The Role of Rumen Fungi in Fibre Digestion*
- Review -

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ABSTRACT : Since the anaerobic rumen fungi were discovered in the rumen of a sheep over two decades ago, they have been reported in a wide range of herbivores fed on high fibre diets. The extensive colonisation and degradation of fibrous plant tissues by the fungi suggest that they have a role in fibre digestion. All rumen fungi studied so far are fibrolytic. They produce a range of hydrolytic enzymes, which include the cellulases, hemicellulases, pectinases and phenolic acid esterases, to enable them to invade and degrade the lignocellulosic plant tissues. Although rumen fungi may not seem to be essential to general rumen function since they may be absent in animals fed on low fibre diets, they, nevertheless, could contribute to the digestion of high-fibre poor-quality forages.
(**Key Words**: Rumen Fungi, Fiber Digestion, Colonization, Diet, Plant Cells, Review)

INTRODUCTION

Until the discovery of the anaerobic fungi in the sheep rumen by Orpin in 1975, the microbial population of the rumen was believed to be made up of bacteria and protozoa only. Since this discovery, rumen fungi have been isolated from a wide range of herbivores. The possible role of rumen fungi in fibre digestion was recognised when large numbers of fungi were observed to colonise fibrous plant materials in the rumen of sheep and cattle (Bauchop, 1979a, b, 1980). All rumen fungi isolated so far are fibrolytic and are able to degrade structural carbohydrate of plant cell walls. They show a preference for the thick-walled sclerenchyma and vascular tissues (Akin et al., 1983; Ho et al., 1988a, b, 1991) and are capable of digesting various fibrous forages and various types of fibrous crop residues (Akin et al., 1983; Gordon and Ashes, 1984; Ho et al., 1988b, 1991, 1996a, Grenet et al., 1989, Trinci et al., 1994 Ushida et al., 1997; Wuliji and McManus, 1988).

All rumen fungi that have been described are classified in five genera (Ho and Barr, 1995). They are *Neocallimastix* (Vavra and Joyon, 1966), *Piromyces* (Gold et al., 1988), *Caecomyces* (Gold et al., 1988), *Orpinomyces* (Barr et al., 1989) and *Anaeromyces* (Breton et al., 1990). The classification is based mainly on the morphology of the zoospores and thallus development of the fungi. The fungal thallus development is either monocentric or polycentric. In the monocentric development, the thallus usually develops a single sporangium and in the polycentric development, the thallus develops a number of sporangia (Ho and Barr, 1995). *Neocallimastix*, *Piromyces* and *Caecomyces* have monocentric development whereas *Anaeromyces* and *Orpinomyces* have polycentric development.

Rumen fungi possess a simple life cycle. It consists of a motile flagellated zoospore stage alternating with a non-motile vegetative and reproductive stage attached to the digesta fragments. It is during the non-motile stage that fungi colonise and degrade fibrous plant materials, thus enabling them to play a role in the digestion of fibre in the rumen.

INVASION AND COLONISATION OF FIBROUS MATERIALS

Fungal zoospores swimming freely in the rumen fluid locate freshly ingested plant fragments by chemotaxis to soluble carbohydrates diffusing from the damaged plant tissues (Orpin and Bountiff, 1978). Four chemoreceptors for carbohydrates have been identified in the zoospores of *Neocallimastix*. They are the glucose, sucrose, mannose and sorbitol receptors (Orpin and Bountiff, 1978). Zoospores of *N. frontalis*, *Piromyces communis*, *Orpinomyces*

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joyonii and *Anaeromyces* sp. also show chemotactic response to phenolic acids such as ρ-coumaric acid, ferulic acids and syringic acid which are often found in the lignified tissues of plants (Wubah and Kim, 1996). It seems that appropriate amount of concentrate may support fungal growth and stimulate zoosporogenesis in the rumen (Matsui et al., 1997).

Once the zoospore has located the plant fragment, it moves in an amoeboid manner over the tissues until it reaches a suitable site for attachment and encystment (Orpin, 1994). Sites for attachment are mainly stomata (figure 1), damaged surfaces (figure 2) and cut ends of plant fragments (Bauchop, 1979b, 1981; Akin et al., 1983; Ho, et al., 1988a, b, 1991; Orpin, 1994). The zoospores shed their flagella and attach on the tissues by means of fibrillar or non-fibrillar materials of unknown nature (Munn et al., 1981; Ho et al., 1988b). Attachment of the zoospores on plant tissues occurs very rapidly, usually within 15-30 min after rumen incubation of the tissues (Bauchop, 1981; Ho et al., 1988a, b, 1991). Soon after attachment and encystment, the zoospore germinates with a single germ-tube which penetrates the plant tissues. The germ-tube elongates and branches very rapidly forming a network of rhizoids which colonises the surrounding tissues. Rhizoids colonising vascular tissues are observed to grow to some length along the cell before branching (Ho et al., 1988b). Rhizoids with appressoria for the penetration of cell walls are sometimes observed (Ho, et al., 1988a, b). The appressoria probably assist the rapid spread of the rhizoids within the plant tissues, resulting in maximum colonisation within a short time.

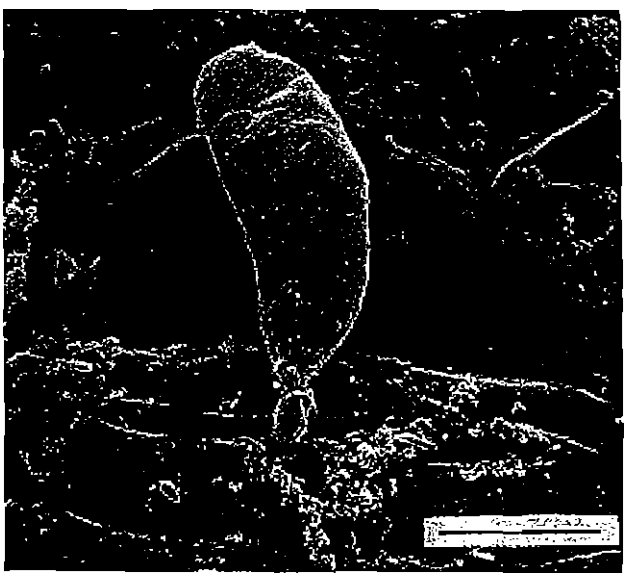

Figure 1. A germinated zoospore in a stoma of guinea grass from the rumen of buffalo 30 min after incubation. Bar = 5 μm.

Figure 2. An attached zoospore on a damaged parenchyma cell of a plant fragment in the rumen of a goat. Bar = 2 μm.

In colonising plant tissues, anaerobic rumen fungi show a preference for lignified tissues. *In vivo* studies of grass fragments in nylon bags suspended in the rumen of cattle and buffalo showed that by 6h lignified tissues such as sclerenchyma and vascular tissues are extensively colonised (Ho et al., 1988b). *In vitro* incubation of the forage *Digitaria pentzii* with rumen fungi also showed colonisation of sclerenchyma by the fungi and penetration of cell walls by rhizoids after 6h of incubation (Akin et al., 1983). Large numbers of fungi have also been observed to colonise the vascular cylinders of lucerne stems and the mesophyll of wheat straw leaf in the rumen of sheep after 2-3 h of incubation (Bauchop, 1979a, b).

Fungal colonisation of plant fragments is very extensive 24 h after rumen incubation (figures 3 and 4). Most of the colonised thin-walled tissues such as the mesophyll, parenchyma and phloem are degraded leaving behind mostly lignified thick-walled tissues such as sclerenchyma and vascular cylinders heavily colonised by fungi (Bauchop, 1979a, b; Akin et al., 1983; Akin, 1989; Ho et al., 1988b, 1991, 1996a). Sections of these lignified tissues showed the cell lumina filled with rhizoids. In those cells with pits (sclerenchyma and vascular fibres), rhizoids invade and colonise the cells not only through direct penetration of the cell walls but also through the pits in the cell walls. Although considerable amount of lignified tissues is left, disruption of cell wall structure and weakening and loosening of the tissues occur in areas with profuse colonisation of fungal rhizoids.

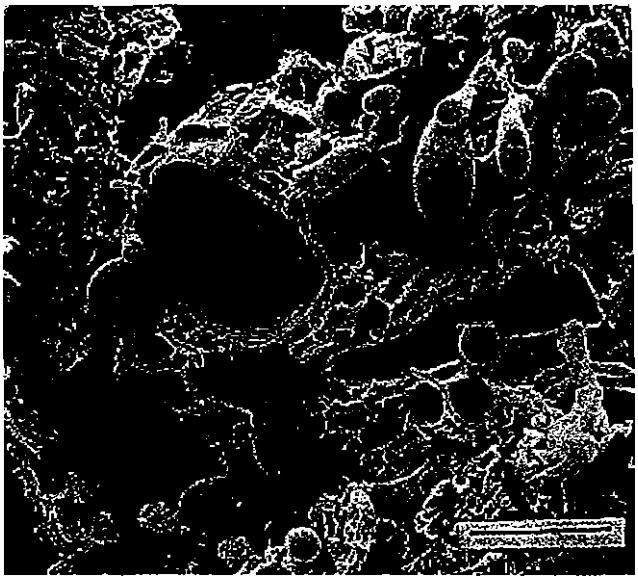

Figure 3. Fungi colonising the thick-walled vascular tissues of a rice straw fragment 24h after incubation in the rumen of a goat. Most of the thin-walled cells have been degraded. Bar = 25 μm.

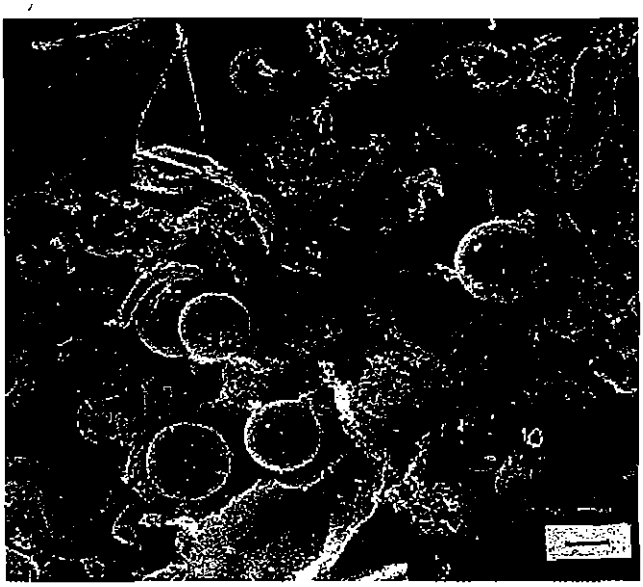

Figure 4. Fungi colonising and degrading a leaf blade of guinea grass 24h after rumen incubation in a cattle. Bar = 10 μm.

The partial degradation of the lignified cell walls, even without apparent removal of the tissues, weakens the plant fragment (Akin et al., 1990b). Tensile strength of stems and leaf blades of various forages has been found to be reduced after fungal colonisation (Akin et al., 1983, 1989, 1990b). Stems of bermuda grass and alfafa incubated with rumen fungi are weakened by 57% and 55%, respectively, whereas those incubated with rumen bacteria are only weakened by 29% and 42%, respectively (Akin et al., 1990b). The dry weight loss of various types of forages by rumen fungi ranged from about 16%-57% (table 1).

Fungal rhizoids degrade plant tissues by the production of extracellular enzymes and the middle lamella is observed to be the last layer to be degraded (Akin et al., 1990a). Compared to rumen bacteria, rumen fungi are able to degrade sclerenchyma walls to a greater extent. The fungal rhizoids could penetrate deep into the recalcitrant tissues and digest the cell walls by means of enzymes, resulting in complete degradation of the sclerenchyma walls. On the other hand, rumen bacteria which are attached to the wall surface could only degrade the peripheral areas, resulting in slight to moderate digestion of the cell walls (Akin, 1994). Calculations of remaining tissue areas after digestion of plant fragments with rumen fungi or bacteria showed that rumen fungi digest significantly more sclerenchyma tissues than bacteria (Akin, 1994). Fibre loss due to the fungi was found to be higher than that due to bacteria at 24 and 48h of incubation (Akin and Rigsby, 1987). Non-filamentous rumen fungi such as *Caecomyces* are able to disrupt and fibrillate plant tissues by the expansion of their spherical or bulbous rhizoids in the plant cell (Joblin, 1989). The ability of rumen fungi to digest or weaken recalcitrant tissues to a larger extent than rumen bacteria indicates that they could play a major role in the physical weakening of fibrous feeds and aid in particle breakdown during rumination.

EFFECTS OF DIET ON FUNGAL POPULATIONS

Despite the absence of an accurate method of measuring fungal biomass in the rumen, comparisons of fungal populations in animals fed different diets have been made. It has been observed that some diets are more conducive to the growth and development of the fungi. In general, diets that are high in fibre content support a higher population of rumen fungi.

Large populations of fungi occur in animals fed stalky diets but they occur in low numbers or are absent when the animals are fed soft leafy diets (Bauchop, 1979a, b), high starch diets (Grenet et al., 1989), high sucrose or high lactose diets (Grenet et al., 1989). Since rumen fungi preferentially colonise thick-walled lignified tissues, diets that comprise large amounts of fibrous materials are favourable to their growth. Furthermore, as the life cycle of rumen fungi is about 24-32 h, (Bauchop, 1981; Lowe et al., 1987a), diets containing lignified tissues with long retention time will ensure the survival of the fungi. In

Table 1. Dry Weight Loss of Intact Forages by Rumen Fungi

Plant	Incubated with mixed rumen fungi[a]		Source
	Plant Part	% Dry Weight loss[b]	
Warm-season grasses			
Cynodon dactylon (Bermuda grass)	Blade	37.6	Akin (1994)
	Sheath	38.5	Akin (1994)
	Stem	31.4	Akin (1994)
Sorghum bicolor (sorghum)	Blade	36.5	Akin (1994)
	Sheath	36.3	Akin (1994)
	Stem	25.7	Akin (1994)
Cool-season grasses	Blade	28.6	Akin (1994)
Phalaris arundinacea (reed canary-grass)	Sheath	40.0	Akin (1994)
	Stem	45.2	Akin (1994)
Festuca arundinacea (tall fescue)	Blade	44.5	Akin (1994)
	Sheath	48.2	Akin (1994)
Legumes			
Medicago sativa (lucerne)	Leaves	14.7	Akin (1994)
	Stems	25.6	Akin (1994)
Lespedeza cuneata (sericea lespedeza)	Leaves	0.9	Akin (1994)
	Stems	46.0	Akin (1994)

Fungus	Bermuda grass incubated with pure rumen fungal culture[c]		Source
	Plant Part	% Dry Weight loss[b]	
Monocentric			
Piromyces MC-1	Blade	29.5	Akin et al. (1990a)
	Stem	15.7	Akin et al. (1990a)
Neocallimastix MC-2	Blade	67.8	Akin et al. (1990a)
	Stem	20.6	Akin et al. (1990a)
Polycentric	Blade	42.5	Akin et al. (1990a)
PC-1 (probably *Anaeromyces*)	Stem	16.2	Akin et al. (1990a)
Orpinomyces PC-2	Blade	56.0	Akin et al. (1990a)
	Stem	23.8	Akin et al. (1990a)
Orpinomyces PC-3	Blade	57.2	Akin et al. (1990a)
	Stem	33.6	Akin et al. (1990a)

[a] Incubated 72 hours with rumen fluid plus streptomycin and penicillin.
[b] Values are corrected for acid-pepsin-soluble materials and for loss in uninoculated controls.
[c] Incubated 72 hours in basal medium.

starch-, sucrose- and lactose-rich diets, the rapid fermentation of carbohydrates lowers the rumen pH to below 5.5 (Grenet et al., 1989) which will decrease zoospore production (Orpin, 1977). Large quantities of soluble carbohydrates in the rumen may also prevent the attachment and germination of zoospores on plant fragments (Orpin and Bountiff, 1978).

The nutritional status of the diet may also exert an effect on the population of fungi in the rumen. The fungi are abundant in sheep fed a diet of sulphur-fertilised forage but are found in low numbers or are absent in sheep fed sulphur-deficient forage (Akin et al., 1983). The

ingestion of sulphur-fertilised forage by the animal probably stimulates the growth of the fungi in the rumen. *In vitro* studies of *N. patriciarum* cultures showed that the fungus requires sulphur for growth (Orpin and Greenwood, 1986).

FERMENTATION OF PLANT CELLS

All the strains of rumen fungi so far examined are capable of degrading structural carbohydrates of plant cell walls. The fungi produce a range of hydrolytic enzymes to utilise these substrates. The cellulolytic activity of rumen fungi was first demonstrated by Bauchop (1979b) and Orpin and Letcher (1979) who showed that the fungi were able to colonise and digest filter paper. Bauchop and Mountfort (1981) later reported that *N. frontalis* was able to ferment cellulose producing acetate, formate, lactate, ethanol, carbon dioxide and hydrogen as end-products. Studies on other species of rumen fungi, such as *N. hurleyensis* (Lowe et al., 1987b), *Caecomyces* sp., *Piromyces* sp. and *Neocallimastix* sp. (Phillips and Gordon, 1989), and various other monocentric and polycentric rumen fungi (Borneman et al., 1989), grown on glucose and cellulose showed similar end-products. In contrast, *N. patriciarum* produces only acetate, lactate, hydrogen and carbon dioxide (Orpin, 1978; Orpin and Munn, 1986), and *P. spiralis*, *P. minutus* and *N. frontalis* (= *N. variabilis*, Ho et al., 1993) from Malaysia produce mainly acetate and formate, and lactate is not detected (Ho et al., 1996b).

Hydrogen production is a common feature of all anaerobic rumen fungal fermentation. The formation of hydrogen is localised in microbodies designated as hydrogenosomes (Heath et al., 1983; Yarlett et al., 1986). The hydrogen production of rumen fungi makes it possible to coculture them with other microbes which utilise hydrogen. In cocultures with methanogenic bacteria, there is a shift in the major fermentation end-products. Formate is produced in trace amounts only, ethanol and lactate are reduced, but acetate is increased and hydrogen is not detected (Bauchop and Mountfort, 1981; Mountfort et al., 1982; Mountfort and Asher, 1985; Joblin et al., 1990; Marvin-Sikkema et al., 1990). The rate and extent of cellulose and xylan fermentation are also increased in coculture and triculture with methanogens (Bauchop and Mountfort, 1981; Mountfort et al., 1982; Joblin et al., 1990; Marvin-Sikkema et al., 1990; Bernalier et al., 1991) or with non-methanogenic bacteria such as *Selenomonas ruminantium*, *Succinivibrio dextrinosolvens* and *Bacteroides ruminicola* (Marvin-Sikkema et al., 1990; Williams et al., 1991; Bernalier et al., 1991). The increase in cellulose fermentation by the rumen fungi in the presence of hydrogen-utilising microbes is probably attributed to the removal of electron-sink products, such as lactate and ethanol, which are inhibitory to fungal growth and development (Mountfort et al., 1982).

Rumen fungi produce cellulases that consist of a complex of enzymes capable of solubilising both the amorphous and crystalline cellulose present in plant tissues. Large amounts of endo-1,4-β-D glucanase (CMCase) have been observed to be released into the culture supernatants by *N. frontalis* (Mountfort and Asher, 1985; Wood et al., 1986), *N. patriciarum*, *P. communis* (William and Orpin, 1987), *P. spiralis*, *P. minutus* (Ho et al., 1996b) and *Anaeromyces elegans* (= *Ruminomyces elegans*, Ho et al., 1990) (Abdullah et al., 1990). Exo-1,4-β-D-glucanase (cellobiohydrolases) which can degrade crystalline cellulose is also produced but in lesser amount than endoglucanase (Mountford and Asher, 1985). Excretion of β 1,3-glueanase by pironyces communis with a possible role for autolysis has been reported by sakurada et al. (1996).

Rumen fungi are among the few fungi which can degrade crystalline cellulose (Pearce and Bauchop, 1985; Hebraud and Fevre, 1988). Wood et al., (1986) found that the cellulase of *N. frontalis* cocultured with methanogenic bacteria was capable of solubilising cotton fibre and avicel and it was more active than the cellulase produced by *Trichoderma reesei* which is considered to be the most active cellulase known. It has been suggested that the cellobiohydrolases act synergistically with endo-1,4-β-D-glucanase and β-D-glucosidase enzymes to solubilise the highly ordered crystalline cellulose (Wood et al., 1986, 1987).

Cellulases of rumen fungi are probably partially constitutive and partially inducible. Considerable amounts of cellulases are produced when the fungi are grown in soluble sugar substrates but much larger amounts are produced when the fungi are grown on cellulosic substrates (Mountfort and Asher, 1985; William and Orpin, 1987; Barichievich and Calza, 1990).

The ability of rumen fungi to degrade fibrous plant tissues indicates that they produce the necessary enzymes for the breakdown of hemicellulose. The xylanases (hemicellulases) of rumen fungi, which include β-xylanase, β-xylosidase and xylobiase, have been studied by many workers (Orpin and Letcher, 1979; Pearce and Bauchop, 1985; William and Orpin, 1987; Lowe et al., 1987c; Hebraud and Fevre, 1988; Mountfort and Asher, 1989; Teunissen et al., 1991; Ho et al., 1996b). The xylanases are probably partially constitutive and partially inducible because low levels of activity are produce when

the fungi are grown on various soluble sugar substrates (William and Orpin, 1987; Lowe et al., 1987c; Mountfort and Asher, 1989) and much higher levels of activity occur when the fungi are grown on xylan (William and Orpin, 1987; Mountfort and Asher, 1989).

The findings that the cellulases and xylanases of rumen fungi are partially inducible and partially constitutive are later supported by the genetic studies on *N. patriciarum* by Xue et al., (1992a, b). They found that the expression of some celulase genes is inducible by the presence of cellulose, but the expression of other genes is constitutive.

Amylases are also produced by the rumen fungi (Pearce and Bauchop, 1985; William and Orpin, 1987; Mountfort and Asher, 1988; Phillips and Gordon, 1988). Similar to the cellulases and xylanases, amylases are also partially constitutive and partially inducible. Some amylase activities are detected in fungal cultures grown on cellulose, xylan and a variety of soluble sugars (William and Orpin, 1987; Mountfort and Asher, 1988) but much higher enzyme activities occur in cultures grown on starch. Mountfort and Asher (1988) found that in the presence of high concentration of starch, α-amylase production by *N. frontalis* was reduced with a corresponding accumulation of glucose. This demonstrates that α-amylase production is suppressed with increased glucose levels.

Although rumen fungi have been observed to preferentially colonise lignified thick-walled sclerenchyma and vascular tissues (Bauchop, 1979b; Akin et al., 1983, 1990; Akin and Rigsby, 1987; Grenet and Barry, 1988; Ho et al., 1988b, 1991, 1996a), they are unable to utilise the lignin moiety (Akin dan Benner, 1988; Gordon and Phillips, 1989). The lignin loss reported in some studies (Orpin, 1981; Akin and Benner, 1988) is probably due to solubilisation of lignin from the plant cell wall (Akin and Benner, 1988). Borneman et al., (1990, 1991, 1992) found that rumen fungi (both monocentric and polycentric) produced esterases (feruloyl and ρ-coumaroyl esterases) capable of breaking linkages and releasing the phenolic acids (ferulic and coumaric acids) from lignified cell walls. This probably enables the fungi to colonise and penetrate lignified plant tissues.

Rumen fungi utilise a wide range of carbohydrates. All rumen fungi are able to utilise glucose, cellobiose, lactose and xylan for growth but they are unable to utilise arabinose (Orpin and Letcher, 1979; Bauchop and Mountfort, 1981; Mountfort and Asher, 1983; Lowe et al., 1978b; Williams and Orpin, 1987; Phillips and Gordon, 1988; Gordon and Phillips, 1989; Breton et al., 1989, 1990; Barichievich and Calza, 1990). Fungi which are able to utilise maltose, without fail, will also utilise starch (Phillips and Gordon, 1988). Very few of the anaerobic fungi can utilise galactose, ribose, mannose, rhamnose, trehalose and melezitose. It is not known why most anaerobic fungi are unable to utilise carbohydrates such as galactose, mannose, ribose and arabinose, all of which are commonly found as essential constituents of plant cells.

Almost all the rumen fungi studied are unable to utilise pectin or polygalacturonate (Gordon and Phillips, 1989; Theodorou et al., 1992). However, high pectinolytic activity has been found in a Neocallimastix isolate and an Orpinomyces isolate and the pectin degradation by the Neocallimastix isolate is probably catalysed by an endo-acting pectin lyase (Gordon and Phillips, 1991, 1992).

CONCLUSION

Anaerobic rumen fungi may not seem to be important to rumen function by itself since they are absent or occur in very small number when the host animal is fed on low-fibre diet, but the widespread colonisation of fibrous plant materials by the fungi indicates that they play a role in fibre digestion and in the digestion of high-fibre poor-quality forages. The cellulases, hemicellulases and phenolic acid esterases produced by the fungi enable them to invade and degrade structural carbohydrates in lignified plant tissues. The fungi are considered initial colonisers of lignocellulose digestion. In tropical regions where most of the forages are fibrous and poor quality, the development of methods to manipulate the fungi in the rumen could offer a means of improving feed efficiency in ruminants fed high-fibre poor quality feeds.

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