Animals' little helpers

To Ingest and to Digest

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Ruminants ingest plants, but they in fact derive the main source of the nutrients they truly utilize from microorganisms inhabiting the first chamber of their complex stomachs

Many vertebrate species feed on grass or leaves, but paradoxically, none of them produce enzymes enabling them to digest large quantities of plant polysaccharides, such as cellulose or hemicellulose. This also holds true for ruminants, a group of herbivores consisting of about 160 species. Over more than 60 million years of evolution, mammals have developed mechanisms that enable them to successfully overcome this inability. In the case of ruminants, the most important such mechanism is a close-knit symbiosis with anaerobic microorganisms that inhabit the rumen – the first compartment of their four-chamber stomach. The weight of rumen content can even exceed 100 kg in cows, or 15 kg in sheep. This *digesta* is inhabited by bacteria, fungi and protozoa that synthesize all types of digestive enzymes. They convert dietary protein to their own protein, of much higher quality than that ingested by the host. It is very important for the host animal that microorganisms are continuously passing down from the rumen to the small intestine. Their protein is digested there, while the amino acids released are absorbed and utilized by the host. Dietary carbohydrates, on the

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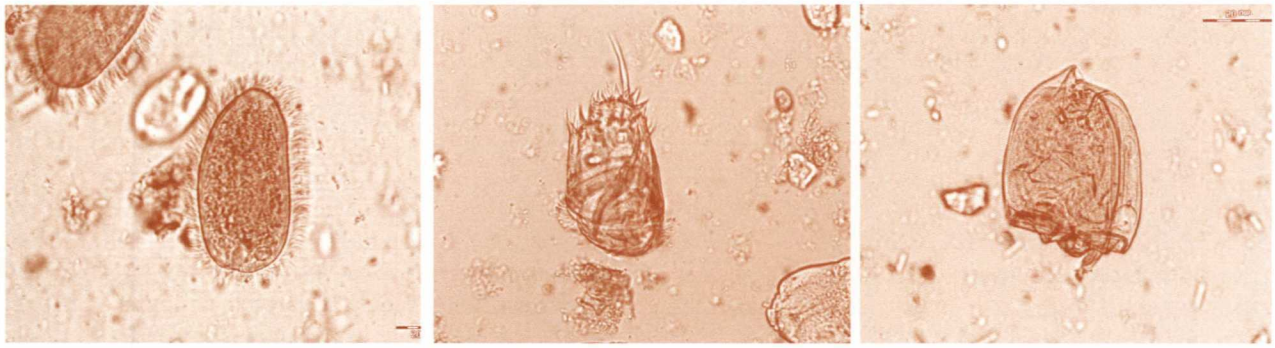
The smallest ruminants, such as the suni antelope, weigh only about 3 kg, while the largest, like cattle or bison, can even weigh as much as one ton or more. Nevertheless, regardless of their body size they all rely on the hard work being done by microbes and protozoans inside their stomachs



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The role of particular species of rumen protozoa remains unclear, however we've found that at least some of them digest crystalline cellulose and dried grass

other hand, are digested and fermented by the rumen microbiota into short fatty acids, which are absorbed and utilized by ruminants to meet their energy requirements.

Microbes *a la carte*

There are three groups of rumen microorganisms that participate in the digestion and utilization of nutrients from the feed ingested: bacteria, fungi and protozoa. Of the bacteria inhabiting the rumen, only *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus* are actually able to degradate cellulose. But such cellulolytic bacteria operate in a spectacular manner. Some cells (called "primary colonizers") start to adhere to a newly-ingested feed particle, and then rapidly multiply. This increase in adhering bacteria results in the formation of a biofilm around the colonized particle. In the second step of this process, non cellulolytic bacteria also join the primary colonizers, leading to the formation of a "cellulolytic consortium," which enables the rapid breakdown of cellulose.

Unlike bacteria, fungi, the second group of microorganisms in the rumen, penetrate the colonized particles themselves. Thus the vegetative stages of these microorganisms actually develop inside the feed particles. Rumen fungi readily colonize feed particles that are rich in lignin.

Among the third group of microorganisms inhabiting the rumen - protozoa - the most numerous are ciliates. In general, about 250 species of ciliates have been described to date. Interestingly, some of the species are very common to numerous domesticated and wild ruminants, while some others only seem to be specific to a single host. On the other hand, we have found great variability in the morphology of individuals developed from single cells. Some of these forms have in the past been described as quite separate species. This suggests that the true number of rumen ciliate species might be lower than the above figure, and genetic studies seem to confirm this hypothesis.

Analyzing protozoan workers

The role of rumen ciliates in the digestion of food components is not yet well understood. Unlike bacteria and fungi, protozoa engulf food particles and digest them within their own cells. They seem to utilize rumen bacteria as their main source of protein, but evidently also participate in the utilization of dietary carbohydrates. Their ability to degrade cellulose has intrigued scientists ever since these organisms were first detected more than 150 years ago. This question remains of current interest insofar as particular species-related studies have been strongly limited due to the difficulties involved in isolating and especially cultivating ciliates out of their natural environment, in order to obtain ciliates from a single species. Such material is necessary for performing enzymatic and especially genetic studies.

Our Institute endeavors to study the biology of rumen ciliates. We have succeeded in isolating and cultivating many species. In *in vitro* experiments we have found that some ciliates ingest, digest and ferment particles of crystalline cellulose and dried grass. We were, however, unable to confirm the significant involvement of one of the most cellulolytic species of ciliates i.e. *Eudiplodinium maggii*.

Visible progress has been made over the last three years due to the project entitled "European rumen ciliates culture collection," under the acronym ERCULE. This study was funded by the European Commission as an infrastructure support project under the 5th EU Framework Programme. It was carried out via close collaboration between microbial laboratories in France, the Netherlands, Poland, Scotland and Slovakia, and resulted in the production of a bank of cryopreserved cultures of pure ciliate species, and also in the identification, sequencing and cloning of their genes that encode certain cellulolytic and hemicellulolytic enzymes. ■

Further reading:

The ERECULE project: www.ercule.com

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