

Paromomycin preserves gut microbiota development while controlling neonatal diarrhea in preweaned dairy calves

¹B.P. Santarosa, ¹M.P. Branco, ¹G.L. Marques, ¹K.N. Da Silva, ¹A.L. Lelis, ²G. M. Januário, ³G.G. Silveira, ⁴L. Queiroz, ⁴T.S. dos Santos, ⁴B. Ilha, ⁴V. Gomes, ⁵R. Esteves Lopes

¹Huvepharma®, Porto Alegre, Brazil ²Department of Internal Medicine, School of Veterinary Medicine and Animal Science, University of São Paulo, Brazil ³Federal University of the Jequitinhonha and Mucuri Valleys, Brazil ⁴Karaja Biosciences, São Carlos, São Paulo, Brazil ⁵Huvepharma® NV, Antwerp, Belgium

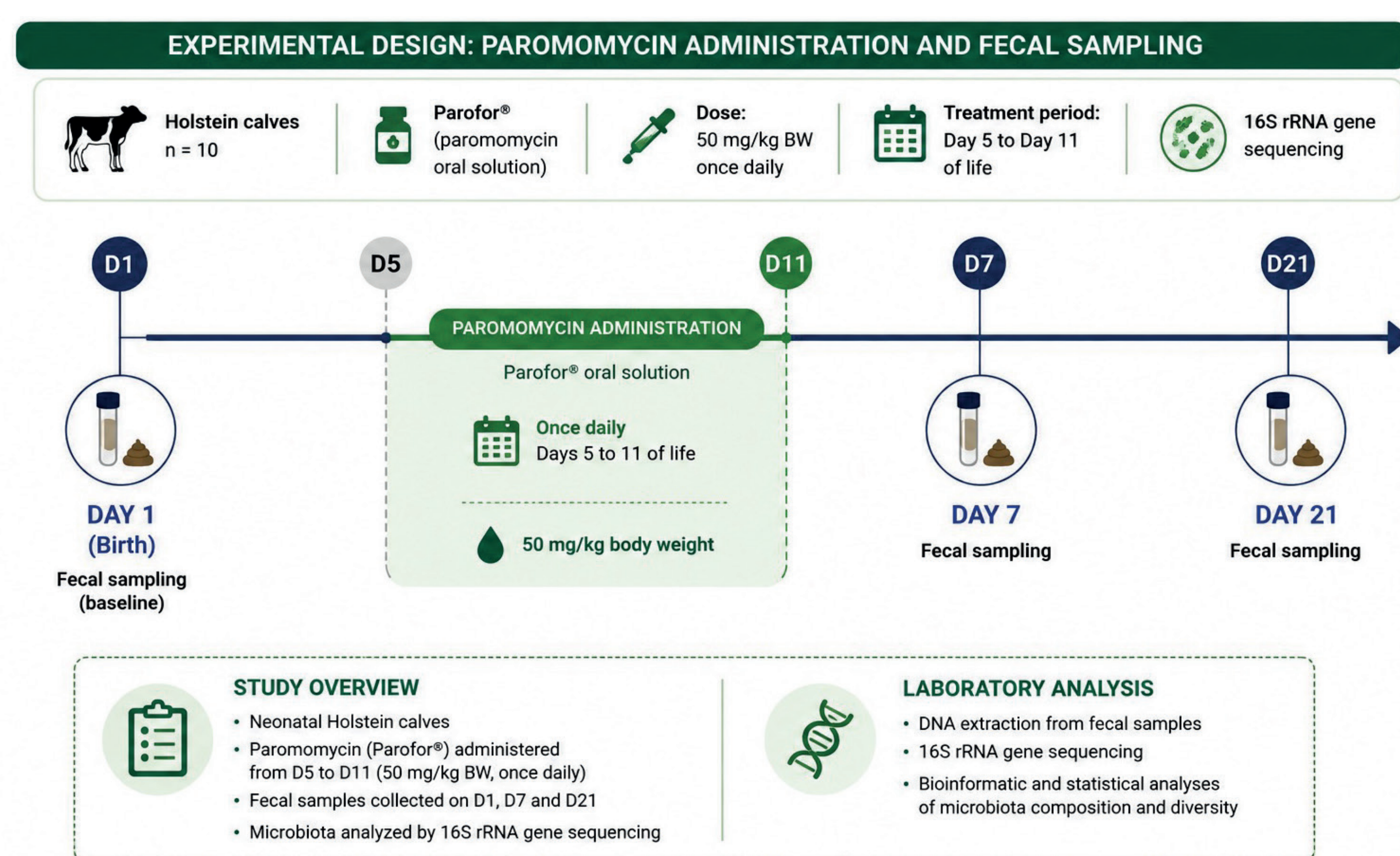
Background and Objectives

Neonatal diarrhea remains a major constraint to calf health and productivity, with early-life gastrointestinal disturbances closely linked to microbiota development. This study evaluated gut microbial dynamics in preweaned Holstein calves receiving paromomycin (Parofo® oral solution) during the neonatal period.

Materials and Methods

The experiment was conducted at a commercial dairy farm in Brazil. Forty newborn calves met inclusion criteria (eutocic calving, adequate vitality, and confirmed passive transfer). Calves were housed individually and fed whole milk (14% total solids) twice daily, with free access to water and a commercial starter (22% crude protein). Paromomycin was administered orally (Parofo oral solution, Huvepharma, 50 mg/kg body weight) once daily from day 5 to day 11 of life.

Fecal samples from a subset of 10 calves were collected at three time points: day 1 (D1; prior to treatment, at 5 days of age), day 7 (D7; end of treatment), and day 21 (D21; 14 days after the end of treatment).



Results

Microbiota composition was analyzed using 16S rRNA gene sequencing, generating 4,801,629 sequences clustered into 6,074 amplicon sequence variants. Alpha diversity indices were primarily influenced by age and individual variation ($p > 0.05$), with a progressive increase over time, consistent with microbiota maturation. Reduced diversity observed in some calves at D21 was associated with diarrhea events, indicating transient dysbiosis. These patterns align with previous studies, reinforcing that early-life microbial succession follows a predictable trajectory. At D1, the microbiota was dominated by facultative anaerobes such as *Escherichia-Shigella* and *Enterococcus*, along with early beneficial genera including *Bifidobacterium* and *Blautia*. By D7, a transitional community emerged, with increased abundance of *Bacteroides*, *Collinsella*, and *Faecalicoccus*, reflecting adaptation to anaerobic conditions. At D21, the microbiota exhibited a more mature and stable profile dominated by *Blautia*, *Lactobacillus*, and *Faecalibacterium*, genera associated with short-chain fatty acid production and gut health. Principal coordinates analysis revealed clear clustering by sampling day (PERMANOVA: $F = 4.983$, $R^2 = 0.249$, $p < 0.001$), confirming age as the primary driver of microbial composition. Differential abundance analysis showed reductions in genera associated with gastrointestinal disturbance and enrichment of commensal fermentative bacteria over time. Importantly, no relevant alterations in microbiota development were attributable to paromomycin.

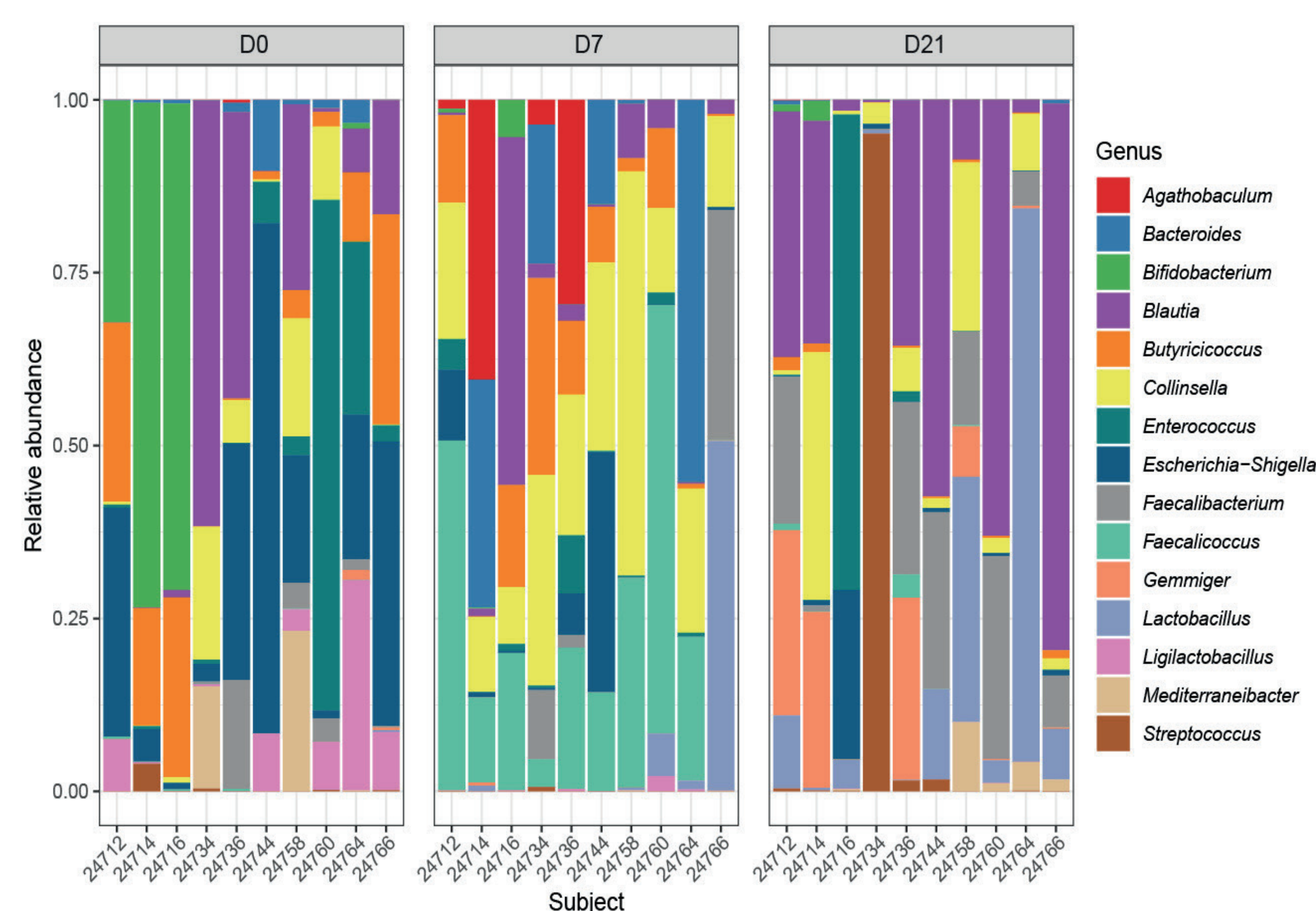


Figure 1. Relative abundance of ASVs classified at the genus level in fecal samples from 10 animals, assessed by 16S rRNA sequencing at days 0 (left), 7 (center), and 21 (right) of the experiment.

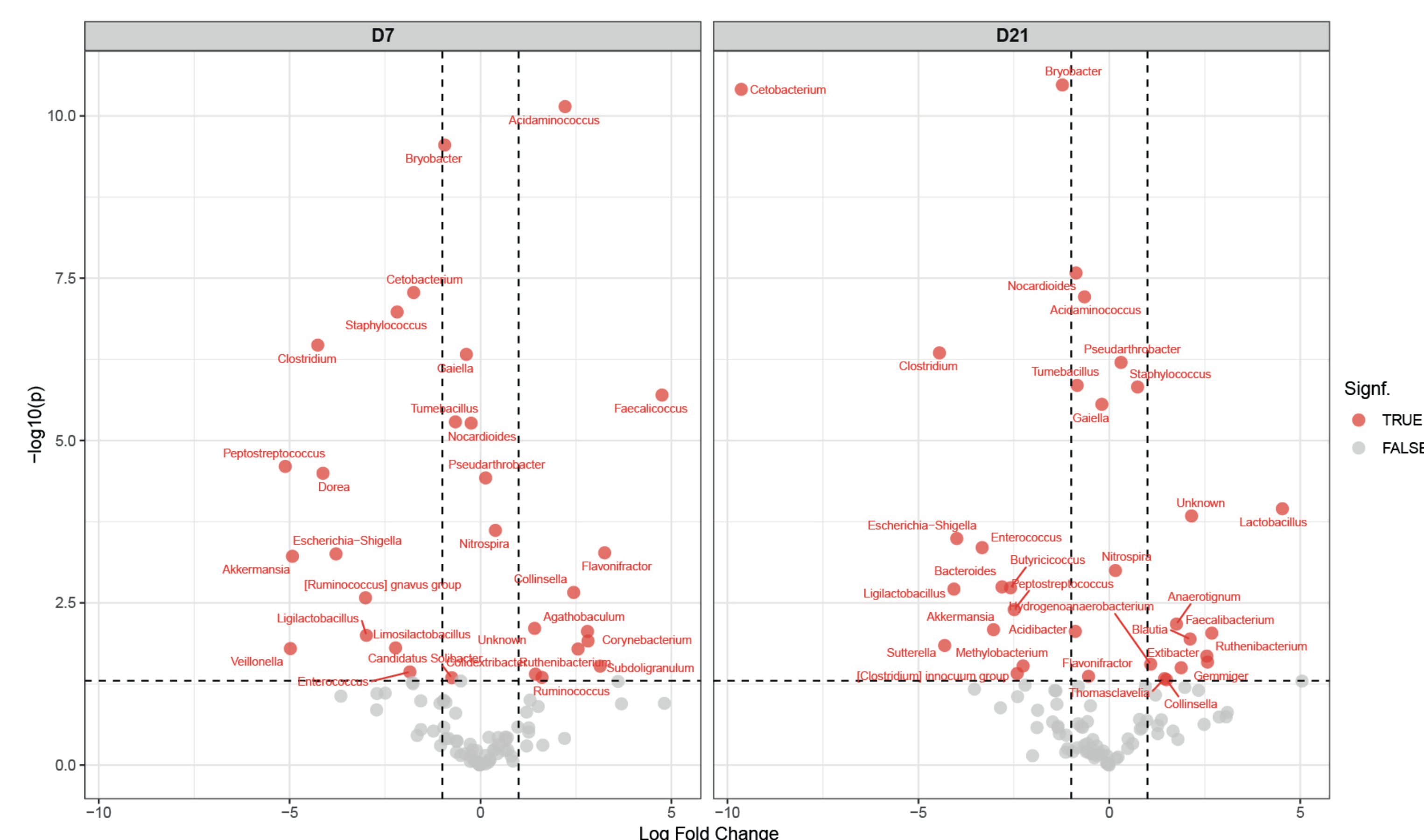


Figure 2. Differential abundance analysis (ANCON-BC2). Red points indicate genera with statistically significant differential abundance ($p < 0.05$), while points to the left and right of zero on the x-axis represent negative and positive log fold change values, respectively.

Conclusion

In conclusion, early-life paromomycin administration does not disrupt gut microbiota maturation and supports the establishment of a stable microbial ecosystem in dairy calves. Therefore, its use is recommended for the treatment and prevention of neonatal diarrhea, as it contributes to reducing diarrhea-associated morbidity and mortality while improving calf performance.

Keywords

dairy cattle • gut microbiota • neonatal diarrhea • microbial maturation