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Manipulation of ruminal fermentation by electrofermentation

Manipulación de la fermentación ruminal mediante electrofermentación

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ABSTRACT

Rumen fermentation (RF) is an anaerobic redox process carried out by a complex microbial community that generates volatile fatty acids (VFAs), the primary energy source for ruminants. However, RF also produces methane, a greenhouse gas that negatively impacts the sustainability of livestock systems. This has intensified interest in strategies aimed at improving rumen energy efficiency by increasing VFA production while simultaneously reducing methane emissions per unit of fermented feed. Electrofermentation (EF) has emerged as an innovative alternative to address these limitations. This approach integrates microbial fermentation with the application of electrical currents through electrodes that act as electron sinks or sources. By modifying the microbial redox balance and overcoming thermodynamic constraints, EF redirects electron flow toward metabolic pathways that enhance VFA synthesis. *In vitro* studies have reported increases of over 80% in VFA production, accompanied by significant reductions in biogas generation and increased microbial biomass. Collectively, these findings position EF as a promising strategy to improve feed efficiency and reduce the environmental impact of livestock production, although its *in vivo* implementation still requires further technical and methodological research.
Keywords: rumen fermentation, electrofermentation, rumen microbiota.

RESUMEN

La fermentación ruminal (FR) es un proceso anaerobio de óxido-reducción realizado por una comunidad microbiana compleja, mediante el cual se generan ácidos grasos volátiles (AGV), principal fuente de energía para los rumiantes. Sin embargo, la FR también produce metano, un gas de efecto invernadero que afecta la sostenibilidad de los sistemas ganaderos. Esto ha impulsado el interés en estrategias que aumenten la eficiencia energética del rumen, lo que favorece una mayor producción de AGV y reduce simultáneamente las emisiones de metano por unidad de alimento fermentado. La electrofermentación (EF) puede considerarse como una alternativa innovadora para abordar estas limitaciones. Este enfoque combina fermentación microbiana con la aplicación de corrientes eléctricas mediante electrodos que

funcionan como sumideros o fuentes de electrones. Al modificar el balance redox microbiano y superar restricciones termodinámicas, la EF redirige el flujo de electrones hacia rutas metabólicas que incrementan la síntesis de AGV. Estudios *in vitro* han demostrado aumentos superiores al 80% en la producción de AGV, acompañados de una reducción significativa del biogás y un incremento en biomasa microbiana. En conjunto, estos avances posicionan a la EF como una estrategia prometedora para mejorar la eficiencia alimentaria y disminuir el impacto ambiental de la ganadería, aunque su implementación *in vivo* requiere mayor investigación técnica y metodológica.
Palabras clave: fermentación ruminal, electrofermentación, microbiota ruminal.

INTRODUCTION

Ruminants are mammals capable of converting fibrous feed into high–biological value products for human consumption, such as meat and milk. This remarkable ability is supported by a complex digestive system, particularly the rumen–reticulum, which functions as a continuous fermentation chamber where feed is broken down by a diverse community of resident microorganisms. Ruminal fermentation is a redox-driven process that supplies energy for microbial growth and generates volatile fatty acids (VFAs), the main energy source for the host animal. However, this process also produces greenhouse gases such as carbon dioxide (CO_2) and methane (CH_4) (Romagnoli *et al*., 2017). Meeting the growing global demand for meat and dairy products while minimizing environmental impact represents a major challenge for livestock production systems. One of the most promising strategies to address this challenge is to enhance the energetic efficiency of ruminants—that is, to improve how effectively they convert ingested feed into usable energy for body maintenance and the production of milk and meat. Achieving higher feed-to-energy conversion efficiency would ultimately support greater food production for human consumption while reducing the environmental footprint of ruminant agriculture.

In view of the fact that ruminal fermentation is a central driver of the energetic metabolism of ruminants, a wide range of research efforts has focused on understanding this process and redirecting it toward greater efficiency. Several strategies have been tested, including adjustments in diet formulation, the use of high-quality concentrates, feed additives, antibiotics (now largely discontinued), genetic improvement, and even vaccine development. However, most of these approaches have proven either impractical or insufficiently effective, or they have disrupted the energetic balance of the rumen, ultimately leading to reduced animal productivity (Lan & Yang, 2019). A new perspective currently under evaluation involves manipulating the metabolic pathways of rumen microorganisms through electrofermentation (EF). EF is a process that integrates microbial electrochemical techniques (MET) with conventional fermentation systems. It relies on the application of anodic or cathodic electrical currents to microbial cultures, helping to overcome redox constraints and enhancing the production of specific metabolites such as volatile fatty acids (VFAs) (Moscoviz *et al*., 2016; Sravan *et al*., 2019).

The rumen as a bioreactor

The rumen is a large chamber where ingested feed undergoes anaerobic microbial digestion. During this process, the rumen functions as a continuous-culture system operating under well-defined conditions that critically regulate the types, abundance, and biochemical activities of its resident microorganisms. This system closely resembles a "chemostat," in which water and nutrients are continuously supplied to support microbial growth, while waste products and fermentation end-products are steadily removed. (Liu *et al*., 2021; Moraïs & Mizrahi, 2019) (Figure 1). The end-products of fermentation, such as volatile fatty acids (VFAs), are removed either by absorption through the rumen wall into the bloodstream or by eructation in the case of biogas (Nagaraja, 2016). Maintaining these conditions is essential for the survival of rumen microorganisms, for preserving the integrity and function of the ruminal epithelium, and ultimately for ensuring the overall health and productivity of the ruminant (Romagnoli *et al*., 2017).

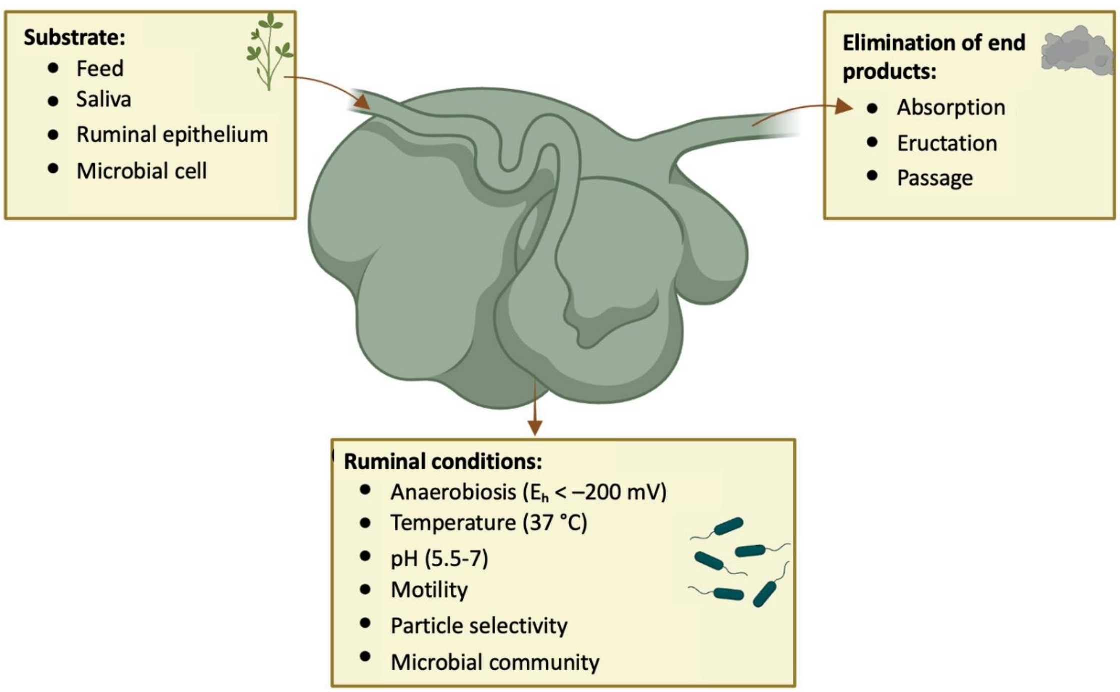

Figure 1. The rumen as a bioreactor

Fermentation in the rumen

To sustain ruminal fermentation, the rumen's microbial ecosystem consists of a symbiotic community of diverse anaerobic microorganisms, including representatives of all three domains of life: Eubacteria (bacteria), Archaea (methanogens), and Eukarya (protozoa and fungi), as well as viruses (Seshadri *et al*., 2018; Newbold & Ramos-Morales, 2020).
In general terms, ruminal fermentation can be divided into three levels of metabolic events, each carried out by different trophic groups. In the first trophic level (I), the structural

components of the plant cell wall are colonized by microorganisms specialized in hydrolyzing complex organic polymers—such as cellulose and hemicellulose—into their monomeric forms (Leahy *et al*., 2022; Sanjorjo *et al*., 2023).

In the second trophic level (II), soluble sugars are fermented by most members of the rumen microbiota through multiple metabolic pathways. This process results in the production of VFAs and gases such as carbon dioxide and molecular hydrogen, the latter of which must be carefully regulated to avoid inhibiting microbial metabolism and disrupting the metabolic cascade. During the oxidation of sugars into intermediates such as acetyl-CoA, NAD^+ is reduced to NADH, which must then be reoxidized back to NAD^+ to sustain continuous fermentation. The balance of the NAD^+/NADH ratio influences specific reactions within the metabolic network, and its regulation plays a central role in shaping the overall fermentation profile (González-Cabaleiro *et al*., 2015).

In the third trophic level (III), microorganisms utilize electrons in the form of hydrogen. At this stage, methanogenic archaea consume most of the H_2 generated during the second phase of fermentation. Under the anaerobic conditions of the rumen, classical electron acceptors such as oxygen are absent. As a result, hydrogenotrophic methanogens primarily use CO_2 as an electron acceptor. Methanogenic archaea can also reduce methylated compounds (methylotrophic methanogenesis) or acetate (acetoclastic methanogenesis) to methane, although these pathways are less common. These microorganisms are essential for maintaining electron sinks within the rumen and serve as a key driving force for the entire trophic network (Figure 2) (Sikora *et al*., 2017; Moraïs & Mizrahi, 2019).

Through this fermentative process, rumen microorganisms obtain the energy required for their maintenance, growth, and reproduction. Because ruminal fermentation occurs under strictly anaerobic conditions, and no terminal electron acceptor such as oxygen is available, ATP is generated primarily through substrate-level phosphorylation or through proton gradients established by the energy differences between electron donors and acceptors via chemiosmosis (Kracke *et al*., 2015; Vassilev *et al*., 2021). These thermodynamic limitations under anoxic conditions lead to lower energy yields and, consequently, reduced biomass production (Buckel, 2021).

Therefore, maintaining redox balance and conserving energy within rumen microorganisms depend on the availability of an electron acceptor. This requirement has posed a major challenge for efforts aimed at redirecting ruminal fermentation toward increased VFA production and reduced methane emissions (Leahy *et al*., 2022).

There are several approaches aimed at improving the efficiency with which ruminants convert feed into usable energy, with the goal of increasing food availability while reducing environmental impact. Some of these strategies include dietary manipulation, the use of antibiotics, the application of plant secondary metabolites, genetic selection, and other targeted interventions (Firkins & Mitchel 2023; Króliczewska *et al.*, 2023).

However, all of these approaches present notable limitations, highlighting the need for new alternatives capable of increasing VFA production while reducing the release of biogases such as methane (CH_4) and carbon dioxide (CO_2). One emerging and innovative alternative is the manipulation of ruminal fermentation through electrofermentation (EF), a strategy that helps regulate electron flow within the fermentation process (Aguilar-González *et al*., 2022; López-Hernández *et al*., 2023).

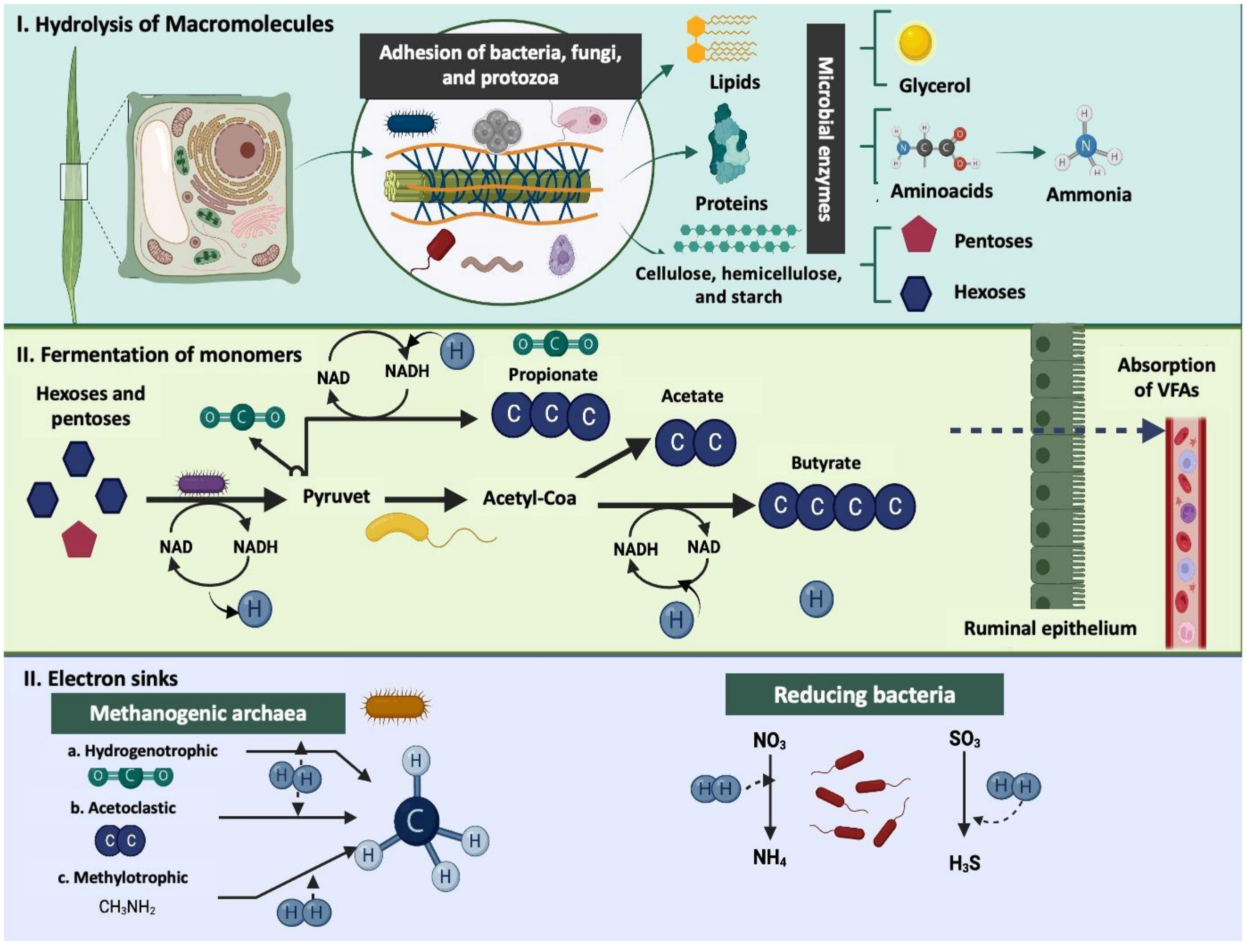

Figure 2. Metabolic cascades of the rumen represented by three stages of metabolic events: macromolecule hydrolysis (I), monomer fermentation (II), and electron sinking (III)

Electrofermentation

Electrofermentation (EF) is a biotechnological process that integrates microbial electrochemical techniques with conventional fermentation systems. In this approach, microbial metabolism is modulated through electrochemical reactions to drive the fermentation of organic substrates and enhance the production of reduced metabolites of productive interest, such as volatile fatty acids (VFAs) (Moscoviz *et al*., 2016; Schievano *et al*., 2016).

In these types of processes, controlled potentials and solid electrodes are used, which can function as electron sinks (anodes) or electron sources (cathodes), thereby modulating the redox reactions that occur during fermentation. These electrical currents may originate from external power sources or can be generated internally by electroactive microorganisms.

As mentioned earlier, ruminal fermentation generates electrons as a result of the redox reactions that occur within microbial metabolic pathways. The application of electrical currents enables the generation of additional electrons. These electrons can be incorporated into microbial metabolism, increasing the efficiency of energy production and stimulating metabolic rates. In addition, the presence of an anode—acting as an electron sink—can help overcome the thermodynamic constraints imposed by internal redox imbalances. This can lead to an unbalanced electrofermentative process or bacterial electrosynthesis, ultimately resulting in an increased formation of reduced compounds (Bhagchandanii *et al*., 2020; Mukherjee *et al*., 2021).

The effectiveness of an electrofermenter will depend primarily on (i) interactions among microorganisms, (ii) dissolved electron carriers present in the medium, (iii) interactions between microorganisms and electrode surfaces through cellular mechanisms of extracellular electron transfer (EET), (iv) the substrate, and (v) the configuration of the electrochemical cell (Bajracharya *et al*., 2022). The efficiency with which microorganisms transfer electrons to the anode, or among themselves, depends on their extracellular electron transfer (EET) mechanisms. To date, detailed information is available for only a limited number of microorganisms capable of performing these processes (Yee *et al.,* 2020).

Ruminal electrofermentation to improve efficiency and mitigate emissions

Most research aimed at manipulating ruminal fermentation has focused on modifying electron flow within redox reactions to reduce methane production without compromising animal health and nutrition, and ideally, to improve overall production efficiency. However, overcoming the thermodynamic limitations of this process has proven to be a major challenge. A full understanding of the flow of key metabolites within the rumen, the interactions between microorganisms and these metabolites, and the physiology, microbiology, and biochemistry of the microorganisms involved is still lacking (Lan & Yang, 2019; Leahy *et al*., 2022).

Recent studies have shown that applying electrofermentation (EF) to in vitro rumen fluid can increase VFA production by up to 80%, while simultaneously reducing total biogas output and enhancing microbial biomass. These changes are associated with an intracellular redox imbalance caused by shifts in the NAD^+/NADH ratio. EF, through the use of solid electrodes that accept electrons derived from oxidative reactions, has proven to be an effective strategy for altering the NADH/NAD^+ balance and generating proton gradients sufficient for ATP synthesis. As a result, biomass yields increase, leading to greater production of microbial protein available to the ruminant (Choi & Sang, 2016; Buckel, 2021; Chandrasekhar *et al*., 2021; Bhagchandanii *et al*., 2020; Aguilar-González *et al*., 2022; López-Hernández *et al*., 2023).

These advances represent a novel and efficient strategy for modifying ruminal fermentation in a way that directly influences electron flow toward greater productive efficiency. In practice, this could translate into a reduced environmental impact by lowering greenhouse gas emissions, as well as improved feed efficiency if applied in vivo. Altogether, this approach offers an important contribution to addressing the economic and environmental sustainability challenges facing the livestock sector.

CONCLUSIONS

Implementing electrofermentation (EF) in animal production faces significant challenges, including the design of systems that can be applied *in vivo* and the need to develop new methodologies or tools to measure electron flows. It is also necessary to evaluate the long-term effects of EF on animal health and productivity.

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