

Adaptation to captivity drives rapid changes in the rhinoceros gut microbiome

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While long-term effects of captivity (*i.e.*, gastrointestinal disease and endocrine dysfunction) have been observed in a number of animal species, the transition from in- to ex-situ and subsequent impacts on host fitness are not fully understood. Here, we examined microbiota extirpation and replacement over a two-year period in wild-born southern white rhinoceros (SWR; *Ceratotherium simum simum*) transitioning into human-care (wild-born/captive) by 16S rRNA sequencing and untargeted mass spectrometry. We also compared these data to measures of rhinos born in captivity (captive-born/captive) and those wild-born and wild-ranging (Kruger National Park, wild-born/wild), finding that both microbiota and metabolome differed significantly across populations (PERMANOVA; $P < 0.001$). Adaptation to captivity rapidly altered the microbiome, reducing both variance between individuals and microbial richness within 100 days. Despite further changes occurring over two years, the wild-born/captive population retained a distinct composition from captive-born/captive individuals even though approximately 60% of bacterial taxa found late in transitioning rhinos are associated with the captive-born/captive population by source tracking. Taxa lost included members of Spirochaetia and well-known fiber degraders such as *Fibrobacter* spp., while taxa gained included the

Coriobacteriales, an order linked to phytoestrogen-associated endocrine disruption in captive-born SWR. After the two-year period, microbes from both wild-born/captive and captive-born/captive populations were metabolically active upon phytoestrogen exposure, as observed by Raman microspectroscopy following heavy water incubations. Altogether, these data indicate that microbial extirpation and replacement occurs rapidly and alters microbiome activity. These findings provide new insight into how environmental change affects gut microbiota composition and function that may subsequently impact long-term fitness.