COMPARISON OF THREE MICROBIOME PRESERVATION METHODS WITHIN MANAGED SOUTHERN WHITE RHINOCEROS (*CERATOTHERIUM SIMUM SIMUM*)

Christina M. Burnham, BS[1]*, Larry J. Minter, MS, DVM, DACZM[2], Kimberly Ange-van Heugten, MS, PhD[1], and Shweta Trivedi, BVSc, MVSc, PhD[1]

[1]Department of Animal Science, NC State University, Raleigh NC 27695, USA.
[2]Hanes Veterinary Medical Center, North Carolina Zoo, 4401 Zoo Parkway, Asheboro, NC 27205, USA.

Abstract

Due to the uncertain future of southern white rhinoceros (*Ceratotherium simum simum*) in the wild, it is critical that reproductively healthy assurance populations be maintained in human care. Reproductive outcomes in managed southern white rhinoceros may be mediated in part by their diet and gut microbiota. While immediate freezing at -80°C is the ideal method of preserving samples for microbiome research, it is impractical for conservation fieldwork and resource-intensive for zoological institutions. The main objective of this research was to examine the efficacy of three different fecal preservation methods for microbiome studies in managed southern white rhinoceros. Fecal samples were collected from the reproductively successful southern white rhinoceros' population at the North Carolina Zoo (n = 10) within 20 minutes of deposition. The daily diet of these rhinoceros included one bale of timothy hay and three pounds of Mazuri® Wild Herbivore High Fiber diet per animal. Limited quantities of timothy hay cubes and pellets, orchard grass hay, and alfalfa hay were supplemented during training and enrichment sessions. Fecal samples were preserved using: immediate freezing at -80°C, PERFORMAbiome•GUT (PB) tubes, or 95% ethanol. Samples preserved in PB tubes and in 95% ethanol were stored at ambient temperature for a minimum of two weeks before processing to replicate field conditions. Microbial DNA was extracted from each sample and sequenced using the 16s rRNA gene. The alpha diversity in 95% ethanol samples was different (P = 0.05) from both the frozen and PB samples. Ethanol preserved a wider range of operational taxonomic units (OTUs) across samples but with far greater variability and a lower median number of OTUs. There was no difference in preserved total OTUs between the frozen and PB samples. This indicates that samples stored in ambient temperatures in PB tubes perform similarly to those frozen immediately at -80°C. Thus, PB tubes may be advantageous in microbiome fieldwork applications. Total OTUs were examined using data from frozen and PB samples. Initial results show that the microbiome of this population of managed southern white rhinoceros is colonized by 99% bacteria and 1% archaea. Of the bacteria present, the main phyla were Firmicutes (56%), Bacteroidetes (22%), and Spirochetes (9%). The archaebacteria were dominated by Euryarchaeota, the majority of which were methane-producing species (93%). Determining the most appropriate microbiome preservation techniques and cataloguing the healthy microbiome in reproductively successful populations is vital for future nutritional husbandry considerations and conservation success in the southern white rhinoceros.