BEHAVIOR AND FECAL MICROBIOME OF GIRAFFES (*GIRAFFA CAMELOPARDALIS*) ENGAGING IN A PUBLIC ENRICHMENT ACTIVITY

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Abstract
The implementation of human-animal interactions by zoological institutions has increased in recent years as it allows zoo guests the opportunity to directly interact with animals' increasing education and conservation efforts. Additionally, it exposes the animals to a form of enrichment activity which can positively affect animal welfare and behavior. Giraffes (*Giraffa camelopardalis*) specifically have been highlighted for usage in these enrichment activities via guest feeding programs (GFP), due to their behavior and prevalence in zoological institutions. However, a pertinent aspect that has been overlooked and undocumented is the effect of these GFP on giraffe health, specifically gut microbiome. Therefore, the current study objectives aimed to characterize the fecal microbiome utilizing 16S rRNA sequencing and evaluate behavior of giraffes engaging and not engaging in a GFP. Giraffes ($n = 6$; 5 female and 1 male) housed at the Fort Worth Zoo were utilized for the outlined trial. Trial consisted of 3 treatment groups and 4 periods in which animals were equally assigned into 3 treatment groups, based upon previous individual involvement in the GFP. Treatments consisted of: High Romaine Eaters (HRE), Intermediate Romaine Eaters (IRE), and Non-Romaine Eaters (NRE). Periods were 7 days in duration, in which the first period and last period only behavior observations were performed, while intermediate periods consisted of both fecal and behavior collections. Behavior was monitored for each giraffe using an instantaneous scan sampling method over a timeframe of 6 days, for a maximum of 1680 minutes observed for each giraffe (7 hours/day). Fecal samples were collected individually on day 1, 3, 4, and 7 for 16S rRNA sequencing analysis. Behavior results were analyzed using JMP (JMP Pro 16.0) and sequencing results performed utilizing R (2023.06.1). Giraffes that did not engage in GFP showed increased ($P \leq 0.01$) time spent foraging from hay-feeder sources, being locomotive, engaging in social interaction, and object exploration as compared to giraffes that engage in GFP. Additionally, giraffes not engaging in GFP had a tendency ($P = 0.09$) for increased time spent object licking as compared to those giraffes that engage in GFP. Bacterial beta-diversity differed ($P \leq 0.01$) as a result of GFP. However, alpha diversity was not impacted ($P \geq 0.38$) by giraffe engagement in GFP. There were distinct alterations in taxonomic compositions as a result of giraffe engagement in GFP. *Firmicutes* (64.2%, 67.7%, 61.8%), *Bacteroidetes* (28.6%, 24.2%, 31.0%), and *Verrucomicrobiota* (1.5%, 1.72%, 2.4%) represented the most predominant phyla (~95%) for HRE, IRE, and NRE, respectively. For which there was a higher ($P \leq 0.01$) *Bacteroidetes:Firmicutes* ratio observed for NRE as compared to HRE and IRE. Therefore, it seems giraffe engagement in guest feeding programs influenced behavior and fecal microbiome parameters, specifically the inter-variability of bacterial communities. These findings indicate that direct contact with individuals of the public via supplementation with romaine leaves seem to be influencing giraffe gut bacterial communities, which could be affecting giraffe digestion.