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**ORIGINAL RESEARCH**

# Effect of Ration Type on Microbial Community in Dromedary Rumen

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**ABSTRACT**
Camel rumen is inhabited by a diverse of microorganisms, bacteria, archaea, protozoa and fungi; this microbial community has the main role in the rumen fermentation. Camel rumen's microorganisms were not been targeted to be enough studied like cattle. The study of Camel rumen microorganisms will help to explain the Camel efficiency in Cellulolytic Material digestion which results in better exploitation and farming of Camel. The main objective of the current study is to investigate the density of the archaea and bacterial Community of Camel's foregut by using Real time PCR. Microorganisms in rumen samples were collected from eleven animals, three camel groups were fed on Clover hay and concentrates mixture (R1) , green clover (R2) , and wheat straw (R3), the microbial community was studied in solid and liquid fraction . Camel group R3 has highest microbial density in comparison with R1 and R2. Changing camel diets has impacted the camel rumen's microbial density in different degrees.

**KEY WORDS:** *The Dromedary, Rumen, Microbial Community, Real Time PCR*

## Introduction

The one humped Camel (*Camelus Dromedaries*) well suited for the life under harsh conditions (Mason, 1979, Wilson, 1984) of hot climates. The camel digestive system is distinct, the third compartment is absent from the honey comb-like structure which is not distinctively separated from the fourth compartment. Camel does not have a gall bladder (Wardeh, 2004). The rumen is inhabited by high density of resident microorganisms, include bacteria, protozoa, archaea and fungi, which has a vital role in degradation of the ingested plant materials. Camel has more efficiency in the digestion of Fiber of range plants, alfalfa, straw and trifolium than other ruminants (Bhattacharya *et al*., 1986). Dromedary Camels were not subjected to modern studies as it was in domestic animals , nutrition studies were least conducted with Camels (Wardeh 2004).

Understanding rumen micro biome composition, adaptation, and function has global implications ranging from climatology to applied animal production (Joshua *et al*., 2014). The adaptive nature of the rumen micro biome allows ruminants to convert a wide array of low- and high-quality feedstuffs into high-quality microbial crude protein via fermentation (Russell *et al*., 1992). Studying the microbial populations associated with the gastrointestinal tract (GIT) holds vast potential for answering questions associated with improving animal production (Mackie and White, 1990) and increasing the efficiency of animal feed (Hegarty *et al* ., 2007 and Zhou *et al.,* 2009). Real-time PCR assays have been used to quantitatively estimate microbial populations in complex environmental samples (McSweeney *et al*., 2007). Tajima et al. (2001a) designed primer sets for 12 ruminal species and quantified these using a real-time PCR assay. Ruminal archaea, fungi and protozoa have also been quantified using real-time PCR (Sylvester et al., 2004; Denman and

McSweeney, 2006 and Jeyanathan *et al*., 2011). Stiverson et al. (2011) reported the first study that quantified uncultured bacteria represented by rrs sequences in the rumen using real-time PCR.

Archaea Bacterial population density in dromedary camels' rumen were not well studied , the main objective of the current study to investigate the Density of the Archaeal and Bacterial Community of Camel's foregut by using Real time PCR.

## Material and Methods

### Sampling

Eleven rumen samples were used in this study, three samples were collected from dromedary camels fed on Egyptian clover hay and concentrates mixture (R1), Six samples were collected from adult dromedary camels fed on green clover (R2), and two samples were collected from Adult dromedary camels fed on wheat straw (R3). Samples were prepared in Animal Biotechnology and Industrial Biotechnology Depts. Laboratories of Genetic Engineering and Biotechnology Research Institute, University of Sadat City (GEBRI-USC). The proximate analysis of feeds (Table 1) was determined according to the procedure of A.O.A.C. (1995).

**Table 1:** The chemical composition (%) of diets fed to experimental animals

| Feeds | DM | Chemical analysis (%) on DM basis | | | | |
|---|---|---|---|---|---|---|
| | | Ash | CP | CF | EE | NFE |
| Concentrate mixture[1] | 92.41 | 9.96 | 13.81 | 14.48 | 2.92 | 58.83 |
| Fresh Berseem[2] | 18.00 | 12.94 | 14.23 | 31.38 | 1.34 | 40.11 |
| Berseem hay | 89.29 | 12.13 | 12.44 | 25.64 | 1.73 | 48.06 |
| Wheat straw | 93.12 | 10.80 | 2.94 | 39.71 | 0.45 | 46.10 |

[1]Concentrate mixture composed of 30% wheat bran, 22% cotton seed meal, 33% yellow corn, 10% sunflower meal, 3% molasses, 1.5% limestones, 0.5% salt .[2]*Trifolium alexandrinum*

### RNA Isolation, reverse transcription, and Real time PCR quantification

The frozen rumen samples for animal groups using liquid nitrogen. Total RNA was isolated The RNA was reverse-transcribed into first strand cDNA by using Super Script III First-Strand Synthesis System for RT-PCR according to Manufacturer instructions. PCR amplification was carried out using the primer set complementary to the V4 region of 16S rRNA gene. PCR reaction volume of 25µl that was applied in this study was consisted of:

- 4µl template cDNA, 12.5µl Hot Start Ready Mix PCR Kit
- 1.25µl of 10 pmol/ µl of forward primer
- 1.25µl of 10 pmol/ µl of reverse primer
- 6µl double distilled deionised sterilised water

Amplification was performed by thermal cycler (Dyad Peltier Thermal Cycler, Roche Molecular system, Inc., USA) under the following program conditions:

- initial denaturation at 95°C for 3 min,
- followed by 30 cycles of denaturation at 94°C for 20s
- annealing at 65°C for 20s and
- Extension at 72°C for 50s with a final extension at 72°C for 3 min.

The PCR products were Gel purified using QIAquick Gel Purification Kit and quantified by Applied Bio-systems 7900HT Fast Real-Time PCR System using NEB Next Library Quant Kit Protocol.

### Statistical Analysis

Data of the present study were statistically analyzed using the method of least squares analysis of variance using software SPSS for windows version 15 (SPSS, 1999).

## Result and Discussion

Rumen microorganisms study helps to understand the rumen fermentation that consequently increases animal efficiency. Many factors were reported that affecting the rumen microbial diversity including host genetics, environmental factors and diets composition as illustrated by Chaucheyras-Durand and Ossa (2014). In Current study, bacterial and archaea community in Camel rumen was studied using Real time PCR by primer complementary to Variable Region 4 (V4) on 16S rRNA gene. The experimental animals were fed different rations .cDNA was synthesized from 22 solid and liquid rumen samples.

Results indicated that, the average of archaeal and bacterial concentration in camel for-stomach was 43067 Pico Molar. results reveal that the nutritional treatment has a significant effect on microbial density in camel rumen, Differences in bacterial and brchaeal concentrations between camel groups

**Table 2:** Concentration of Archaeal and Bacterial community in camel rumen by Pico Molar (PM)

| | R1 | R2 | R3 | Overall Mean |
|---|---|---|---|---|
| **Concentration Average** | 20614.36[a] | 43770[ab] | 74640[b] | 43067 |
| **Standard Error(SE)** | ±4587.29 | ±6421.06 | ±24721.30 | ±6649.13 |
| **Concentration Av./RLF** | 20327.18[a] | 46097.09[a] | 100157.9[b] | 48898.18 |
| **Standard Error(SE)** | ±9092.82 | ±9894.08 | ±18257.44 | ±10379 |
| **Concentration Av./RSF** | 20901.54 | 41442.91 | 49122.05 | 37236.92 |
| **Standard Error(SE)** | ±4738.604 | ±9019.61 | ±45068 | ±8435.26 |

[a, b , c] Means in the same row with different superscripts differ significantly ($P$<0.05). R1: clover hay plus concentrates mixture; R2: Fresh clover; R3: wheat straw; Av.: Average; A: Animal;S: solid; L: liquid.

were significant (P<0.05). Group R3 of Camel was that fed on wheat straw had the highest concentration (74640 pM) followed by group R2 (43770pM) and R1 (20614pM).The same trend found between the groups in liquid fraction and solid fraction, however the difference solid fraction was not significant.

Diet is a key determinant of microbial composition in the rumen, influenced by the complexity of available substrates within feed. Most ruminant diets are plant-based and rich in complex polysaccharides that enrich the rumen microbial community that capable of breaking down specific polymeric components in the diet (Krause et al., 2003). This primary degradation produces a range of organic acids together with hydrogen and carbon dioxide (Stewart et al., 1997).

Microorganisms concentrations on the fraction level were higher in liquid rumen fraction (48898 pM) than in solid rumen fraction (37236 pM) as shown in Table (2) and Figure (1) which illustrate the Bacteria and Archaea concentrations quantified by Pico Molar (pM) in camel fed on different rations and in solid and liquid fractions. Abundance of microbial communities (bacteria and archaea,) was differed between the groups as a result of changing the type of ration.

The direct microscopic count is the chief method for ascertaining the total number of bacteria in the rumen. It is subjected to a high percentage error but in experienced hands could give consistent and reproducible values (Hungate ,1960),. The direct counts reported in the literature range roughly from $1 \times 10^{10}$ to $1 \times 10^{11}$ cell per gram of rumen contents. The abundance of rumen bacteria has been reported to be $10^{10}$ to $10^{12}$ individuals per gram of rumen content (Hungate, 1966 and Chaucheyras-Durand and Ossa, 2014).

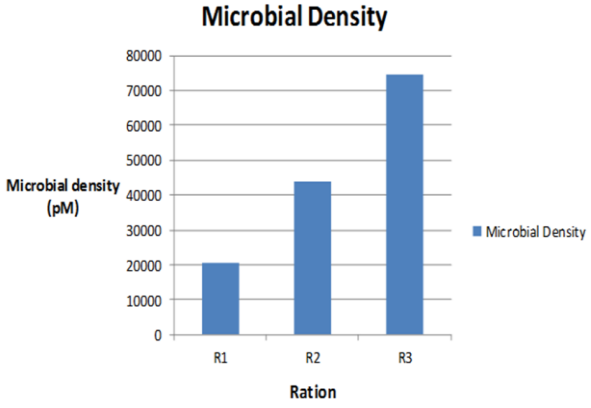


**Figure 1:** The density of bacteria and Archaea in Camel fed different rations

In dairy cows, the high forage rations has higher diversity than high concentrates rations (Kumar *et al.*, 2015), and this finding is similar to the result in current study. Seeka deer that fed high tannin ration showed lower Chao1 and Shannon indices than that fed high fiber ration (Li *et al.*, 2013). The Fibrolytic bacteria such as Fibrobacteres tend to increase in solid phase comparing to liquid phase (Gharechahi *et al*., 2015).

The archaeal population especially the Methanogens is an important factor when investigating methane mitigation

strategies. The rumen Methanogen species differ depending on diet and geographical location of the host. Methanogens can be reduced by modifying dietary composition or by supplementation of monensin, lipids, organic acids, and plant compounds within the diet (Hook *et al*., 2010).The shifting of archaeal community by changing the type of diet might be explained as a result of alteration the fermentation pattern due to the changing the fermented substrates which change the proportions of produced volatile fatty acid and hydrogen (Carberry *et al*., 2014).

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