLE AND BUFFALOES
Ⅱ. RICE STRAW AND PALM PRESS FIBRE

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Summary

Degradation of rice straw was observed to be higher ($p < 0.01$) in the buffaloes than in cattle. At 48 h, the dry matter (DM) loss of straw for buffaloes was $53.6 \pm 0.8\%$ and that for cattle was $48.7 \pm 2.6\%$. Palm press fibre (PPF) was poorly degraded in the rumen of both animal species. A loss of about 21% DM was observed in both cattle and buffaloes after 48 h of incubation in the rumen. The pattern of bacterial and fungal colonization of straw and PPF seemed to be similar in both cattle and buffaloes. Microbial colonization was restricted by plant structures like the silica crystals in both straw and PPF. The predominant bacteria colonizing both straw and PPF fragments were the rods. Eroded zones and digestion pits were pronounced in straw fragments after 1 h of incubation. The PPF fragments appeared undegraded even after 6 h of incubation. Fungal colonization of straw was rapid and extensive in both cattle and buffaloes. The sporangia observed in straw were mainly spherical or oval in shape, but fusiform sporangia with acuminate tip were predominantly seen in PPF fragments.

(**Key Words**: Straw, Palm Press Fibre, Digestion, Microbial Colonization, Cattle, Buffaloes)

Introduction

The bacteria, fungi and, to some extent, the protozoa are involved in feed degradation in the rumen. The rumen microbes are well-equipped enzymatically to hydrolyse the carbohydrate polymers to small saccharides for their own metabolic needs. The initial process in the digestion of structural carbohydrates is microbial colonization as a close proximity is required between the microbes and the substrates.

It has been reported that buffaloes (*Bubalus bubalis*) are able to digest fibrous feed materials more than cattle (*Bos indicus*) (Abdullah et al., 1990; Vijchulata et al., 1985). The reason(s) for the superiority of buffaloes over cattle in fibre digestion is not well understood. The pattern of microbial colonization of fibrous feed materials has not been investigated before in these two animal species. Hence, an investigation on the colonization and degradation pattern by rumen bacteria and fungi of cattle and buffaloes on rice straw and palm press fibre (PPF) exposed to the rumen environment at different time intervals was carried out to determine whether there were any apparent differences in the microbial population between the two animal species.

Materials and Methods

Feed Materials

Rice straw and PPF (after removing the shell debris) were dried at 65℃ for 48 h and then ground through a 4 mm screen in a Hammer mill. Palm press fibre was refluxed with petroleum ether to remove residual fat for 8 h and dried at 65℃ for 48 h before used.

Degradation of Rice Straw and PPF by Nylon Bag Technique

The loss in dry matter (DM) for straw and PPF (treated and untreated) were determined by the nylon bag technique described by Ørskov et al. (1980). Four male Kedah-Kelantan (KK)

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Received May 20, 1991

Accepted February 10, 1992

cattle and 4 male swamp buffaloes, about 12 months old, each fitted with a rumen cannula were used. The animals were fed guinea grass (*Panicum maximum*) *ad libitum*. Each animal had free access to mineralized-cobalt salt blocks and drinking water. The nylon bags (9 × 12 cm, 44 μm mesh size) containing 3-4 g of straw or PPF were incubated in the rumen for 8, 24, 32, 48, 56 and 72 h. The percentage DM loss was calculated after the incubated bags were washed and dried to constant weights at 65°C. The percentage DM loss at various incubation periods was compared between animal species by the 2-way analysis of variance. Percentage DM loss was plotted against time for each animal. Using the equation $p = a + b\ (1 - e^{-ct})$ given by Ørskov and McDonald (1979), c, the degradation rate in h, was calculated by using a computer programme developed by Owczkin (pers. comm. 1987).

Preparation of Samples for Microbial Colonization Study

About 1 g samples of straw, ether-extracted and untreated PPF were placed separately in nylon bags (mesh size 50 μm) and incubated in the rumen of 2 Kedah Kelantan cattle and 2 swamp buffaloes fed guinea grass *ad libitum* as mentioned above. All bags were placed in the rumen just before feeding. Bags containing straw were withdrawn at 15 and 30 min, 1, 3, 6, 24, 48 and 72 h, while those that contained PPF were withdrawn at 6, 24, 48 and 72 h. After incubation, the bags were rinsed under tap water and the samples were processed for light microscopy and scanning electron microscopy (SEM) study. The methods for preparing samples for SEM and light microscopy were similar to that described by Ho et al. (1988).

For each sample collection, at least 10 fragments of straw and PPF were investigated. Control samples (unincubated straw and PPF) were also processed following the same procedure and studied for the presence of any microbial colonization.

Results and Discussion

Degradability of Straw and PPF

Figure 1 shows the percentage DM loss of straw, ether-extracted PPF and untreated PPF with

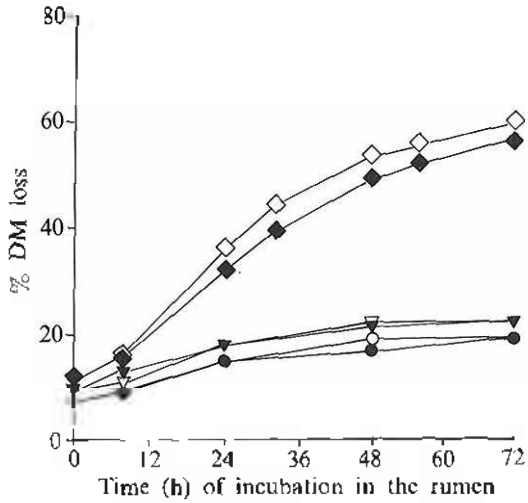

Figure 1. Percentage loss of dry matter of rice straw and palm press fibre incubated in the rumen of cattle and buffaloes. Straw in cattle (◆) and buffalo (◇): unextracted-PPF in cattle (▼) and buffalo (▽): ether-extracted PPF in cattle (●) and buffalo (○).

different incubation times in the rumen of both cattle and buffaloes. Two-way analysis of variance with % DM loss and times of incubation as sources of variation showed that % losses of straw DM was significantly ($p < 0.01$) higher in the buffaloes than in cattle. The % DM loss at 48 h was 53.6 ± 0.8 for buffaloes and 48.7 ± 2.6 for cattle (table 1). There was no significant difference in the rates (c) of straw digestion between animal species.

Degradability of both treated and untreated PPF was very low, ranging from 17 to 22% at 48 h of incubation in both cattle and buffaloes. There was no significant difference between the two animal species in percentage loss of DM or degradation rates of both PPF samples. This is contrary to the observation reported by Vijchulata et al. (1985), where degradability of PPF was better in buffaloes than in cattle when fed PPF-based diet. Depressed PPF digestion in the present study could be the effect of rumen environment which is determined by the animal's diet. The PPF used contained 4% ether-extract. Degradation of ether-treated samples was reduced significantly ($p < 0.05$) in both cattle and buffaloes. However, untreated or ether-extracted PPF showed little potential for supporting growth and other pro

TABLE 1. PERCENTAGE DRY MATTER LOSS OF STRAW, ETHER-EXTRACTED AND UNTREATED PPF AT 48 H OF RUMEN INCUBATION IN CATTLE AND BUFFALOES

Animals	Straw		Ether-extracted PPF		Unextracted-PPF	
	Degradation (% DM Loss)	Rates (/h)	Degradation[1] (% DM Loss)	Rates (/h)	Degradation[1] (% DM Loss)	Rates (/h)
Cattle	48.7 ± 2.6	0.028 ± 0.003	17.3 ± 0.8	0.035 ± 0.006	20.7 ± 0.7	0.045 ± 0.002
Buffaloes	53.6 ± 0.8	0.031 ± 0.002	19.6 ± 0.6	0.034 ± 0.002	21.6 ± 0.2	0.040 ± 0.004
Significance	$p < 0.01$	NS	NS	NS	NS	NS

Straw contained 86.0% DM, 6% crude protin, 73% NDF. PPF contained 70.6% DM, 6.2% crude protein, 77.6% NDF.

Each value is a mean ± S.E. of 4 samples.

[1] Ho et al. (1991).

duction systems as their degradability was low even after 48 h incubation in the rumen.

Colonization of Rice Straw by Rumen Microbes

There seemed to be no apparent differences in the morphology of bacteria and fungal sporangia on the straw fragments between cattle and buffaloes. Microbial colonization pattern was also similar between the two animals species. Very few ciliates or flagellates were observed on fragments from both animal species.

Control samples (not incubated in the rumen) of rice straw did not show any bacterial or fungal colonization. A number of straw fragments showed numerous surface structures. The structures consisted of prickle hairs, small and large papillae and rows of silica crystals that may cover the whole surface or parts of the straw fragments.

Colonization by bacteria and fungal zoospores took place 15 min after incubation in the rumen. Bacterial colonies consisting of rods and cocci and germinated zoospores (figure 2) were observed on the surface of the plant fragments, along cut ends and damaged surfaces. The rapid colonization of straw fragments by a large number of fungal zoospores soon after incubation (15 and 30 min) was probably due to their increase in numbers soon after feeding. Orpin (1977) observed that plant materials can trigger zoosporogenesis and zoospores were liberated within 15-45 min of feeding. The rhizoids that developed from the germinated zoospores then rapidly branched and ramified the other tissues.

Straw samples incubated for 1 and 3 h showed varying degrees of bacterial density and colonization. Some samples showed an increase in bacterial colonization, whereas a number of fragments were still uncolonized. At these times, bacterial population was usually a mixture of rods and cocci. The plant cell walls would be ruptured by bacterial digestion and the bacteria formed microcolonies within the cells. At 3 h, small spherical sporangia (about 14-20 μm in diameter) could be observed on the straw fragments.

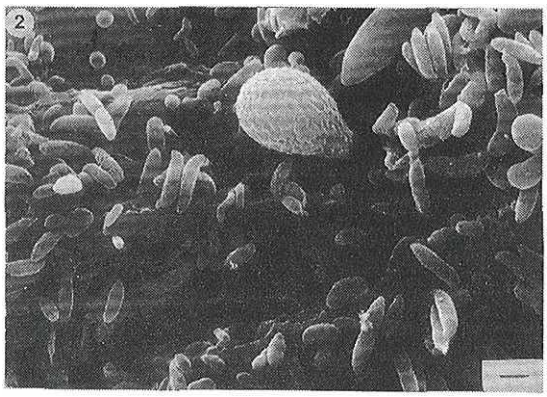

Figure 2. A mixed population of bacteria and a germinated zoospore on a straw fragment, 15 min after incubation in a buffalo rumen. Bar = 1 μm.

Straw fragments at 6 h of incubation showed an increase in bacterial population density and tissue digestion. Digestion pits or zones with dense population of bacteria were more pronounced (figure 3). Fairly homogenous microcolonies of rods were frequently observed (figure 4). The fungal sporangial size had not increased very much at 6 h and was relatively the same as those observed at 3 h. Numerous 'appresoria' which were produced by the fungus for penetrating the plant cell wall could be seen on the rhizoids or on the unbranched germ tube of some fungi. The

Figure 3. Bacterial colonization and degradation of plant cell wall (rice straw) by bacteria, 6 h after incubation in a cattle rumen. Bar = 10 μm.

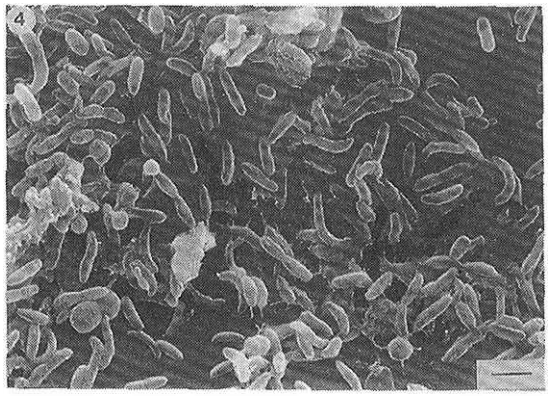

Figure 4. Curved rods on a straw fragment, 6 h after incubation in a cattle rumen. Bar = 1 μm.

detailed structure and occurrence of the 'appressoria' had been described by Ho et al. (1988).

Samples at 24 h showed further bacterial colonization and digestion. A mixture of thick or curved rods was usually observed. Fragments that were covered with cuticle layer appeared undegraded, except at regions where this layer had peeled off and bacterial colonization could be seen in the inner tissues. Fungal colonization was also extensive at 24 h. The extensive network of rhizoids penetrated and disrupted plant tissues. Most of the sporangia had reached maximum size of 20-30 μm in diameter (figure 5) by 24 h. The spherical sporangia seemed to predominate in straw fragments, whereas the other forms of sporangia (filiform, fusiform and clubbed-shaped) were lesser in numbers.

Bacterial colonization was still extensive at 48 h in some parts of the straw fragments. Signs

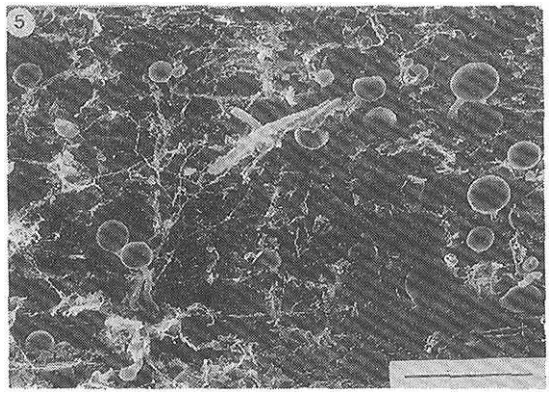

Figure 5. Fungi with spherical sporangia colonizing and degrading a straw fragment, 24 h after incubation in a cattle rumen. Bar = 100 μm.

of bacterial activity were indicated by the disorganised tissue surfaces and partially degraded cell walls with fairly dense bacterial population, predominated by rods. Some parts of the cuticle were detached to expose the tissue underneath, which could be colonized or uncolonized by bacteria. Most of the fungal sporangia had collapsed and disintegrated at 48 h, with the remnants scattered on the surface of the straw fragments. Zoospores could have been released by this time. Lowe et al. (1987) observed that liberation of zoospores of rumen fungal isolate R1 occurred at 27 h after inoculation *in vitro*.

Some straw samples showed the presence of small (5-10 μm diameter) pyriform or ovoid sporangia with short thick rhizoids lying or half hidden in the empty plant tissue cells (figure 6).

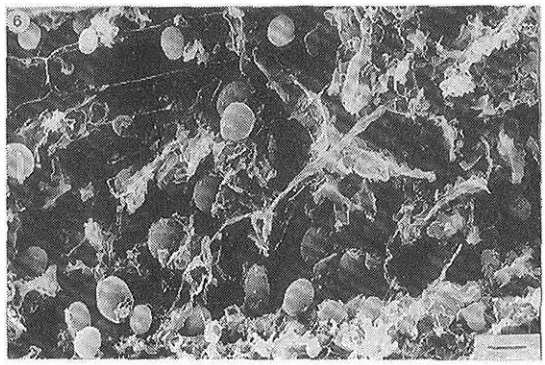

Figure 6. Small ovoid sporangia on a straw fragment, 48 h after incubation in a cattle rumen. Bar = 10 μm.

Some of them had a single pore on the wall at 48 h (figure 7). Besides the oval sporangia, other sporangial shapes (cylindrical, filiform of fusiform with elongated pointed tip) were of lesser occurrence. Straw fragments after 72 h of incubation were not much different in terms of bacterial and fungal colonization from those observed at 48 h.

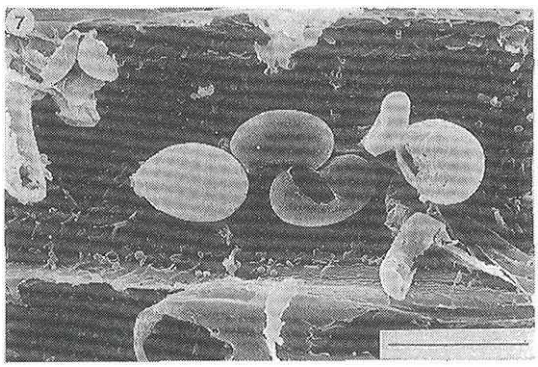

Figure 7. Small ovoid sporangia with pores on a straw fragment, 48 h after incubation in a buffalo rumen. Bar = 10 μm.

Invasion stages did not take place simultaneously in all regions of the plant fragments as different tissues showed variable stages of bacterial and fungal colonization. The cell walls of different plant tissues had shown distinct differences in the extent to which they were colonized and digested by bacteria and fungi. The plant cuticle in particular was not colonized and digested by rumen microbes.

SEM study showed that bacteria were in close proximity with plant cell walls either at a close distance or embedded to the substrates within the tissues. According to Akin et al. (1974), bacteria degraded thin, primary cell walls of mesophyll and phloem apparently by extracellular enzyme and without prior attachment, but thick walled bundle sheath cells were apparently degraded after bacterial attachment by an extracellular substance to the plant cell walls. This adhesion resulted in a sequestration of bacterial population in the rumen.

Generally, the bacteria seen attached to the straw particles were mainly a mixture of rods (thick or curved or crescent-shaped rods). Cocci were usually seen in samples with shorter incubation periods (up to 6 h). The mixed bacterial population consisting of different morphological types at shorter incubation periods indicated that various plant substrates were available for hydrolysis. When all soluble materials (probably sugars and leachates substances) had been digested, the ones left were the cellulose digesting bacteria that adhered to the solid matrices. The bacterial species in these studies were not identified. However, the predominant bacterial species active in cellulose digestion and normally adhere to the substrates are of four types. They have been identified by Bryant (1973) as *Bacteroides succinogenes* which has been reclassified as *Fibrobacter succinogenes* by Montgomery et al. (1988), *Butyrivibrio fibrisolvens*, *Ruminococcus albus* and *R. flavifaciens*. Cheng et al. (1983/84) reported that with wheat straw, *F. succinogenes* was the most active bacterium for cell wall digestion.

Colonization of PPF by Rumen Microbes

No apparent difference in bacterial and fungal colonization and development was observed between the ether-extracted and the untreated PPF fragments. Difference in bacterial and fungal population between animal species was also not detected. As in straw, very few ciliates or flagellates were seen on the PPF fragments.

Control samples (not incubated in the rumen) of PPF fragments did not show any bacterial or fungal colonization.

Like straw, PPF also showed the presence of silica bodies that limit the surface area for microbial colonization and digestion. Ether-extracted and untreated PPF samples after 6 h incubation showed slight colonization of bacteria associated with the tissue surrounding the crsytals. Most of the bacteria were rods. Groups of spiral bacteria were also seen. At this time, a number of attached and encysted zoopores were scattered over the surface of the fragments. They were rather small in size (about 5-8 μm in diameter) and were attached to damaged surfaces and cavities left by disloged silica crystals. 'Appresoria' were also produced by fungi colonizing the PPF fragments.

By 24 h, some of the fibres showed light to heavy bacterial colonization in the empty zones left by detached crystals. The straight and curved rods seemed to predominate (figure 8). The curved rods with thread-like structures, observed on

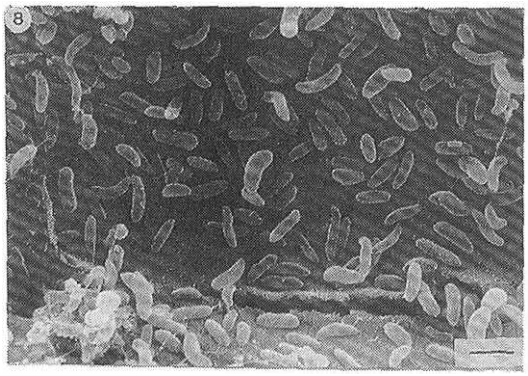

Figure 8. Thick rods on a PPF fragment, 24 h after incubation in the rumen. Bar = 1 μm.

ether-extracted PPF fragments from the buffalo rumen, occurred singly or in clumps (figure 9). Chains of rods were also observed on ether-extracted PPF fragments from the cattle rumen. Fungal sporangia which were either ovoid or elongated with pointed tips were of various sizes. The elongated sporangia may possessed stalks, about 30 μm long, which raised them above the surface of the fibre (figure 10). They resembled the *Ruminomyces* sp. (Ho et al., 1990).

Palm press fibre fragments at 48 h showed varying degrees of bacterial attachment. A number of strands were devoid of any bacterial colonization. Fungal sporangia with pointed tips were more abundant with the 48 h fragments. Groups of egg-shaped or ovoid sporangia (similar to figure 6) could be seen at some of the digested zones of the PPF fragments. Extensive network of rhizoids for this fungi was not observed.

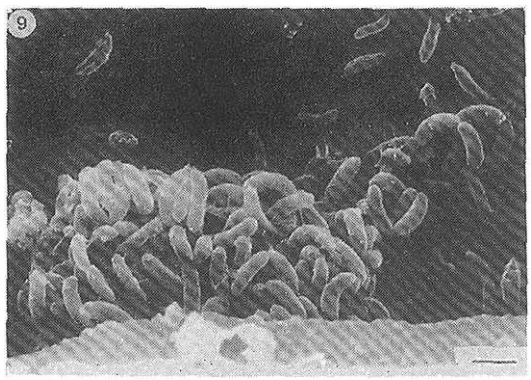

Figure 9. Clumps of thick rods with hair-like structures on a PPF fragment, 24 h after incubation in a buffalo rumen. Bar = 1 μm.

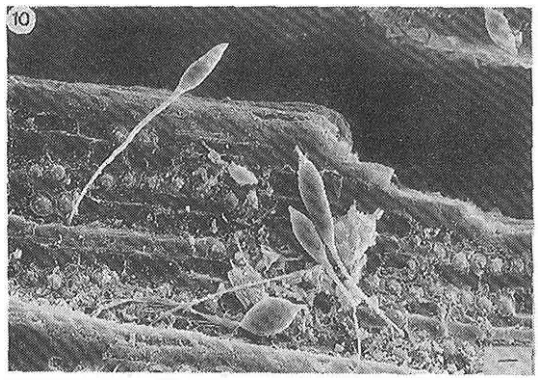

Figure 10. Fusiform sporangia with long sporangiophores on a PPF fragment, 48 h after incubation in a cattle rumen. Note the rows of silica crystals and empty cavities left by dislodged crystals. Bar = 10 μm.

Samples at 72 h incubation showed similar bacterial and fungal colonization to those observed at 48 h.

The higher % loss in dry matter of straw when incubated in the buffalo rumen indicated a more intense microbial activity in the buffalo than in the cattle. However, the present investigations with the light microscope and SEM on the bacteria and fungal morphologies and their colonization pattern could not indicate any apparent differences in microbial populations and colonizing activity between the two animal species.

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