MICROBIAL COLONISATION AND DEGRADATION OF SOME FIBROUS CROP RESIDUES IN THE RUMEN OF GOATS

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Summary

An investigation was carried out to study the microbial colonisation and degradation of five crop residues, viz., sago waste, rice straw, oil palm trunk shavings, untreated palm press fibre and palm press fibre treated with 3% ammonium hydroxide in the rumen of goats. Colonisation by rumen bacteria and fungi was already established on all the five crop residues 8 h after incubation. However, the extent of colonisation varied among the crop residues. Microbial colonisation was poor on palm press fibre (treated and untreated) but more extensive on sago waste, oil palm trunk shavings and rice straw. By 24 h, most of the soft-walled tissues in sago waste, rice straw and oil palm trunk shavings were degraded leaving the thick-walled tissues extensively colonised by bacteria and fungi. Degradation on palm press fibre was still limited. At 48 h, the thick-walled tissues of sago waste, oil palm trunk shavings and rice straw showed various degrees of degradation - from small erosion zones to large digested areas. Bacterial growth was similar to that at 24 h but fungal growth was less. On palm press fibre, microbial colonisation was more extensive than at 24 h but degradation of the fibres was still limited. Degradation of all the five crop residues at 72 h was somewhat similar to that at 48 h. Overall, microbial colonisation and degradation were the most extensive on sago waste, followed by rice straw and oil palm trunk shavings, and the least on palm press fibre (treated and untreated). Dry matter loss of the five crop residues at the various incubation periods also showed the same order of degradation.

(**Key Words** : Goats, Rumen Fungi, Rumen Bacteria, Fibrous Crop Residues, Microbial Degradation)

Introduction

Goats in Malaysia and other Southeast Asian countries are mainly managed by small farmers for subsistence production. Under such a farming system, feeding materials are inadequate and the farmers do not have the resource nor the capacity to increase feed availability. A possible means of increasing feed resources is by utilising fibrous crop residues since goats have been reported to have a high digestive efficiency for fibrous materials (Devendra, 1977, 1978, 1991; Gihad et al., 1980; Houston et al., 1988). However, it is not known how the fibrous materials are colonised and degraded by rumen microbes. Besides the cellulolytic rumen bacteria, rumen fungi are also found to be cellulolytic and they form an integral part of the fibre-degrading microbial population in the rumen. Rumen fungi readily colonise plant tissues, including the fibrous thick-walled tissues and have been found to contribute to the overall digestion of various forages and straw (Akin et al., 1983; Gordon and Ashes, 1984; Akin and Rigsby, 1987; Ho et al., 1988, 1991). This investigation was carried out to study the microbial colonisation and degradation of five fibrous crop residues in the rumen of goats with a view to determine the potential of the crop residues as a feed resource for goats.

Materials and Methods

Animals

The animals used were four indigenous male goats

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(Kambing Katjang) (*Capra hircus*), about 3-year old, each fitted with a permanent rumen cannula and housed separately. The animals were fed once daily with guinea grass (*Panicum maximum*) *ad libitum* and had free access to water and mineral blocks.

Fibrous crop residues

The five fibrous crop residues used were palm press fibre, untreated; palm press fibre, treated with 3% ammonium hydroxide; oil palm trunk shavings; sago waste and rice straw. Palm press fibre is the residual mesocarp fibres of oil palm fruits after oil extraction. Treatment of palm press fibre with 3% ammonium hydroxide (to soften the fibres) was similar to that described by Ho et al. (1991). Oil palm trunk shavings (from cut trunks of oil palm over 30 years old) were obtained from the Malaysian Agricultural Research and Development Institute, Serdang, Malaysia. Sago waste is the residue from the sago palm (*Metroxylon sagu*) stem after starch extraction. The rice straw used was untreated.

The five fibrous crop residues were dried and then ground through a 4 mm screen in a hammer mill.

Determination of digestibility of the fibrous crop residues

In situ digestibility of each crop residue after 8, 24, 48 and 72 h of rumen incubation was determined by the nylon bag technique (Ørskov et al., 1980). The percentage of dry matter (DM) loss of the five fibrous crop residues at various incubation periods are given in table 1.

TABLE 1. PERCENTAGE DRY MATTER LOSS OF SAGO WASTE, OIL PALM TRUNK SHAVINGS, RICE STRAW AND PALM PRESS FIBRE (TREATED AND UNTREATED)

Incubation period (h)	Percentage dry matter loss (%)*				
	Sago waste	OPT	Rice straw	PPF (untreated)	PPF (3% NH_4OH)
8	26.25 ± 1.07[a]	19.81 ± 0.87[b]	17.95 ± 0.59[b]	9.62 ± 0.31[c]	9.87 ± 0.31[c]
24	39.94 ± 0.99[a]	26.02 ± 1.46[b]	25.38 ± 0.70[b]	13.61 ± 0.78[c]	18.64 ± 0.77[d]
48	56.19 ± 1.24[a]	34.42 ± 1.27[b]	42.57 ± 1.02[c]	17.81 ± 0.94[d]	25.19 ± 0.72[e]
72	60.46 ± 1.03[a]	42.39 ± 0.96[b]	47.28 ± 0.49[c]	18.70 ± 0.72[d]	25.25 ± 0.74[e]

* Values presented are the means of four replicates.
mean ± standard error; OPT, oil palm trunk shavings; PPF, palm press fibre.
Means in the same row followed by different superscripts are significantly different ($p < 0.05$).

Incubation of fibrous crop residues in the rumen

About 1 g of each crop residue was placed separately in nylon bags (90 × 60 mm, mesh size 50 μm). The methods for tying the bags and inserting them into the rumen were the same as those described by Ho et al. (1988). The bags were incubated in the rumen for 8, 24, 48 and 72 h, after which they were retrieved and washed with tap water until the washings were clear. The samples in the bags were then fixed in appropriate solutions for scanning electron microscopy (SEM) and light microscopy.

Preparation for SEM and light microscopy

Samples for SEM and light microscopy were prepared according to the methods of Ho et al. (1988). For each crop residue, at each incubation period, 20 fragments were examined using SEM (JSM 35C JEOL scanning electron microscope) and about 30 fragments were studied using light microscopy (Leitz, Aristoplan).

Statistical analysis

The percentages of DM loss of the five crop residues were analysed by analysis of variance using the SAS programme (SAS Institute Inc., Railgh, North Carolina, USA).

Results and Discussion

Microbial colonisation of all the five types of fibrous crop residues was already established 8 h after rumen incubation. However, the extent of microbial colonisation varied with the different crop residues. Fungal colonisation on palm press fibre (treated and untreated) was very limited and was mainly confined to the cut-ends, cracks and cavities formed by dislodged silica crystals. Fungal colonisation was more extensive on sago waste, oil palm trunk shavings and rice straw. On sago waste, encysted fungal zoospores and young sporangia attached predominantly on cut-surfaces, fissures between fibres and

empty cells with some residual starch. On oil palm trunk shavings, the zoospores and young sporangia were mainly attached on cut-surfaces, cavities formed by dislodged silica crystals and damaged parenchyma cells. For rice straw, fungal attachment was found mostly on stomata, cut-ends of fragments and damaged surfaces.

Bacterial colonisation was also found on these sites (figure 1), particularly on cells with residual starch in sago waste, and digestion pits or zones occurred in areas with dense population of bacteria. The morphology of bacterial colonies on all the five crop residues was quite similar and the bacteria consisted mostly of mixed rods (curved rods resembling *Butyrivibrio* sp. or thick rods resembling *Fibrobacter* sp.), cocci, diplococci (resembling *Ruminococcus* sp.) and spirochetes (figure 2). The variety of bacteria colonising the plant tissues during this time suggests that various types of substrates suitable for different bacterial species are present in the tissues. Similar forms of bacteria have also been observed colonising palm press fibre and rice straw in the rumen of cattle and water buffaloes 6 h after rumen incubation (Abdullah et al., 1992a). Encysted fungal zoospores and young sporangia were also similar on all fibrous crop residues. The manner by which the encysted zoospores attached on the residue fragments resembled those found in the rumen of cattle and buffaloes described by Ho et al. (1988). The young sporangia were mostly globose, ovoid or columnar, 10-16 × 12-20 μm. Some of the fungi, particularly those colonising the thin-walled parenchyma cells of rice straw, developed extensive hyphal system.

Figure 2. A mixture of bacteria consisting of rods (thick and curved), cocci, diplococci and spirochetes on a palm press fibre fragment (treated with 3% NH_4OH) 8 h after rumen incubation. Bar, 5 μm.

Disc-shaped appressoria with penetration pegs produced by hyphae for penetrating cell walls were not present in any of the fungi colonising the five fibrous crop residues but appressoria were commonly found in fungi colonising rice straw, guinea grass and palm press fibre in the rumen of cattle and buffaloes (Ho et al., 1988; 1991; Abdullah et al., 1992a,b).

By 24 h, microbial colonisation of sago waste (figure 3), oil palm trunk shavings and rice straw was extensive

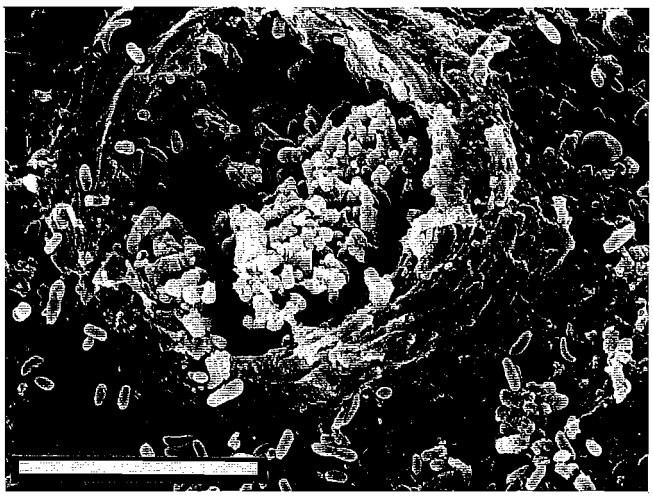

Figure 1. Bacteria colonising a cavity from dislodged silica crystals on a palm press fibre (untreated) fragment 8 h after rumen incubation. Bar, 10 μm.

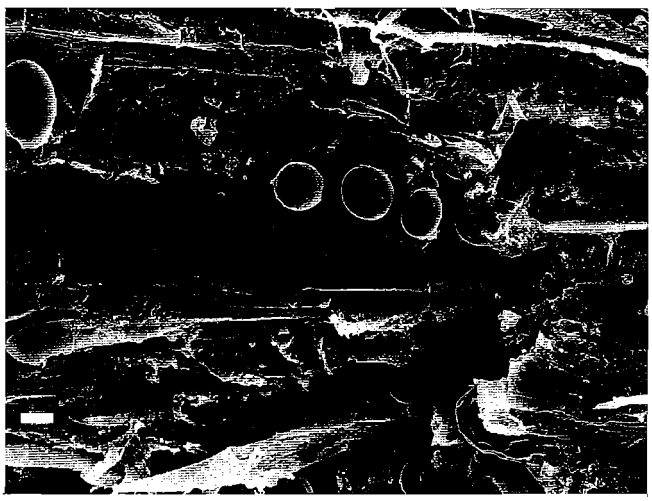

Figure 3. Sago waste fragment showing degraded tissues and fungal sporangia attached to some of the tissues 24 h after rumen incubation. Bar, 10 μm.

and degradation of tissues occurred in areas with profuse microbial colonisation. A lot of the thin-walled tissues such as the parenchyma cells (in rice straw, sago waste and oil palm trunk shavings) and mesophyll cells (in rice straw) were degraded leaving mostly the thick-walled sclerenchyma and vascular tissues which were colonised by dense bacterial colonies and extensive network of fungal hyphae (figure 4). The bacterial populations could be a mixture of rods, cocci and diplococci or homogenous colonies of curved or thick rods. The fungi were predominantly those that produced globose, pyriform or oval sporangia. The sporangia were much larger (20-40 × 30-100 μm) than those observed at 8 h. On some oil palm trunk shavings, the profuse fungal colonisation was evident by the presence of closely packed sporangia forming a sporangial mat on the tissues which were extensively degraded (figure 5). Polycentric fungi belonging to *Anaeromyces* sp. (= *Ruminomyces* sp.) which produce fusiform sporangia or sporangia with elongated apical tips were not found on any of these three crop residues. Fungi producing appressoria were also absent on all the three fibrous crop residues during this time. In contrast, polycentric *Anaeromyces* sp. and fungi with appressoria are commonly found colonising grass fragments, rice straw and palm press fibre in the rumen of cattle and buffaloes (Ho et al., 1988, 1990, 1991; Abdullah et al., 1992a,b).

Fungal colonisation of palm press fibre, both treated and untreated, at 24 h of rumen incubation was still limited mainly to cut-ends, cavities, cracks and fissures

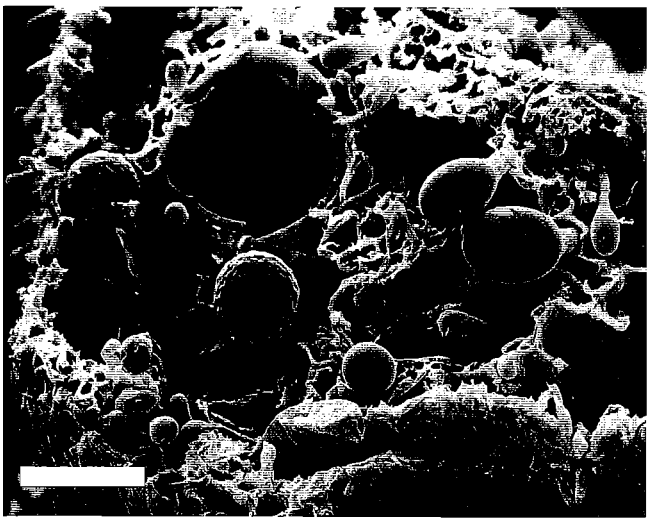

Figure 4. Rice straw fragment showing degraded thin-walled-cells and fungal colonisation on the thick-walled vascular tissues 24 h after rumen incubation. Bar, 25 μm.

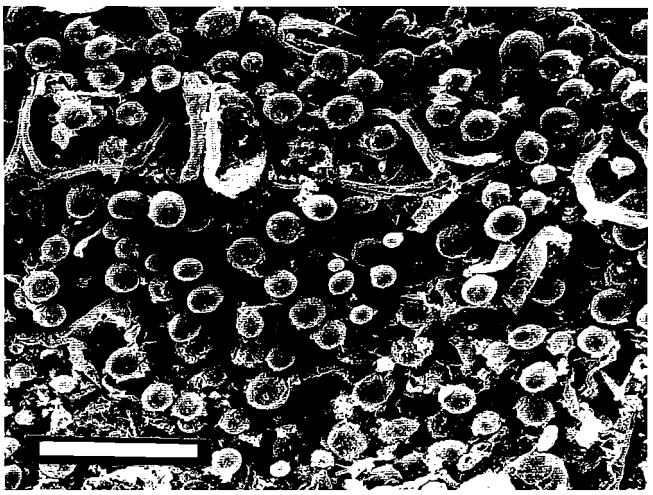

Figure 5. Profuse fungal colonisation on an oil palm trunk shaving 24 h after rumen incubation. Bar, 100 μm.

between fibres. Sporangia produced by the fungi were also globose, oval or pyriform and their sizes were similar to those found on the other crop residues mentioned above. Like the other three fibrous crop residues, palm press fibre, treated or untreated, did not show any colonisation by polycentric *Anaeromyces* sp. It is interesting to note that polycentric *Anaeromyces* sp. is not found on palm press fibre in the rumen of goats; it is particularly prevalent on palm press fibre in the rumen of cattle and buffaloes (Ho et al., 1991; Abdullah et al., 1992a). Bacterial colonisation at this time was more extensive than at 8 h, but like fungal colonisation, it was mainly confined to cavities and damaged areas. The bacteria were predominantly thick and curved rods, and some diplococci; either scattered or in clumps in the colonised sites.

At 48 h, degradation of the tissues of sago waste, oil palm trunk shavings and rice straw was more extensive than at 24 h. Most of the thin-walled tissues were already degraded and the remaining thick-walled tissues showed various degrees of degradation; from being pitted with small erosion zones only, to being fragmented by large digested areas. Although degradation of tissues was more extensive, fungal growth on sago waste and rice straw seemed to be the same as that at 24 h, but fungal growth on oil palm trunk shavings was much less, as evident by the smaller number of sporangia present. The size of sporangia on the three crop residues was smaller (8-14 × 10-30 μm) than those at 24 h and their rhizoidal system less extensive. Remnants of empty sporangia after the release of zoospores were commonly found among the

sporangia (figure 6). The presence of empty sporangia during this time is not surprising since the life cycle of anaerobic rumen fungi is in the range of 27-32 h (Orpin, 1975; Bauchop, 1980; Lowe et al., 1987). Sporangia were spherical or ovoid; fusiform sporangia of *Anaeromyces* sp. were not detected in all the three crop residues. Bacterial colonisation on the three crop residues during this period was generally the same as that at 24 h but the types of bacteria were predominantly the curved and thick rods. The rather limited variety of bacterial species found at 48 h (compared to 8 h) could probably be due to the depletion of most non-structural carbohydrates available in the fibrous crop residues and the remaining cellulosic materials were colonised mainly by the cellulolytic bacteria which were mostly the curve and thick rods.

In contrast to the other three crop residues, microbial colonisation and degradation of palm press fibre, both untreated and treated, at 48 h was more than at 24 h. Bacterial colonisation, in particular, was extensive. Some fragments were covered by a dense layer of bacteria interspersed with fungal sporangia (figure 7). The bacteria and fungi present on the fragments were morphologically similar to those present at 24 h. Collapsed empty sporangia or remains of sporangial walls after zoospore discharge were occasionally found.

The extent of degradation of all the five crop residues at 72 h was somewhat similar to that at 48 h. Bacterial colonies, mainly rods, and small fungal sporangia were observed among the degraded tissues.

Overall, among the five crop residues, sago waste showed the most amount of degradation, followed by rice straw and oil palm trunk shavings. Degradation of palm press fibre, both untreated and treated was limited. Results on the DM loss of the five crop residues at the different incubation periods (table 1) corresponded to the above order of degradation observed.

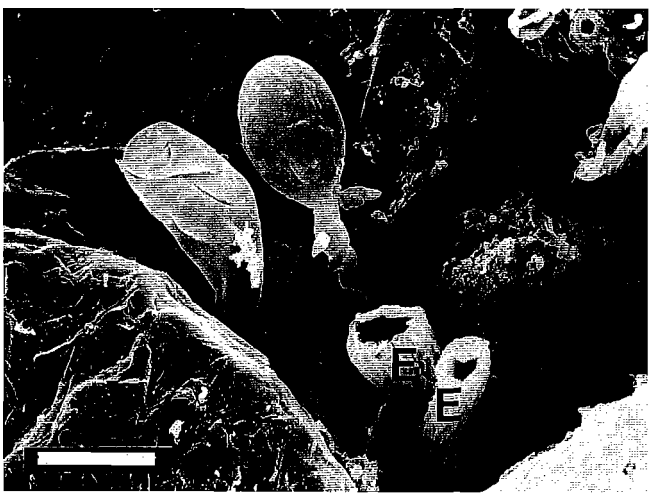

Figure 4. Rice straw fragment showing degraded thin-walled-cells and fungal colonisation on the thick-walled vascular tissues 24 h after rumen incubation. Bar, 25 μm.

Figure 7. A dense layer of bacteria interspersed with fungal sporangia on a palm press fibre (treated with 3% NH_4OH) 48 h after rumen incubation. Bar, 10 μm.

In conclusion, this study shows that, of the five fibrous crop residues studied, sago waste has the highest digestibility. It has the potential to be utilised as a source of feed for goats but this has to be confirmed with further feeding trials using sago waste as a basal diet for goats.

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