MICROBIAL COLONIZATION AND DIGESTION OF FEED MATERIALS IN CATTLE AND BUFFALOES
I. GUINEA GRASS

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Summary

An experiment was conducted to determine whether there were any apparent differences in the microbial population, colonization pattern and digestion of guinea grass *in situ*, between cattle and swamp buffalo. Percentage losses in dry matter (DM), nitrogen (N) and neutral detergent fibre (NDF) of guinea grass were significantly ($p < 0.01$) higher when incubated in the rumen of buffalo than in cattle. Buffalo also showed significantly ($p < 0.05$) faster degradation rates than cattle for each grass component (DM, N, DNF). Light microscopy and SEM examination of the incubated grass materials showed that there were no apparent differences in the pattern of bacterial and fungal invasion and colonization of the grass materials between cattle and buffalo. Attachment of bacteria and fungal zoospores on the grass fragments occurred at 15 min after rumen incubation. After 3 h of rumen incubation, dense population of bacteria was observed in the thin-walled mesophyll and parenchyma tissues, whereas root-like fungal rhizoids were observed in both thin-walled and thick-walled cells. By 6 h, eroded zones were apparent in the thin-walled tissues and in thick-walled tissues with profuse rhizoids. After 24, 48 and 72 h of rumen incubation, most thin-walled tissues were degraded leaving mostly the thick-walled tissues. The predominant bacteria were the curved rods resembling *Butyrivibrio* sp., the thick rods resembling *Fibrobacter* sp., the diplococcoids resembling *Ruminococcus* sp. and spirochetes. Fungi were predominantly those with spherical or oval sporangia. Fusiform sporangia with acuminate apices which resembled *Ruminomyces* sp. were of lesser occurrence. Few protozoa were found on the grass fragments at all incubation times.

(Key Words: Rumen Microbes, Degradation of Grass, Degradation Rates, Cattle, Buffaloes)

Introduction

Within the rumen, an intensive microbial degradation of feedstuff takes place. Plant carbohydrates are hydrolyzed to small saccharides which in turn are fermented to numerous end-products. The anaerobic fermentation of the plant materials in the rumen is the function of bacteria, protozoa and fungi. It has been demonstrated that buffalo is able to utilize low quality fibrous feed more effectively than cattle (Vijchulata et al., 1985) and this may be due to the differences in digestive physiology of the animals or differences in the microbial population involved in fibre digestion. So far, there has been limited studies to compare the microbial population involved in degradation of fibrous feed materials between cattle and buffalo.

This investigation was carried out to determine whether there were any apparent differences in the microbial population, colonization and digestion pattern of guinea grass between the cattle and buffalo.

Materials and Methods

Animals

Four Kedah-Kelantan (KK) cattle (*Bos indicus*) and four swamp buffaloes (*Bubalus bubalis*), all male, about 12 months old and each fitted with a permanent rumen canulae, were used. The animals were kept separately and had free assess to water and cobalt mineralized salt blocks, and were fed guinea grass (*Panicum maximum*) once daily *ad libitum*.

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Degradation of guinea grass by nylon bag technique

Potential degradability and degradation rates of guinea grass were determined by the nylon bag technique (Ørskov et al., 1980). Guinea grass was dried at 65°C for 72 h and ground through a 2 mm screen. About 3 g of the ground grass and a glass marble (acting as weight) were placed in dried, preweighed nylon bags (measuring 9 × 16 cm with mesh size 44 μm). The bags with the contents were dried again at 65°C for 24 h, reweighed and then placed in the ventral section of the rumen with complete immersion and wetting. Control bags containing marbles, but without grass materials, were also included in one cattle and one buffalo to check for any increase in weight from materials entering the bags. The bags were withdrawn from the rumen after 8, 24, 32, 48, 56 and 72 h incubation and were washed and dried at 65°C to constant weights. The percentage dry matter (DM) loss at various incubation periods was calculated and compared between animal species by the 2-way analysis of variance. Percent 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 per h was determined by using a computer programme developed by Owezkin (pers. com. 1987).

Both incubated and unincubated samples of guinea grass were analysed for nitrogen (N) by Kjeldahl digestion (AOAC, 1980). Neutral detergent fibre (NDF) for the grass samples (both incubated and unincubated) was determined by the method described by Goering and Van Soest (1970).

Preparation of grass sample for microbial colonization studies

Thirty pieces of freshly-cut leaves and stems (1.0 × 0.5 cm) of guinea grass and a glass marble were placed in nylon bags (9.0 × 6.0 cm). The bags were put into the ventral section of the rumen about 30 min after the animals were fed, and were removed after 15, 30 min, 1, 3, 6, 24, 48 and 72 h of rumen incubation. After removal, the bags were washed, and the grass samples fixed and processed for scanning electron microscopy (SEM) and light microscopy. Grass samples for SEM and light microscopy were prepared according to the methods described by Ho et al. (1988a).

Results and Discussion

Degradability of guinea grass

Degradability curves for DM, N and NDF of guinea grass incubated for various periods in the rumen of cattle and buffaloes are shown in figure 1. Two-way analysis of variance with

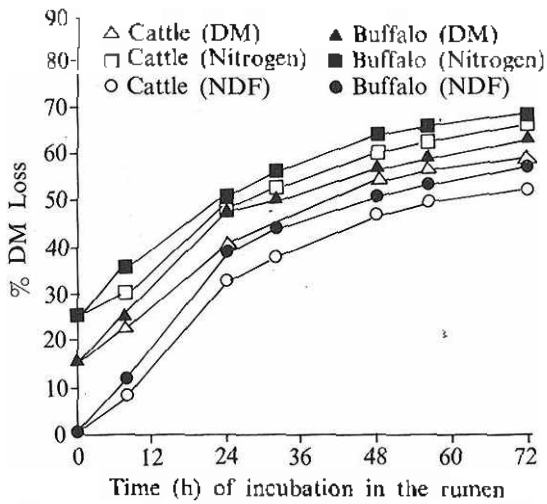

Figure 1. Percentage loss of dry matter (DM), nitrogen (N) and neutral detergent fibre (NDF) of guinea grass incubated in the rumen of cattle and buffaloes.

species and incubation times as sources of variation showed percentage losses in DM, N and NDF to be significantly ($p < 0.01$) higher in the buffaloes. Table 1 shows the degradation rates and potential degradability of each component of guinea grass (DM, N and NDF) after 48 h incubation. Buffaloes showed significantly ($p < 0.05$) faster degradation rates than cattle for each grass component. The faster rates were indicative of a more intense microbial activity possibly resulting from the ability of buffaloes to maintain higher rumen ammonia. Abdullah et al. (1990) have reported that with guinea grass diet and straw-based diet, rumen ammonia was significantly higher in buffaloes than in cattle. Other workers have also observed higher fibre digestion in buffalo when compared to cattle (Radzan et al., 1971; Grant et al., 1974; Bhatia et al., 1979; Reddy and Das, 1980; Langar et al., 1984 and Sangwan et al., 1987).

TABLE 1. DEGRADATION RATES AND POTENTIAL DEGRADABILITY (48 H) OF DM, N AND NDF COMPONENTS OF GUINEA GRASS INCUBATED IN THE RUMEN OF CATTLE AND BUFFALO

Animals	DM		N		NDF	
	Degradation (% Loss)	Rates (/h)	Degradation (% Loss)	Rates (/h)	Degradation (% Loss)	Rates (/h)
Cattle	53.7±0.8	0.032±0.005	59.4±1.7	0.024±0.002	47.1±1.0	0.037±0.005
Buffaloes	55.8±1.2	0.044±0.004	63.5±1.4	0.037±0.005	49.6±0.8	0.047±0.003
Significance (species)	$p < 0.01$	$p < 0.05$	$p < 0.01$	$p < 0.05$	$p < 0.01$	$p < 0.05$

% Potential degradation was tested by two-way analysis of variance (% loss and sampling times as sources of variation), while degradation rate was tested by t-test.
DM = dry matter; N = nitrogen; NDF = neutral detergent fibre.
The grass contained 31% DM, 14 g N and 810 g NDF per kg DM.
Each value is a mean ± S.E. of 4 samples.

Colonization of guinea grass by rumen microbes

Direct observations of incubated grass fragments by SEM and light microscopy showed that there were no apparent differences in the microbial population between the cattle and buffaloes. The colonization pattern of the grass by the rumen microbes was also very similar between the two animal species.

The predominant bacterial species adhering to the guinea grass fragments in the rumen of cattle and buffaloes were the rods, curved ones resembling *Butyrivibrio* sp. and thick ones resembling *Fibrobacter* sp.; diplococci which resembled *Ruminococcus* sp.; and the spiral bacteria, with loose or tight helical coils.

The fungi were mostly those which produced spherical, oval or fusiform sporangia. Very few protozoa were seen attached to the guinea grass fragments at all incubation times.

Bacterial and fungal colonization in both stems and leaves of guinea grass was established after 15 min of incubation in the rumen of cattle and buffalo. Attachment of bacteria was mainly on isolated areas below the epidermis, on the parenchyma cells of the stem and mesophyll cells of the leaf. The bacteria could be in mixed populations or present as pure colonies of either rods or diplococci. Attachment of fungi was by means of zoospores. Sites for attachment were the stomatal openings of the leaf, damaged surfaces and cut ends. The zoospores at the attachment sites germinated very rapidly producing rhizoids which spread into the adjacent cells.

The density of bacterial colonies, the root-like systems of fungal rhizoids and degradation of plant tissues (seen as eroded zones and cavities) increased with longer incubation times (30 min, 1, 3 and 6 h) at the thin-walled tissues (mesophyll, phloem and parenchyma). Bacteria invading these tissues proliferated until they filled the cellular compartment with a tightly-packed microcolony of bacterial cells. Very large population of bacteria, with a limited number of morphological types, also formed coherent microcolonies in the intercellular spaces. The bacterial colonies could be *en masse* (figure 2) or spread in isolated

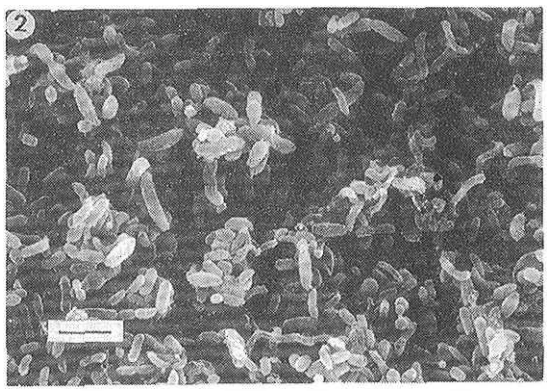

Figure 2. En masse of bacteria on a stem fragment 3 h after incubation in the rumen of buffalo. Bar = 2 μm.

colonies over the plant surface. The spirochetes were usually found together with the rods. At 3 h after rumen incubation, the fungal rhizoids not only colonized the thin-walled parenchyma and mesophyll cells but also the thick-walled

sclerenchyma and vascular tissues. Some rhizoids possessed special structures which resembled "appressoria" for penetrating cell walls. A description of the "appressoria" has been given by Ho et al. (1988a). By 6 h, the stomatal openings showed dense aggregation of mixed bacteria (figure 3). The thick-walled vascular tissues were mostly uncolonized. However, sclerenchyma and vascular tissues were extensively colonized by fungal rhizoids after 6 h and areas of degradation were apparent in the sclerenchyma tissues with profuse rhizoids.

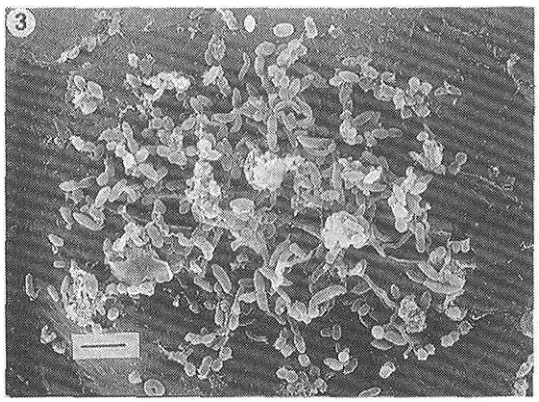

Figure 3. A mixed population of bacteria at a stomatal opening (arrow) on a leaf blade fragment 6 h after incubation in the rumen of buffalo. Bar = 2 μm.

After 24 h of rumen incubation, the cuticle layer could be easily detached from the plant fragments. In many of the leaf fragments, the thin-walled tissues were extensively degraded (figure 4) leaving mostly the thick-walled vascular cylinders which were easily detached. The stem fragments showed similar degradation pattern as the leaf at 24 h of incubation. Bacterial colonization could be observed either in small numbers or dense populations scattered over the stem surface. The bacterial population could be a mixture of rods and diplococci or homogenous colonies of rods resembling *Fibrobacter* sp. Fungal colonization was also very extensive, and breakdown and degradation of cell walls were apparent in areas with profuse rhizoids. The fungi were predominantly those with spherical or oval sporangia (figure 5). Fungi with cylindrical or fusiform sporangia with acuminate apices which resembled *Ruminomyces* sp. (Ho et al., 1990) were

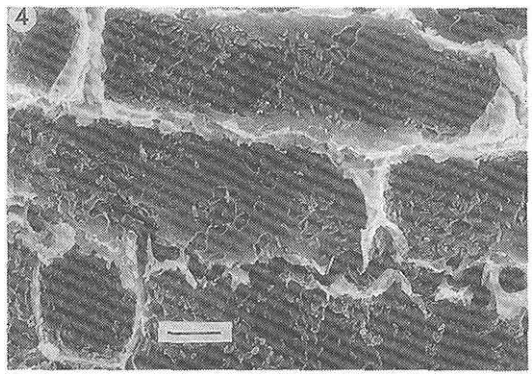

Figure 4. Extensive degradation of cell walls of a leaf blade fragment by bacteria 24 h after incubation in the rumen of buffalo. Bar = 5 μm.

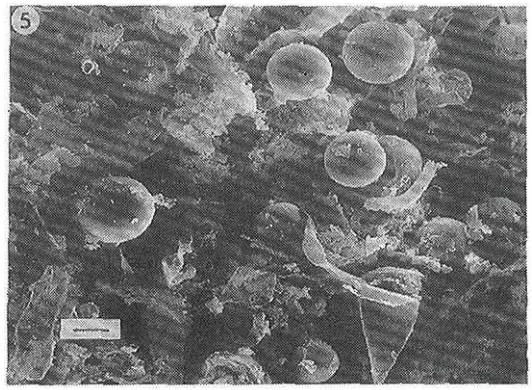

Figure 5. Fungi with spherical or oval sporangia colonizing and degrading cell walls of a leaf blade fragment 24 h after incubation in the rumen of cattle. Bar = 10 μm.

of lesser occurrence.

Grass samples at 48 h of rumen incubation showed further degradation. Most of the thin-walled cells around the vascular cylinders were digested resulting in the vascular bundles appearing as detached or semi-detached strands running parallel along the fragments. The predominant bacteria were a mixture of rods. Small cocci and spirochetes were of lesser occurrence. Fungal sporangia (spherical, oval and fusiform) were observed to be attached to the vascular cylinders which were still intact. Fusiform sporangia with acuminate apices were more abundant than at 24 h. Collapsed sporangia with pores or disintegrating walls were observed occassionally (figure 6).

These are probably the remnants of sporangia after the release of zoospores. Similar sporangial remnants have been reported by Ho et al. (1988b).

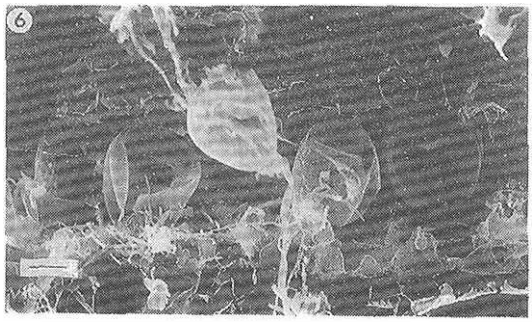

Figure 6. Collapsed sporangia with pores or disintegrating walls after the release of zoospores, 48 h after incubation in the rumen of buffalo. Bar = 10 μm.

Grass samples after 72 h of incubation in the rumen were not very much different from those at 48 h. Generally, the samples contained undigested vascular tissues and cuticle layers. Fungal sporangia were less abundant than that at 48 h. Some of the spherical sporangia were small (around 10 μm) in diameter. Bacteria observed were similar to those at 48 h.

Although the degradation rates and percentage losses in DM, N and NDF of guinea grass were significantly higher in buffaloes than in cattle, the microbial population and pattern of microbial colonization, as observed by SEM and light microscopy, were not distinctly different between the two species of animals. Hence, it is not possible, to conclude from the present study that differences in the ability to utilize fibrous feed by the two species of animals are due to differences in microbial population and digestion.

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