





Review on Effect of the Rumen Protozoa on the Productivity Performance of Some Ruminant

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Abstract:

One of three groups of microorganisms that can be found in the rumen of ruminants is the ciliate protozoa. Isotrichid and Entodiniomorphid are the two categories into which ciliate protozoa can be separated in the rumen. This mini-review briefly describes the role of protozoa in ruminant metabolism and fiber digestion, as well as the influence on animal performance. The role of the rumen is carried out by diverse groups of microorganisms residing within it, including protozoa. Rumen protozoa are responsible for digesting approximately 19% to 28% of the total fibrous material in the rumen.

These microorganisms within the rumen have the capability to

acclimate to an anaerobic environment through a fermentation process that occurs in the absence of oxygen. Forages that contain cellulose can be transformed by rumen protozoa into easily absorbed chemicals and energy. For a variety of reasons, protozoa are beneficial to their hosts. Metabolic process is the first, while fiber digestion is the second. The amounts of fermentation products including methane, ammonia, lactate, propionate, butyrate, and others are maintained in large part by protozoa. When ciliate protozoa are eliminated from the rumen, the amount of microbial protein produced can rise by up to 30% while methane production can fall by up to 11%. The purpose of this review is to elucidate the involvement of rumen protozoa in the degradation of forage within the rumen, as well as their potential to enhance ruminant growth, reduce methane emissions, and positively contribute to the fermentation process in herbivores.

Keywords: Ruminant, Ciliate, Protozoa, microbial protein, metabolism.

Introduction

There are three different groups of microorganisms that are present in the rumen of the digestive track of the ruminant. The rumen represents the first part of the gastrointestinal track. In the rumen, herbivorous mammals, including cattle, buffalo, sheep, and goats, are

able to transform forages that contain cellulose into materials that can be absorbed simply in the rumen and produce energy. The rumen's function has been performed by the various groups of microbial organisms that live in the rumen, which include bacteria, fungi, and protozoa (Patel & Ambalam, 2018; Takenaka et al., 2004). The rumen's microorganisms are able



to adapt to an anaerobic ecosystem in the rumen by a process of fermentation that occurs in the absence of oxygen (Leahy et al., 2013). Rumen protozoa were first discovered in 1843 by Gruby and Delafond. Ciliated protozoa that exist in the rumen possess two types of nuclei, like all ciliates that are micronucleus and macronucleus, whether those are dependent on parasitic life or free-living (Newbold et al., 2015). There are hundreds of ciliated protozoa found in numerous herbivores, but all these microbial organisms belong to two kinds, which are *isotrichid* and *entodiniomorphid* (Imai, 1998; Takenaka et al., 2004). The first group feeds on sugars and other soluble carbohydrates, while the second group consumes feed particles and bacteria that have protein. Ruminant protozoa account for half of the rumen microbe biomass (Williams & Coleman, 1992; Regensbogenova et al., 2004). These microorganisms contribute to rumen function, but their role in rumen function remains unclear (Newbold et al., 2015; Williams et al., 2020). As a result, opinions differ and there is discussion about the function of ruminal protozoa in the metabolism of proteins, feeding, or nourishment of their host. In other words, protozoa make up the majority of the rumen's biomass. However, it is still debatable and unknown how protozoa contribute to their host's metabolism, shared feeding, and fermentation process. Therefore, if there are a lot of ciliated protozoa present in the rumen, it may have a negative impact on the protein metabolism, methanogenesis or methane emission rate, and nutrition of herbivores (Newbold et al., 2015). reported that Ruminant performance and survival are negatively impacted by the superfluous presence of rumen protozoa; there is a conflict of interest between the ruminal protozoa and their herbivore hosts. This paper instead aims to assemble the most pertinent publications through an exceptional and thorough assessment by several references rather than to undertake an extensive review of the impacts of defaunation. The rumen microorganisms possess hundreds of protozoan types that have been classified; the majority of them can be divided into two groups (Williams & Coleman, 1997). These two groups have been classified into one phylum called Ciliophora. The

first one is *Isotrichidae*, which has fur-like cilia that beat like oars and allow protozoa to move. This species ingests sugars and other soluble carbohydrates. The second one is *Entodiniomorphida*, and this species also possesses cilia. The first type means *isotrichidae* do not ingest feed particles, but they can connect to them to gain access to the carbohydrates contained within. It ingests fewer bacteria than the second most common type of protozoan. While the second type implies that *entodiniomorphida* are capable of ingesting feed particles and bacteria (Belanche et al., 2012). These two groups of ciliate protozoa are most important for rumen metabolism and fibrolytic activity (Veira, 1986).

The metabolism in the rumen is vital in ruminant nutrition because it influences the host's supply of energy and valuable nutrients. As a result, the rumen microbiota is of particular interest in this multidisciplinary research field. It is widely accepted that the ruminal ecosystem is made up of various distinct microbial groups, such as bacteria, protozoa, archaea, fungi, and viruses (Firkins & Yu, 2015). The ruminant digestive system is made up of four parts: the reticulum, the rumen, the omasum, and the abomasum. The rumen is primarily where the fermentation processes take place. Microorganisms produce the enzymes found in the rumen. Because these enzymes are used to digest and ferment the food eaten by ruminants, the rumen is thought of as a fermentation vat (Tharwat et al., 2012; Aschenbach et al., 2011). Temperature, pH, buffering capacity, osmotic pressure, and redox potential are the primary factors influencing the growth and activity of ruminal microbial populations. Environmental conditions determine these factors. The temperature of the rumen is kept between 39 and 39.5 °C (Wahrmund et al., 2012). The rumen is a complicated system in which microorganisms such as bacteria, protozoa, and fungi digest nutrients consumed by them anaerobically. Rumen protozoa can account for up to 50% of microbial biomass and play an important role in ruminal nitrogen (N) recycling due to bacterial predation (Belanche et al., 2014). Ruminants can digest fiber-rich but low-protein food, ruminal

microorganisms are able to produce required enzymes for fermentation processes that enable ruminants to obtain the energy contained in forages more efficiently. The rumen's environment stimulates ciliated protozoa and bacteria to create the digestive enzymes needed for materials nutrition absorption. The primary products of fermentation are free fatty acids and biomass production, both of which are used by the host ruminant. Subsequently, it is a symbiotic relationship between microbes and the host animal (Hall, 1997).

Literature Review

Ruminants can convert low-quality fibrous materials into products that humans can use, such as meat, milk, and fibers (Castillo et al., 2013). The ruminant diet is based on the consumption of plant-based feed. Because cellulose is the major element of these plants' cell walls, cellulolytic ruminal microbial organisms play an important role in animal nutrition through their ability to cellulose assimilation (Castillo et al., 2013). Ciliated protozoa can account for up to half of all microorganisms in the rumen ecosystem and up to 50% of total fermentation products (Newbold et al., 2015; Nguyen et al., 2020).

Ruminant feeds are all exposed to fermentative activity in the rumen, the site of more or less complete microbial fermentation of dietary components. Ruminal fermentation begins by converting carbohydrates and protein into short-term intermediates such as sugars and amino acids. This initial degradation produces microbial mass and carbon dioxide, methane, ammonia, and volatile fatty acids, primarily acetate, propionate, and butyrate, and to a lesser extent, branched chain volatile fatty acids and lactate. The rate and extent of fermentation are important parameters that determine the animal's supply of protein, vitamins, and short-chain organic acids (Koenig, Beauchemin, & Rode, 2003; Hall, 1997). The animal excretes the gases, which are essentially waste products, but the acids are taken up by the circulation through the rumen wall and eventually transformed into the carbohydrates and lipids needed by the

animal for energy and tissue growth. Lipids are hydrolyzed to produce long-chain fatty acids, which are hydrogenated if they are unsaturated, and then passed on to be absorbed in the small intestine. Proteins are broken down into peptides and amino acids, which are subsequently individually deaminated to produce ammonia and a fatty acid. The bulk of the ammonia is absorbed through the rumen wall to be converted into urea. The accompanying liquid, which contains some ammonia and acids, exits the rumen and enters the omasum, where water and some acids are absorbed. Microbial fermentation of feed particles provides approximately 70% of the metabolic energy for ruminant animals, and microbial protein accounts for up to 90% of the amino acids reaching the small intestine (Hall, 1997; Nocek & Russell, 1988; Rabee et al., 2020).

Protozoa affect the methane emission and productivity performance of the ruminant either positively or negatively. The pure effect depends on the nutrients that the animal gets during its feeding. The ruminant's growth that it gets on energy-limited feeding probably has negatively impacted their growth and productivity, while animals that are feeding on protein-limited nutrition probably have positive effects (Newbold et al., 2015). The forage degradation process in herbivores could be associated with fermentation by microorganisms in the rumen. Therefore, these microorganisms' digestion of forages may give rise to increased growth production capacity and decrease methane emission of the ruminant, particularly in camels (Rabee et al., 2020). Ruminants produce the largest amount of methane compared with other livestock species through a fermentation process that occurs within the rumen during feeding digestion, and this is also considered a negative impact (Hook, Wright, & McBride, 2010). According to Tapio, Snelling, & Strozzi, (2017) previous research has found a link between ruminant methane emissions and protozoa concentrations in the rumen. Methanogens in the rumen produce methane (CH₄) primarily from carbon dioxide and hydrogen (H₂) released during feed fermentation by bacteria, protozoa, and fungi. Protozoa contribute to

methanogenesis through their high production of butyrate (C₄) and acetate (C₂), two volatile fatty acids (VFA) whose biosynthesis yields 2 and 4 moles of H₂ per mole of fermented glucose, respectively. Methanogens use half of this H₂ to produce CH₄ inside or in close proximity to protozoan cells. As a result, it was hypothesised that reducing rumen protozoa concentrations could be an effective way to reduce CH₄ emissions (Guyader, et al., 2014). Any direct or indirect effects that ciliate protozoa may have on their host's nutrition are caused by their effects on ruminal function. The presence or absence of ciliates has been shown to affect ruminal factors such as pH, ammonia concentration, volume, and dilution rate, as well as bacterial numbers and types, all of which can influence the rate and extent of digestion (Veira, 1986). The rumen protozoa are significant, but they are not necessary for the ruminal ecosystem (Williams & Coleman, 1992). Herbivore animals contribute to increasing atmospheric temperatures through their production of gas, because methane gas is considered stronger than carbon dioxide by about 23 times. Furthermore, the methanogenesis in the rumen causes nutritional energy loss (Rira et al., 2015). Moreover, there is a study that suggests that the cleaning of the rumen from ciliate protozoa leads to a decrease of up to 11 percent of methane emission and an increment of up to 30 percent of supply of microbial protein (Newbold et al., 2015; Bird, Hill, & Leng, 1979; Eugène, Archimède & Sauvant, 2004).

Protozoa Feeding Performance in the Rumen

Entodiniomorphid protozoa are especially adept at absorbing compounds containing matter suspended in the fluid of the rumen. They have a vestibulum that is encircled by cilia. The cilia trap particles, which are then driven into a vestibulum and then into cytostomes. A cytopharynx extends from the cytostome in *Ophryoscolecidae*. The migration of feed particles inside the cell's digestive vacuoles is thought to be aided by membrane and cytoplasmic movements across the cytopharyngeal

microtubular ribbons. *Entodiniomorphid* protozoa can also absorb substances. Although evidence suggests that their ectoplasm is porous to low-molecular-weight substances, a permeability barrier between the ectoplasm and the endoplasm may limit protozoa's utilisation of soluble compounds (Coleman, 1986). When different proteins were tested on the growth of entodiniomorphid protozoa in vitro, it was discovered that these ciliates do not metabolise soluble proteins and do not grow unless insoluble proteins are supplied (Michalowski, 1989). Isotricha protozoa possess a high capacity for absorbing soluble matters, primarily sugars, from the medium. They are, however, less active than *entodiniomorphs* at the consumption rate of plant substances. This is supported by increased holotrich numbers when animals are fed diets high in readily soluble carbohydrates and decreased isotricha numbers when animals are fed diets high in digestible cellulosic materials (Jouany, 1989).

Discussion

The interaction of microorganisms with the host animal results in a symbiotic relationship that allows ruminants to digest fiber-rich, low-protein diets. The environment in the rumen encourages microorganisms to produce the enzymes required for nutrient digestion. Ruminants can convert low-quality fibrous materials into products that humans can use, such as meat, milk, and fiber. Ruminal microorganisms' ability to produce the enzymes required for fermentation processes enables ruminants to efficiently obtain the energy contained in forages (Burns, 2008). Ruminant animals are fed plant-based feed. Because cellulose is the main component of these plants' cell walls, they are digested in the rumen. The ability to degrade cellulose is primarily determined by forages and the members of the cellulolytic microorganisms such as ciliate protozoa (Castillo et al., 2013). Protozoa-associated methanogens are one of the most active populations in rumen methanogenesis, where methanogenesis represents the primary hydrogen sink in the rumen and leads to more

complete oxidation of substrates by removing hydrogen produced by fermentation and greater energy recovery by rumen microorganisms (Belanche et al., 2014). The ciliate protozoa appear to play an important role in aspects of ruminal metabolism that are related to their host's health and well-being. It has been discovered that the ciliate protozoal fraction of ruminal contents is more important than the bacterial fraction in reducing nitrates and nitrites in the rumen and degrading some mycotoxins. Such detoxification would benefit faunated ruminants that consume contaminated feed. Ciliate protozoa actively participate in ruminal digestion by ingesting plant particles and their complement of enzymes for the digestion of complex proteins and carbohydrates (Veira, 1986). Ciliate protozoa actively participate in the ruminal digestion process through plant particle invasion and ingestion, as well as the addition of enzymes for the digestion of complex proteins and carbohydrates (Coleman, 1983; Coleman, 1985; Forsberg et al., 1984). The genus *Entodiniomorpha* contains nearly 90% of all protozoa, many of which are involved in cellulose hydrolysis and fermentation. It was discovered that crystalline cellulose is degraded primarily by protozoa from the genera *Polyplastron* and *Eudiplodinium*, and to a lesser extent by *Epidinium*. *Diploplastron* affine also has amylolytic activity; due to its ability to produce amylolytic enzymes, including two isoforms of amylase and maltase, it produces maltose, maltotriose, and glucose (Castillo et al., 2013). Despite the fact that protozoa make up a large portion of the rumen biomass, their role in ruminal fermentation and contribution to the host's metabolism and nutrition remains a source of considerable debate. Rumen protozoa have a variety of fibrolytic activities, including glycoside hydrolases and polysaccharide depolymerases, which degrade polysaccharides that form the plant cell wall structure (Patel & Ambalam, 2018). Protozoa-produced enzymes account for a significant portion of the hydrolytic enzymes in the rumen, highlighting the importance of this microbial group in the degradation of organic matter in the rumens of ruminants fed grains and forages. The forages are the foundation of the ruminant diet, and cell

wall degradation carbohydrates are required for ruminant digestion, survival, and production (Duarte et al., 2018). Nearly all rumen protozoa are amylase-active. The concentration of amylase, on the other hand, varies between protozoan species. Maltase, a less active enzyme than amylase, is also present in all species (Patel & Ambalam, 2018). In addition, about 30 to 40 percent of microbial fiber digestion in the rumen may be carried out by ciliate protozoa, which contribute about 19 to 28 percent of cellulose activity (Patel & Ambalam, 2018). These ciliates play an important role in rumen functions, but in excess, they can increase methane emissions, which is harmful to the environment (Patel & Ambalam, 2018). Eventually, according to a study conducted by Forsberg et al., (1984), who reported that these rumen protozoa have fibrolytic activities such as establishing the capacity of protozoan types to express their own enzymes for digestion of plant material, *Epidiniumcaudatum* was found to have this activity. Similarly, *E. Ecaudatum ecaudatum* was discovered to have cellulase and hemicellulase activities. Ten different enzyme activities for plant cell wall degradation have been identified, and their catalytic activities include glycoside hydrolases.

Conclusion

Rumen protozoa are not necessary for the animal's survival and defecation. The removal of ciliated protozoa from the rumen has been shown to increase microbial protein supply rates by upto 30% while decreasing methane production rates by upto 11% (Newbold et al., 2015). Getting rid of protozoa can both be useful and harmful. Because protozoa degrade forage protein and prey on bacteria that are also high in protein, eliminating protozoa can increase the animal's supply of metabolisable protein. As a consequence, getting rid of protozoa leads animals to grow rapidly, but eliminating protozoa reduces fiber digestibility because bacteria cannot recover the loss of protozoal activity towards fiber (Williams & Coleman, 1992). As per a recent study published by Newbold et al. (2015) *holotrich* protozoa play a

disproportionate role in supporting methanogenesis, whereas small *Entodinium* are responsible for much of the bacterial protein turnover. Although no safe and practical method of controlling protozoa in the rumen has been developed, a variety of plant extracts capable of controlling, if not completely removing, rumen protozoa have been described. Irrespective, all ruminants rely on the microorganisms that live in their rumen to convert ingested feed into a form that the host animal can use. Ciliated protozoa are part of this complex ruminal population and are necessary for the host ruminant's nutritional well-being and productivity (Williams & Coleman, 1992). The present author also agrees with the role of ruminant protozoa in metabolism and fiber digestion processes as well as the influence on animal performance.

References

- Aschenbach, J.R., Penner, G.B., Stumpff, F. & Gäbel, G. (2011). Ruminant nutrition symposium: Role of fermentation acid absorption in the regulation of ruminal pH. *Journal of Applied Animal Research*, 89, 1092-1107.
- Belanche, A., Abecia, L., G. Holtrop, G., Guada, J. A., C. Castrillo, C., de la Fuente, G. and Balcells, J. (2014). Study of the effect of presence or absence of protozoa on rumen fermentation and microbial protein contribution to the chyme. *Journal of animal science*, 89, 4163–4174. <https://doi.org/10.2527/jas.2010-3703>
- Belanche, A., de la Fuente, G., Moorby, J. M., & Newbold, C. J. (2012). Bacterial protein degradation by different rumen protozoal groups. *Journal of animal science*, 90(12), 4495–4504. <https://doi.org/10.2527/jas.2012-5118>
- Bird, S. H., Hill, M. K., & Leng, R. A. (1979). The effects of defaunation of the rumen on the growth of lambs on low-protein-high-energy diets. *The British journal of nutrition*, 42(1), 81–87. <https://doi.org/10.1079/bjn19790091>
- Burns J. C. (2008). ASAS Centennial Paper: utilization of pasture and forages by ruminants: a historical perspective. *Journal of animal science*, 86(12), 3647–3663. <https://doi.org/10.2527/jas.2008-1240>
- Castillo, A., Burrola-Barraza, M.E., Viveros, J. & Chavez-Martinez, A. (2013). Rumen microorganisms and fermentation. *Archivos de medicina veterinaria*, 46, 349-361. <https://doi.org/10.4067/S0301-732X2014000300003>
- Coleman, G. (1985). The cellulase content of 15 species of entodiniomorphid protozoa, mixed bacteria and plant debris isolated from the ovine rumen. *The Journal of Agricultural Science*, 104(2), 349-360. <https://doi.org/10.1017/S0021859600044038>
- Coleman, G. S. (1986). The metabolism of rumen ciliate protozoa. *FEMS Microbiology Letters*, 39: 321-344. <https://doi.org/10.1111/j.1574-6968.1986.tb01864.x>
- Coleman, G.S. (1983). Hydrolysis of Fraction 1 leaf protein and casein by rumen entodiniomorphid protozoa, *Journal of Applied Bacteriology*, 55(1), 111–118. <https://doi.org/10.1111/j.1365-2672.1983.tb02654.x>
- Duarte, E.R., Abrao, F.O., Ribeiro, I.C.O., Vieira, E.A., Nigri, A.C., Silva, K. L., & Geraseev, L.C. (2018). Rumen protozoa of different ages of beef cattle raised in tropical pastures during the dry season. *Journal of Applied Animal Research*, 46(1), 1457-1461. <https://doi.org/10.1080/09712119.2018.1530676>
- Eugène, M., Archimède, H., & Sauvant, D. (2004). Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants. *Livestock Production Science* 85, 81-97. [https://doi.org/10.1016/S0301-6226\(03\)00117-9](https://doi.org/10.1016/S0301-6226(03)00117-9)
- Firkins, J.L. & Yu, Z. (2015). Ruminant nutrition symposium: How to use data on the rumen microbiome to improve our understanding of ruminant nutrition. *Journal of Applied Animal Research*, 93, 1450–1470.

- Forsberg, C. W., Lovelock, L. K., Krumholz, L., & Buchanan-Smith, J. G. (1984). Protease activities of rumen protozoa. *Applied and environmental microbiology*, 47(1), 101–110. <https://doi.org/10.1128/aem.47.1.101-110.1984>
- Guyader, J., Eugène, M., Nozière, P., Morgavi, D. P., Doreau, M., & Martin, C. (2014). Influence of rumen protozoa on methane emission in ruminants: a meta-analysis approach. *Animal: an international journal of animal bioscience*, 8(11), 1816–1825. <https://doi.org/10.1017/S1751731114001852>
- Hall, M.B. (1997). The Rumen Microbial Ecosystem. 2nd edition *Journal of animal science*, 81, 3226–3232.
- Hook, S. E., Wright, A. D., & McBride, B. W. (2010). Methanogens: methane producers of the rumen and mitigation strategies. *Archaea (Vancouver, B.C.)*, 2010, 945785. <https://doi.org/10.1155/2010/945785>
- Imai, S. (1998). Phylogenetic Taxonomy of Rumen Ciliate Protozoa Based on Their Morphology and Distribution. *Journal of Applied Animal Research*, 13, 17-36.
- Jouany, J. P. (1989). Effects of diet on populations of rumen protozoa in relation to fibre digestion. In: *The Role of Protozoa and Fungi in Ruminant Digestion*, Nolan, J. V., Leng, R. A. & Demeyer, D. I., (eds.), pp. 59-74. Penambul Books, Annidale, Australia.
- Koenig, K. M., Beauchemin, K. A., & Rode, L. M. (2003). Effect of grain processing and silage on microbial protein synthesis and nutrient digestibility in beef cattle fed barley-based diets. *Journal of animal science*, 81(4), 1057–1067. <https://doi.org/10.2527/2003.8141057x>
- Leahy, S. C., Kelly, W. J., Ronimus, R. S., Wedlock, N., Altermann, E. & Attwood, G. T. (2013). Genome sequencing of rumen bacteria and archaea and its application to methane mitigation strategies. *Animal*, 7(2) 235–243. <https://doi.org/10.1017/S1751731113000700>
- Michalowski, T. (1989). The importance of protein solubility and nature of dietary nitrogen for the growth of rumen ciliates in vitro. In: *The Role of Protozoa and Fungi in Ruminant Digestion*. Nolan, J. V., Leng, R. A. & Demeyer, D. I., (eds.), pp. 223-232. Penambul Books, Annidale, Australia.
- Newbold, C. J., de la Fuente, G., Belanche, A., Ramos-Morales, E., & McEwan, N. R. (2015). The Role of Ciliate Protozoa in the Rumen. *Frontiers in microbiology*, 6, 1313. <https://doi.org/10.3389/fmicb.2015.01313>
- Nguyen, S. H., Thi Nguyen, H. D. and Hegarty, R. S. (2020). Defaunation and its impacts on ruminal fermentation, enteric methane production and animal productivity. *Livestock Research for Rural Development*, 32(4).
- Nocek J. E., Russell J. B. (1988). Protein and energy as an integrated system: Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *Journal of Dairy Science*, 71, 2070–2107. [https://doi.org/10.3168/jds.S0022-0302\(88\)79782-9](https://doi.org/10.3168/jds.S0022-0302(88)79782-9)
- Patel, S. & Ambalam, P. (2018). Role of Rumen Protozoa: Metabolic and Fibrolytic. *Advances in Biotechnology & Microbiology*, 10(4), 2474-7637. https://doi.org/10.19080/AIBM.2018.10.5557_93
- Rabee, A. E., Forster, R., Elekwachi, C., Sabra, E., & Lamara, M. (2020). Comparative analysis of the metabolically active microbial communities in the rumen of dromedary camels under different feeding systems using total rRNA sequencing. *PeerJ*, 8, e10184. <https://doi.org/10.7717/peerj.10184>
- Regensbogenova. Semelakova, M., Kisidayova, S., Michalowski, T., Javorsky, P., DerStaay, S. Y., ... & Pristas, P. (2004). Rapid Identification of Rumen Protozoa by Restriction Analysis of Amplified 18S rRNA Gene. *Acta Protozoologica*, 43, 219 – 224.
- Rira, M., Chentlia, A., Boufenerab, S., and Boussebouaa, H. (2015). Effects of plants containing secondary metabolites on ruminalmethanogenesis of sheep in vitro. *Energy Procedia*, 74(2015), 15-24. <https://doi.org/10.1016/j.egypro.2015.07.513>

Takenaka, A., Tajima, K., Mitsumori, M. and Kajikawa, H. (2004). Fiber Digestion by Rumen Ciliate Protozoa. *Microbes and Environments*, 19(3), 203–210.

<https://doi.org/10.1264/jsme2.19.203>

Tapio, I., Snelling, T.J., Strozzi, F. (2017). The ruminal microbiome associated with methane emissions from ruminant livestock. *Journal of Animal Sci Biotechnol*, 8, 7 (2017).

<https://doi.org/10.1186/s40104-017-0141-0>

Tharwat, M., Al-Sobayil, F., Ali, A., & Buczinski, S. (2012). Transabdominal ultrasonographic appearance of the gastrointestinal viscera of healthy camels (*Camelus dromedaries*). *Research in veterinary science*, 93(2), 1015–1020.

<https://doi.org/10.1016/j.rvsc.2011.12.003>

Veira D. M. (1986). The role of ciliate protozoa in nutrition of the ruminant. *Journal of animal*

science, 63(5), 1547–1560.

<https://doi.org/10.2527/jas1986.6351547x>

Wahrmund, J. L., Ronchesel, J. R., Krehbiel, C. R., Goad, C. L., Trost, S. M., & Richards, C. J. (2012). Ruminal acidosis challenge impact on ruminal temperature in feedlot cattle. *Journal of animal science*, 90(8), 2794–2801.

<https://doi.org/10.2527/jas.2011-4407>

Williams, A. G., & G. S. Coleman. (1997). The rumen protozoa. In Hobson, P. N. and Stewart, C. S. (Eds). Springer, Netherlands, pp. 73–139.

Williams, A., & Coleman, G. (1992). *The Rumen Protozoa*. New York: Springer-Verlag.

Williams, C. L., Thomas, B. J., McEwan, N. R., Rees Stevens, P., Creevey, C. J., & Huws, S. A. (2020). Rumen Protozoa Play a Significant Role in Fungal Predation and Plant Carbohydrate Breakdown. *Frontiers in microbiology*, 11, 720.

<https://doi.org/10.3389/fmicb.2020.00720>