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## **Relationship between protozoa, digestibility and methane production in rumen: a molecular comparative approach between ruminants and camels protozoal fauna**

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**ABSTRACT** Recently, Canada made an agreement to reduce CH<sub>4</sub> emissions. In the agricultural sector, methane is largely produced by cattle and a large part come from the animal itself due ruminant. Improving ruminal efficiency could help reduce emission and thus contribute to the national reduction effort. Ruminants and camels evolved to use fibrous roughage. Fibrous feed remains in their stomach where microbial population like bacteria and protozoa ferments it to produce microbial protein and as a fermentation by-product, volatile fatty acids. These provide an energy source that the host animal can use for maintenance as well as for production functions, such as growth and milk production. Protozoa species in camelids are different and allow better

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cell-wall degradation in the rumen, also reducing methane production as compared to other ruminants. Thus, it seems that the presence of such protozoa in the rumen may increase forage digestibility by up to 30%. Molecular characterization of camel microflora and its comparison with those of other ruminants will provide information about their diversity, along with their effectiveness. This paper is an overall literature review related with the main objective of our study: the characterization of the population of ciliated protozoa and methanogens population in the camel and bovine rumen by the PCR technology.

**Keywords:** Protozoa, digestibility, methane emissions, camelids, ruminants

**INTRODUCTION** Protozoa that inhabit the rumen represent up to 50% of the microbial biomass (Williams and Coleman, 1992). They contribute significantly in ruminal digestion of animal hosts (Ushida, 2011). The ciliated protozoa are the most abundant (Ushida, 2011). In camelids such as camel, the anatomy has been the subject of numerous studies but information on its microbial flora is rare especially those protozoa as opposed to bacteria. Kayouli et al. (1991 and 1993) indicated that the population of protozoa in camels and llamas was lower than in ruminants. However, Dehority (1986) and Jouany (2000) reported a similar numbers in ruminants, camels and camelids of South America such as llamas and alpacas. On the other hand, Rouissi and Guesmi (1996); Rouissi and al. (2008); Del Valle et al. (2008) reported that the dromedary homed only a population type B of protozoa (Epidinium and Eudiplodinium) (Eadie 1962), known for their highest cell wall degradation compared to the population type A in the classics ruminants. Moreover, they added that a specific genre named Butchlia (type B) was observed only in camelids (Kayouli et al, 1993; Rouissi and Guesmi 1996; Rouissi et al, 2008). However, no reference has presented data that support these conclusions by more advanced techniques than microscopic observations such as molecular techniques. The classification of protozoa was always based on morphological criteria (Ogimoto and Imai, 1981 Williams and Coleman, 1992). However, particularly in the in vitro conditions, some species change in morphology or even lose some morphological criteria on which they were originally classified (Williams and Coleman, 1992). The classification based on DNA or DNA is the newest alternative for a better characterization of microbial flora (Huggett et al., 2005; Skillman et al., 2006). In recent years, molecular biology has become essential in the characterization of rumen microorganisms predominately the bacteria (Ghali et al., 2004; Ozutsumi et al., 2006; Mosoni et al., 2007; Ghali et al., 2011). There are few existing data about ciliate protozoa in especially those inhabit the rumen of camels as compared to other ruminants. In the fact no molecular characterization was illustrated for some species of protozoa that inhabit the rumen of camels and were identified microscopically.

However, protozoa were often overlooked in studies examining the microbial ecosystem as a complex population assuming that their presence in the rumen varies with the nature of feed and the presence of other microorganisms in their environment. Thus, traditional techniques to study microorganisms appear no longer effective especially with a microbial population tangled as protozoa. Molecular biology has become the last twenty years, the recent alternative for better study of the microbial flora.

**OBJECTIVE** The objective for this study is to reveal the impact of ruminal microflora types, particularly protozoa, on fiber digestibility and methane production involving in both processes. The study will be done principally on camelin protozoa to reveal the fact that camelids produce less methane and digest better the forage. The present paper will present the result of the literature review as the first part of the overall project.

## COMPARISON OF RUMEN CILIATE PROTOZOA POPULATIONS OF CAMELS AND SMALL RUMINANTS

The protozoa population is dominated by Entodinium genera in all animal species. The rumen content of dromedaries has fewer protozoa compared to small ruminants. It may contain some protozoa genera that are very efficient in cell wall degradation. Dromedaries have type "B" population (Epidinium, Eudiplodinium) while sheep have type "A". The rumen content of dromedaries contains specific genera: Butchlia (Rouissi and Guesmi, 1996) (Table 1). The fact that type "B" protozoa are found in the dromedary rumen (Table 1) is very interesting as these protozoa are very active in the degradation of cell wall (Jouany, 1989).

Table 1. Total count ( $10^5$ ) and various genera of protozoa in % (Rouissi and Guesmi, 1996)

| Diets    | Specie | N  | Total | Ento.  | Epid.  | Eudip. | Butch |
|----------|--------|----|-------|--------|--------|--------|-------|
| Hay      | D      | 16 | 2.7a  | 59.6a  | 20.0aA | 10.1a  | 8.9   |
|          | C      | 8  | 4.6b  | 71.2b  | 12.1b  | 2.9b   | *     |
|          | O      | 16 | 4.1bA | 81.8cA | *      | *      | *     |
| Hay<br>+ | D      | 20 | 3.3a  | 62.9a  | 17.5aB | 10.0a  | 9.6   |
|          | C      | 10 | 5.3b  | 81.1bB | 7.9b   | 1.6b   | *     |
|          | Conc   | O  | 19    | 5.0bB  | 83.7cB | *      | *     |

a, b, c: The values, for the same diet, for the same column, with different letters are statistically different ( $P < 0.05$ ). (Species effect).

A, B: The values, for the same species, for the same column, with different letters are statistically different ( $P < 0.05$ ). (Diet effect).

N: Sample number

D: Dromedaries (n=4), C: Goats (n=2), O: Sheep (n=4)

\*: Genera was not found

Conc: Concentrate

Ento.: Entodinium; Epid.: Epidinium; Eudip.: Eudiplodinium; Butch.: Butchlia

## MICROBIAL ECOSYSTEM AND METHANOGENESIS IN THE RUMEN

Methane production in the rumen occurs by a synergy within a community of microorganisms such as bacteria, protozoa, fungi and particularly methanogenic Archaea (Popova et al., 2011).

Methanogens represent a small population in the rumen (1 to 4%) (Lin et al., 1997; Janssen and Kirs, 2008).

A symbiotic relationship between methanogens and protozoa is also well documented. Ciliates protozoa and non-methanogenic microorganisms can exist in a large proportion in the rumen when the pH is between 6 and 7. They are hosts for methanogenic Archaea who found themselves in their intracellular and extracellular environment. Up to 20% of methanogens are related to protozoal (Ushida and Jouany, 1996). Methanogenic Archaea benefit of volatile fatty acids produced by these protozoa such as acetate and mostly butyrate: two metabolic pathways for H<sub>2</sub> production and consequently methane production (Jouany and Thivend, 2008) (Figure 1).

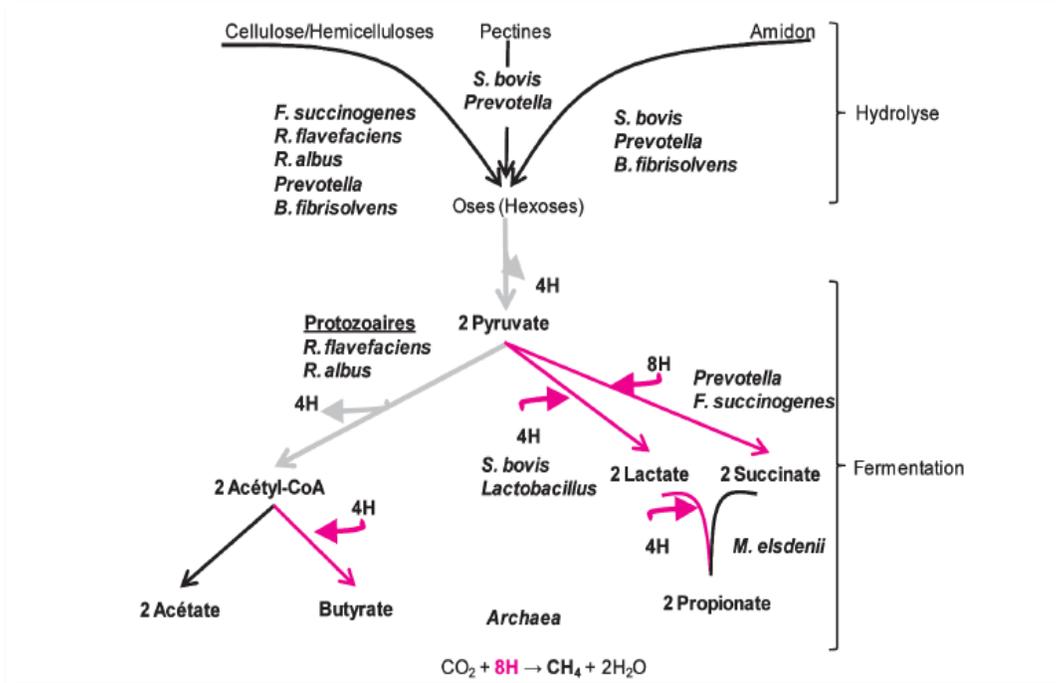


Figure 1. Hydrolysis and microbial fermentation in the rumen. The reactions that produce hydrogen are gray; hydrogenotrophic reactions are red.

Methane produced by methanogenic Archaea attached to the ciliated protozoa can represent up to 37% of the total methane produced by the methanogenic Archaea free in rumen (Newbold and al., 1995). Therefore, studying the relationship between Archaea and protozoa can bring important information that support our study.

## RESEARCH PERSPECTIVES

The molecular characterization of microbial flora in camelids, animal species with excellent efficiency in fiber degradation and reduced methane production, and its comparison with those of cattle, provide interesting and important conclusions about their taxonomy, diversity and their role.

The purpose of this research lie in the highlights they could lead, in the short term, by understanding the mode of action of protozoa and, in the long term, the effectiveness of different species of protozoa to lift some missing data on the ability to transfer a particular microorganism to its environment to another. These prospects will affect the achievement of the ultimate aim of this research resulting, first, the improving the digestibility of low quality forages so decrease the cost of ruminant feed and, secondly, the possibility of reducing their methane production. However, many questions remain as and experimental trail should be done to confirm.

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