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Rumen anaerobic fungi and fibre degradation: An overview

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Abstract
The rumen is a fermentative chamber that is the place to a variety of microorganisms, including bacteria, fungi, protozoa, archaea, bacteriophage and Oscillospira. The rumen fungi are obligate anaerobes, zoospore producers, and extremely molecular oxygen sensitive. Rumen fungi is one of the rumen microorganisms that can break down fibre because it produces extremely potent lignocellulose-degrading enzymes. The number of flagella in zoospores, thallus morphology and rhizoid type are used for classifying rumen fungi into several genera. Fungi can produce rhizoid, which can penetrate feed particles and physically and chemically leads to breakdown of plant cell walls thus, the presence of rumen fungi is crucial. The degradability of feed particles is also increased by the production of fiber-degrading enzymes by rumen fungi, including cellulase, hemicellulase and pectinase. So, this review paper aims to discuss the potential of rumen anaerobic fungi for improving fiber digestibility in livestock.

Keywords: Rumen, anaerobic fungi, fibre degradation, lignocellulose bond

Introduction
Rumen is the first and largest ruminant stomach compartment, followed by reticulum, omasum, and abomasum. Microbial fermentation is the primary distinction between ruminant and non-ruminant animals. Rumen is considered as a chamber for fermentation (Aschenbach *et al*., 2011) [1]. Bacteria, Bacteriophages, Protozoa, Fungi and Oscillospira make up the majority of the rumen microbiota. To ferment and digest the nutrients in feed, the rumen microbial ecosystem performs many functions such as proteolytic, fibrolytic and lipolytic activities. The normal population of rumen bacteria is 10^{10-11} numbers/g, protozoa is 10^{4-6} numbers/g, archaea is 10^{7-8} numbers/g, fungi is 10^{3-5} numbers/g and bacteriophage are 10^{8-9} numbers/g rumen content (Kumar, 2015) [10]. Ruminants' primary source of nutrition is forage. Low feed quality is one of the major constraints in increasing ruminant businesses, particularly during the dry season. Feed with a high fibre content reduces digestion and reduces livestock production. Rumen microbes are one of the elements that may influence livestock performance in fibre degradation because of the presence of cellulolytic microorganisms such as bacteria and fungi. Rumen anaerobic fungi are crucial because they produce extremely active enzymes that breakdown lignocellulose linkages. Furthermore, fungi have the potential to break down and penetrate fibre particles via mycelium growth, increasing the surface area for the action of other bacteria for enzymatic degradation (Paul *et al*., 2004) [16].

Rumen anaerobic fungi: Discovery, taxonomy, identification and isolation techniques
Discovery of fungi
Rumen anaerobic fungi were identified extremely late since they had previously been considered to be flagellate zoospores. Orpin had originally identified anaerobic rumen fungi in sheep and named them *Neocallimastix.* Flagellate zoospores were true anaerobic rumen fungi. (Orpin, 1975) [14]. Rumen fungi are anaerobic, form zoospores, have chitin in their cell walls, colonize plant cell walls and are extremely sensitive to molecular oxygen (Orpin, 1977) [15]. Rumen anaerobic fungi establish spontaneously in the rumen within the first two weeks after birth and reach adult ruminant levels by 6-8 weeks.

The infestation of fungi in rumen through different routes

- Direct mouth-to-mouth contact between young animals and adults during licking and grooming.
- Grazing in the same pasture or feeding from the same manger.
- By aerial contamination with rumen fluid aerosol.
- Because the spore can persist in dried faeces, aerial contamination via dust and soil is possible.

Taxonomy: The anaerobic rumen fungi's unusual morphology and physiology have raised some taxonomic uncertainties. Rumen anaerobic fungi are classified as part of the Phylum *Neocallimastigomycota*, which contains eight genera of rumen fungi (*Neocallimastix* sp., *Piromyces* sp., *Caecomyces* sp., *Anaeromyces* sp., *Orpinomyces* sp., *Cyllamyces* sp., *Oontomyces* sp., *Buwchfawromyces* sp.) (Dollhofer *et al*., 2015) [5]. This is given below in table 1.

Table 1: Classification of rumen anaerobic fungi

Genus	Species	Isolated from
Neocallimastix	*N. Frontalis*	Sheep
	N. Patriciarum	Sheep
	N. Hurleyensis	Sheep
	N. Variabilis	Cow
Piromyces	*P. Communis*	Sheep
	P. Mae	Horse
	P. Dumbonica	Elephant
	P. Rhizinflata	Ass
	P. Minutus	Deer
	P. Spiralis	Goat
	P. Citronii	Horse
Caecomyces	*C. Communis*	Sheep
	C. Equi	Horse
	C. sympodialis	Cow
Anaeromyces	*A. Elegans*	Cow
	A. Mucronatus	Sheep
Orpinomyces	*O. Joyonii*	Sheep
	O. Bovis	Cow
	O. Intercalaris	Cow
Cyllamyces	*C. Aberensis*	Cow
Oontomyces	*O. anksri*	Camel
Buwchfawromyces	*B. eastonii*	Buffalo

Identification

Genera of anaerobic rumen fungi were identified primarily based on the number of flagella per zoospore, rhizomycelium structure and growth patterns of the rumen fungi (Nagpal *et al*., 2011) [13]. Fungi are divided into monoflagellate and polyflagellate groups based on the number of flagella per zoospore. Fungi are divided into filamentous and bulbous types based on the structure of their rhizomycelium. Fungi are categorized as either monocentric or polycentric depending on their growth pattern. Morphological variations in each genus and species of rumen fungi make morphological and phenotypic categorization challenging, hence molecular analysis might be employed to determine the genus of rumen fungi. This is given below in table 2.

Table 2: Morphological characteristics

Genera	Zoospores	Mycelium	Growth pattern
Neocallimastix	Polyflagellate	Filamentous	Monocentric
Cyllamyces	Monoflagellate	Filamentous	Monocentric
Orpinomyces	Monoflagellate	Bulbous	Monocentric
Piromyces	Monoflagellate	Filamentous	Polycentric
Caecomyces	Polyflagellate	Filamentous	Polycentric
Anaeromyces	Monoflagellate	Bulbous	Polycentric
Oontomyces	Monoflagellate	Filamentous	Monocentric
Buwchfawromyces	Monoflagellate	Filamentous	Monocentric

Isolation techniques

Plate culture technique

For the first time, Orpin isolated N. frontalis from a fistulated sheep rumen in 1975 using this technique by overlaying sloppy agar medium (Molten agar) with no enrichment culture. This method was combined with enrichment medium by Lowe *et al.* in 1985 [11] to isolate rumen fungi. They generated enrichment media in molten agar using three subcultures of strained rumen fluid.

Roll bottle technique

Direct isolation of anaerobic microorganisms from the rumen was successfully carried out using this method. By using this method, it is possible to count fungal zoospores and separate fungal genus from rumen fluid without enrichment. Roll tubes kept at 39 °C can be used to sustain cultures for a long time (Joblin, 1981) [9]. The isolation medium is supplemented with Penicillin, Streptomycin, Neomycin and Chloramphenicol to inhibit bacterial growth.

Mechanism of action

After the feed enters the rumen, the zoospores begin to be released from the sporangium 15 to 30 minutes later. Zoospores aggressively swim towards newly ingested plant tissues, employing flagella to respond chemotactically to soluble sugars and phenolic acids (Wubah and Kim, 1996) [25].

Flagellate zoospores travel across plant tissue in an ameboid fashion until they find an appropriate location for attachment. For attachment, zoospores typically favour cut ends, stomata and damaged tissues. Using appressorium-like penetration structures, the rhizoidal system enters plant tissue by a combination of enzyme activity and hydrostatic pressure (Ho and Bauchop, 1991) [8]. The expanding holdfast developed inside the substrate that causes breaking of the plant fibre in non-rhizoidal bulbous species. Following attachment, fungi cause the physical breakdown of feed particles, increasing their surface area and helping other microorganisms. In addition, fungi also produce the various fibrolytic exogenous enzymes like, cellulases (Endoglucanase, Exoglucanase, β-glucosidase), hemicellulase (Xylanase), pectinases (Endocellular pectin lyase, Polygalacturonase), esterases (Acetyl esterase, Feruloyl esterase, p-coumaroyl esterase) and protease (Metalloprotease) which leads to enzymatic degradation of feed particles.

Effect of diet or rumen environment on fungal population

Diet composition

The dietary fibre or lignocellulose content is an important element in determining the existence of ruminal populations of anaerobic fungi. The population of rumen anaerobic fungi grows in response to a high fibre diet. The fungal population is reduced by feeding a high starch and water-soluble carbohydrate diet (Bauchop, 1979) [2]. Lactate is the end product of anaerobic starch fermentation, which lowers rumen pH and as a result, suppresses the fungal population. Because of fungal protein synthesis, Sulphur-fertilized grass or a diet high in methionine enhances the fungal population (Gordon *et al*., 1983) [7].

Feed Pre-treatment

The most used alkali for treating feed are sodium hydroxide and ammonium hydroxide. Because it enhances the fungal counts in the rumen, ammoniating straw increases the dry matter digestibility of the feed (Romulo *et al*., 1986) [17].

Defaunation

The process of removing ciliate protozoa from the rumen is known as defaunation. Dioctyl sodium sulphosuccinate is the defaunation agent that is most frequently employed. Defaunation of animals increases the microbial biomass of rumen fungus, which is associated with an increase in the decomposition of low-quality forage and low-nitrogen herbage (Soetanto *et al*., 1985) [22].

Antifungals

Polyoxin is the most widely used antifungal. Polyoxin works as a chitin synthesis inhibitor, which prevents the development of fungal cell walls and lowers the number of rumen fungi (Cann *et al*., 1993) [3].

Ionophores

Ruminal fermentation has been discovered to be modified by ionophores. Ionophores have been found to significantly slow ruminal fungi's growth and metabolism (Stewart *et al*., 1987) [23]. Monensin, Salinomycin and Tetronasin are the three most often employed ionophores.

Feeding frequency: The population of fungi is increased by feeding them a high-fiber diet, such as hay or straw, two to three times each day. While feeding significant amounts of grain two to three times each day, the rumen's fungal population declines (Gordon, 1985) [6].

Table 3: Meta analysis of supplementation of rumen fungi on digestibility, performance parameters and rumen fermentation pattern:

Sr. no.	Fungi species	Experimental animal	Results	Scientist/ Researcher
1	*Orpinomyces* sp.	Holstein calves	Increased digestibility of dry matter, CF, CP, NDF and ADF. Increased the production of TVFA, total-N and TCA-N. FCR and DWG were numerically improved.	Dey *et al.* (2004) [4]
2	*Orpinomyces* sp. C-14 and *Piromyces* sp. WNG-12	Buffalo calves	Digestibility of CF, CP, NDF and ADF were significantly increased. FCR and DWG were significantly improved. pH and NH_3-N were significantly decreased whereas, TVFA, total-N and TCA-N were significantly increased.	Tripathi *et al.* (2007) [24]
3	*Neocallimastix* sp. GR-1	Murrah buffalo calves	Digestibility of DM, CF, CP, NDF and ADF were significantly increased. pH and NH_3-N were significantly decreased whereas, TVFA, total-N and TCA-N were significantly increased. The feed efficiency (%) and daily weight gain (kg/d) were significantly improved.	Sehgal *et al.* (2008) [19]
4	*Orpinomyces* sp. C-14 and *Piromyces* sp. WNG-12	Lactating Murrah buffalo	Digestibility of DM, cellulose, NDF and ADF were significantly increased. TVFA, total-N and TCA-N were significantly increased whereas. The milk yield (kg/d) and 6% FCM (kg/d) were significantly increased	Saxena *et al.* (2010) [18]
5	*Orpinomyces joyonii*	Murrah buffalo	Digestibility of DM, CF, CP, NDF and ADF were significantly increased. The NH_3-N was significantly decreased whereas, TVFA, total-N and TCA-N were significantly increased.	Sehgal *et al.* (2012) [20]
6	*Neocallimastix* sp. GR-1	Murrah calves	Increased digestibility of dry matter, CF, CP, NDF and ADF. FCR and DWG were significantly improved. TVFA, total-N and TCA-N were significantly increased whereas, pH and NH_3-N were significantly decreased.	Kumar *et al.* (2015) [10]
7	*Orpinomyces* sp. B-16 and *Piromyces* sp. C-15	Nil	*in vitro* degradability of dry matter and disappearance of NDF and ADF were significantly increased.	Manikumar *et al.* (2004) [12]
8	*Orpinomyces* sp. C-14 and *Anaeromyces* sp. B-6	Nil	*in vitro* degradability of dry matter and disappearance of NDF and ADF were significantly increased.	Manikumar *et al.* (2004) [12]
9	*Neocallimastix* sp. GR-1	Nil	IVDMD, IVNDFD and IVADFD were significantly increased	Shelke *et al.* (2009) [21]

Note: ADF- acid detergent fibre, CF- crude fibre, CP- crude protein, DM- dry matter, DWG- daily weight gain, FCR- feed conversion ratio, FCM- fat corrected milk, IVADFD- *in vitro* acid detergent fibre degradability. IVDMD- *in vitro* dry matter degradability, IVNDFD- *in vitro* neutral detergent fibre degradability, NDF- neutral detergent fibre, NH_3-N- ammonia nitrogen, TVFA- total volatile fatty acids.

Conclusions

Naturally occurring rumen anaerobic fungi increase the digestion of fibrous material. Comparatively speaking, fungi are more effective at degrading lignin than other microorganisms. The digestibility of cellulose and hemicellulose in low-quality roughages is increased by rumen anaerobic fungus. Rumen anaerobic fungus increase body weight gain and feed conversion ratio. Additionally, they play a significant function in raising milk production. Fungi increase the production of total volatile fatty acids (TVFA), total nitrogen (TN) and TCA precipitable nitrogen (TCA-N).

Future prospects

There is a need to study the fungal flora of the rumen micro environment of different domestic and wild ruminants. Study of interaction of fungi with other rumen microbes. Efforts should be made to enhance the fungal population in the rumen for better fibre degradation.

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