Abstract

Current methods of evaluating the digestibility and nutritional value of animal feed utilize in vitro Assessments and in vivo studies, that provide a wide range of information on nutrient compounds, kinetic and digestibility. Nucleic acid-based methods with a specific application in ruminants are cellular and molecular methods that can be used to identify an animal microbial collection. The aim of this study is to identify the structure and composition of symbiotic microbial flora in the ruminal liquid and solid contents of the extinct Sistani cattle. For this purpose, ruminal liquid and solid samples from 10 Sistani cattle from Sistan geographical region that kept in a group with similar breeding conditions and fed forage diet, have collected by oral stomach tube before feeding in the morning. Extraction of microbial DNA has performed using the kit. The 18S rRNA gene of the protozoa was amplified and Isolated by specific primers by PCR and then cloned using the T/A cloning method in the PTZ plasmid and the recombinant product has transferred to E.coli. Bacteria have examined by the culture-dependent method and then, their 16S rRNA gene has amplified and sequenced with dedicated primers. Next, the 16S rRNA gene sequencing of the bacteria has performed in a non-cultured method using the Miseq system. The results of this study indicate that the Entodinium genus is the dominant protozoan group in the rumen of Sistani cattle. In the library under assessment, OTUs show over 98.50% similarity to the ciliated protozoa sequences identified in the database. The results of bacterial culture have shown Species Ruminococcus albus, Ruminococcus flavefaciense, Clostridium colinum, Streptococcus equinus, and Butyrivibrio genus have more than 96.54% similar to the bacterial sequences identified in the database. Miseq metagenomics sequencing also has identified 17 phylum, 32 classes, 50 orders, 62 families, 76 genera and 27 species. Over 90% of all sequences are attributed to OTUs belonging to phylums Firmicutes (73.51%), Bacteroidetes (13.56%), Proteobacteria (5.10%), Verrucomicrobia (2.22%), TM7 (2.02%) from the bacteria domain and Euryarchaeota (1.39%). Also in this study, sequences were identified that were eventually identified in the databases up to the level of the order or family. Overall, the results of the study the rumen microbial structure of the Sistani cattle show that the symbiosis microbial in the rumen of the Sistani cattle is structurally similar to the microbial coexisting in the rumen of other ruminants, but shows a large difference in composition and frequency. The rumen microbiome composition of the Sistani's cattle's provides the ability for the host to feed low-nutritious and roughage.

Keywords: Rumen flora, 16S rRNA, 18S rRNA, Phylogeny, Metagenomics.

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The Dissertation Submitted for the Degree of Ph.D in

Animal Nutrition

Title:

Phylogenetic analysis of ruminal protozoa and bacteria in Sistani cattle fed with forage diets using laboratory and molecular techniques

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January 2020