Captive management of many wildlife species has proven to be challenging, with individuals displaying health disorders that are not generally described in the wild population. Retrospective studies have identified gastrointestinal (GI) diseases, in particular inflammatory bowel disease (IBD), as the second leading cause of captive adult red wolf (Canis rufus) mortality. Recent molecular studies show that imbalanced gut microbial composition is tightly linked to IBD in the domestic dog. The goal of the present study was to answer two main questions: (1) How do red wolf gut microorganisms differ among fecal consistency scores? and (2) How do red wolf gut microorganisms differ among diet types? Fresh fecal samples were collected from 53 captive wolves housed in eight facilities and from two wild wolves living in Alligator River National Wildlife Refuge. Each individual sample was given a fecal consistency score (FCS) as a proxy for GI health. Gut microbiome composition from each sample was characterized using a targeted amplicon sequencing approach with the 16S rRNA gene. A higher relative abundance of the bacterial phylum Bacteroidetes was observed in samples obtained from captive wolves compared to the samples from wild wolves. Additionally, an increase in relative abundance of Bacteroidetes and a decreased relative abundance in Firmicutes was seen in red wolves with an FCS of 0 compared to wolves with an FCS of 2. In summary, there are differences in gut microbiome composition between a FCS of 0 and a FCS of 2 and among wild, whole meat, mixed and kibble diet types. Findings from this study increase the understanding of the interplay between diet and GI health in the red wolf, a critical piece of information needed to maintain healthy wolves in this captivity sustained species.