

Supplementary Materials

Effects of maternal rumen microbiota on the development of the microbial communities in the gastrointestinal tracts of neonatal sika deer

Yan Zhang ^{1,2}, Shuang Liang ³, Seongho Choi ^{4,*} and Guangyu Li ^{1,*}

¹ College of Animal Science and Technology, Qingdao Agricultural University, Qingdao, 266109, China

² College of Animal Science and Technology, Jilin Agricultural Science and Technology University, Jilin, 132109, China

³ Department of Animal Science, College of Animal Sciences, Jilin University, Changchun, 130062, China

⁴ Department of Animal Science, Chungbuk National University, Cheongju, 28644, South Korea

*Corresponding author: tcsly@126.com (G.L.); seongho@cbnu.ac.kr (H.S.)

Materials and methods

Animal

All animal procedures were approved by the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences Institutional Animal Care and Use Committees (No. ISAPSAEC-2021-37).

Sampling and analysis

The body weight (BW) of sika deer calves was weighed weekly, and the average daily gain (ADG) was calculated by the difference in BW from experimental day 1 (BW₁) to 35 (BW₂) weighing and divided by the number of days. The ADG was calculated by the following formula:

$$\text{ADG} = (\text{BW}_2 - \text{BW}_1)/35$$

where BW₁ and BW₂ are the body weights of calves on days 1 and 35 of the experiment, respectively.

For rumen fermentation of deer calves, the pH values of the rumen fluid were measured immediately using a pH meter (Hanna Instruments Edge Dedicated pH/ORP meter HI2002). At the same time, an aliquot of rumen fluid (two 1 mL) was taken to determine the volatile fatty acid (VFA) and ammonia-N concentrations. The 1 mL aliquot for VFA analysis was determined as described previously [1], with slight modification. For each rumen fluid sample, a 1 mL aliquot was extracted with 100 μ L phosphoric acid solution (15%), 20 μ L isocaproic acid solution (75 μ g/mL; internal standard), and 280 μ L ether, followed by 10 min centrifugation at 12,000 rpm. The supernatant layers were transferred to glass tubes for GC quantitation. One microliter of rumen fluid extract was injected under a split ratio of 10:1 and separated by an Agilent HP-INNOWAX column (30 m*0.25 mm ID*0.25 μ m, Santa Clara, CA, USA) with 1 mL per min helium flowing through the column. The initial oven temperature was set at 90°C, programmed to 120°C by 10°C/min, to 150°C by 5°C/min, to 250°C by 25°C/min, and finally held at 250°C for 2 min. The other 1 mL aliquot was used for ammonia concentration analysis using the colorimetric method [2]. In brief, the 1 mL aliquot was centrifuged at 12,000 rpm for 10 min, and then 10 μ L of the

supernatant layers were transferred to a 96-well plate and mixed with 100 μ L phenol solution (50 μ g/mL of sodium nitroprusside and 10 mg/mL of phenol) and 90 μ L sodium chlorate solution (5 mg/mL NaOH, 37.85/mL Na₂HPO₄, and 5% sodium chlorate). After 30 min of incubation at 37°C, the absorbance was measured at 550 nm, and the ammonia concentration was calculated based on the standard curve.

DNA extraction and microbiota analysis

Total DNA was extracted from rumen fluid and fecal samples according to the manufacturer's instructions. Briefly, rumen fluid and fecal samples were collected and stored at -80°C. DNA was isolated by incubating samples at 55°C overnight in nuclear lysis solution (200 mM NaCl, 100 mM Tris, 20 mM EDTA, SDS, and proteinase K). Then, protein precipitation solution was added and centrifuged at 15,000 \times g at 4°C for 6 min. Supernatants were collected and incubated with isopropanol. The precipitated DNA was obtained by centrifugation at 15,000 \times g at 4°C for 6 min, washed twice with 70% cold ethanol and resuspended in TE buffer. The quality of the DNA samples was assessed by 1.2% agarose (Invitrogen, United States) gel electrophoresis, and the concentrations were quantified by a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, United States).

Rumen fluid and fecal DAN samples were amplified by PCR using barcoded primer pairs targeting V3-V4. The PCR cycling conditions were as follows: initial denaturation at 98°C for 2 min, followed by 27 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. The amplicons were then sequenced with 2 \times 250 paired-end sequencing using an Illumina MiSeq platform. Amplicon sequencing variants (ASVs) were classified into taxa based on the Greengenes database (version 13.8) classifier with 99% similarity.

Table S1. Sequence data statistics of rumen fluid samples.

	CON (n=5)	FRF (n=6)	MCON (n=5)	MFRF(n=6)
No. of input paired reads	70,935 \pm 3,507	71,149 \pm 6,798	84,991 \pm 4,871	84,110 \pm 6,882
Quality-filtered reads	64,145 \pm 3,068	64,885 \pm 6,433	76,979 \pm 4,820	76,399 \pm 6,468
Denoised reads	61,998 \pm 3,050	62,196 \pm 5,986	74,352 \pm 4,679	73,718 \pm 6,277
Merged reads	55,705 \pm 3,880	53,998 \pm 4,561	64,898 \pm 3,620	64,579 \pm 6,408
Chimera-filtered sequences	42,081 \pm 3,648	41,912 \pm 3,498	45,414 \pm 1,623	46,638 \pm 3,564
Singleton-filtered sequences	41,144 \pm 3,812	40,682 \pm 3,441	43,262 \pm 1,274	44,799 \pm 3,536

CON, control group sika deer calves inoculated without fresh rumen fluid and were grown separately from mother deer over the trial; FRF, fresh rumen fluid group sika deer calves inoculated with fresh rumen fluid and were grown separately from mother deer over the trial; MCON and MFRF represent deer mothers corresponding to CON and FRF groups sika deer calves, respectively; n=the number of sika deer calves.

Table S2. Sequence data statistics of fecal samples.

	T1CON (n=5)	T1FRF (n=6)	T2CON (n=5)	T2FRF (n=6)	T3CON (n=5)	T3FRF (n=6)
No. of input paired reads	90,017 \pm 25,164	78,922 \pm 15,160	67,994 \pm 17,563	71,914 \pm 5,702	64,842 \pm 6,597	72,773 \pm 5,231
Quality-filtered reads	81,896 \pm 22,815	72,177 \pm 14,193	60,741 \pm 14,713	65,399 \pm 5,350	59,209 \pm 6,437	66,279 \pm 4,614
Denoised reads	80,429 \pm 22,630	71,173 \pm 13,840	58,944 \pm 13,829	63,703 \pm 5,335	57,697 \pm 6,491	64,747 \pm 4,668
Merged reads	77,732 \pm 22,562	69,436 \pm 12,833	54,270 \pm 11,154	59,381 \pm 5,502	53,711 \pm 7,339	60,335 \pm 5,293
Chimera-filtered sequences	62,551 \pm 19,913	57,080 \pm 10,517	42,891 \pm 8,498	45,848 \pm 4,821	39,741 \pm 5,353	48,129 \pm 4,657
Singleton-filtered sequences	62,076 \pm 20,154	56,903 \pm 10,514	42,245 \pm 7,852	45,318 \pm 4,847	39,257 \pm 5,565	47,659 \pm 4,848

CON, control group sika deer calves inoculated without fresh rumen fluid and were grown separately from mother deer over the trial; FRF, fresh rumen fluid group sika deer calves inoculated with fresh rumen fluid and were grown separately from mother deer over the trial; T1, T2, and T3 represent days 1, 28, and 35 of the experiment, respectively; n = the number of sika deer calves.

Supplemental figure legends

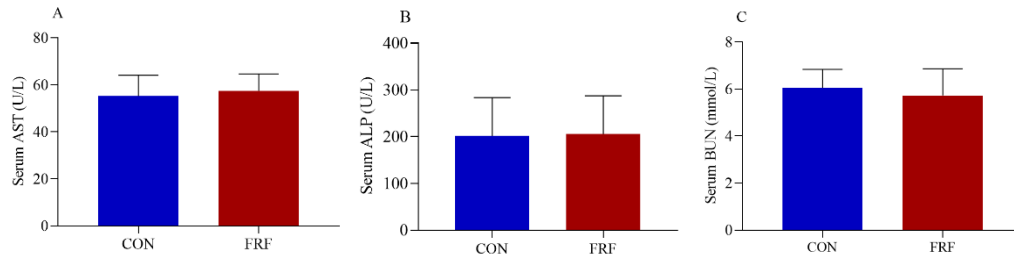


Figure S1. Effect of maternal rumen microbiota transplantation on serum biochemical parameters of sika deer calves. The levels of serum AST (A), ALP (B), and BUN (C) were determined using commercial ELISA kits. CON (n = 5), control group sika deer calves inoculated without fresh rumen fluid and were grown separately from mother deer over the trial; FRF (n = 6), fresh rumen fluid group sika deer calves inoculated with fresh rumen fluid and were grown separately from mother deer over the trial; n = the number of sika deer calves.

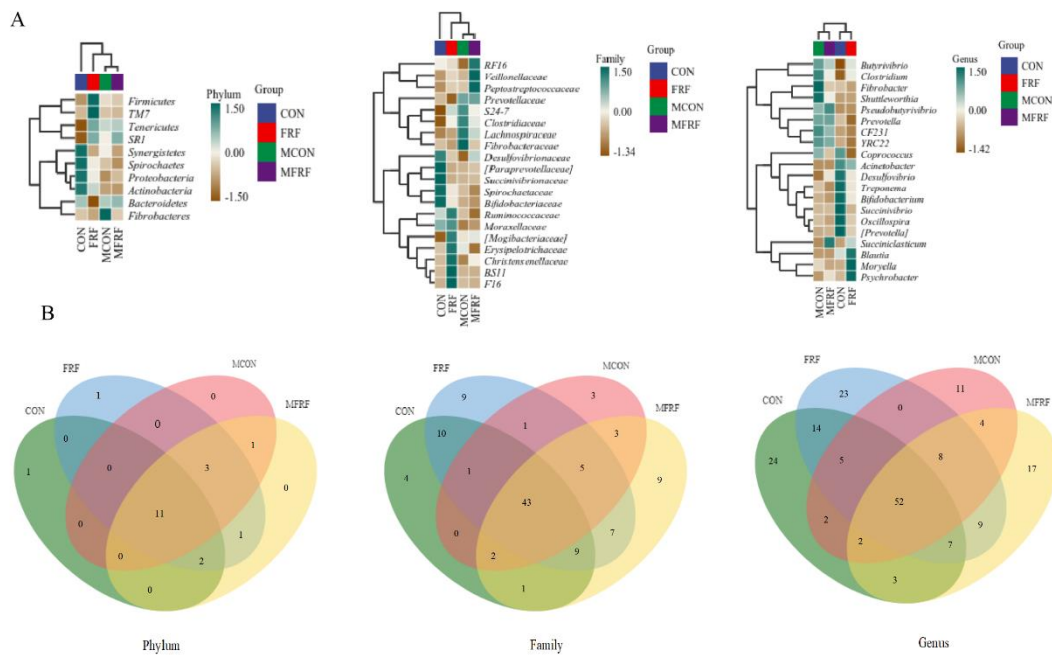


Figure S2. Effect of maternal rumen microbiota transplantation on the rumen microbial taxa of sika deer calves based on heatmaps and Venn diagrams. Heatmaps displaying the microbial taxa at the phylum, family, and genus levels (A). Venn diagrams displaying the numbers of shared microbial phyla, families, and genera within the sample categories at experiment day 35 (B). CON (n = 5), control group sika deer calves inoculated without fresh rumen fluid and were grown separately from mother deer over the trial; FRF (n = 6), fresh rumen fluid group sika deer calves inoculated with fresh rumen fluid and were grown separately from mother deer over the trial; MCON and MFRF represent deer mothers corresponding to CON and FRF groups sika deer calves, respectively; n = the number of sika deer calves.

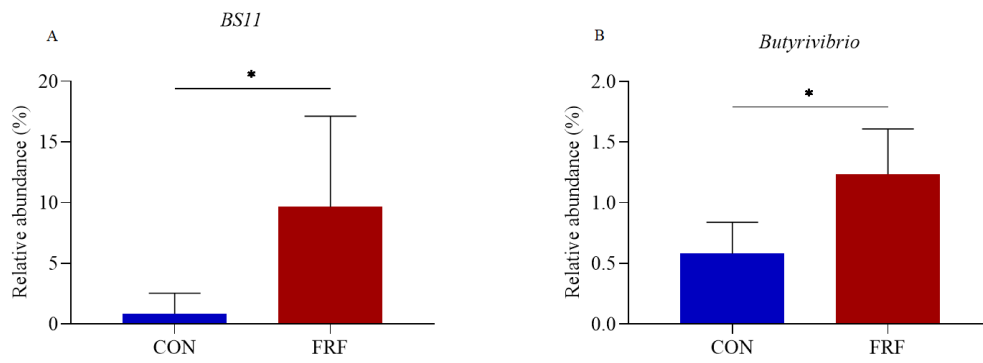


Figure S3. The relative abundances of rumen microbiota on experimental day 35.

CON (n = 5), control group sika deer calves inoculated without fresh rumen fluid and were grown separately from mother deer over the trial; FRF (n = 6), fresh rumen fluid group sika deer calves inoculated with fresh rumen fluid and were grown separately from mother deer over the trial; n = the number of sika deer calves; * $P < 0.05$.

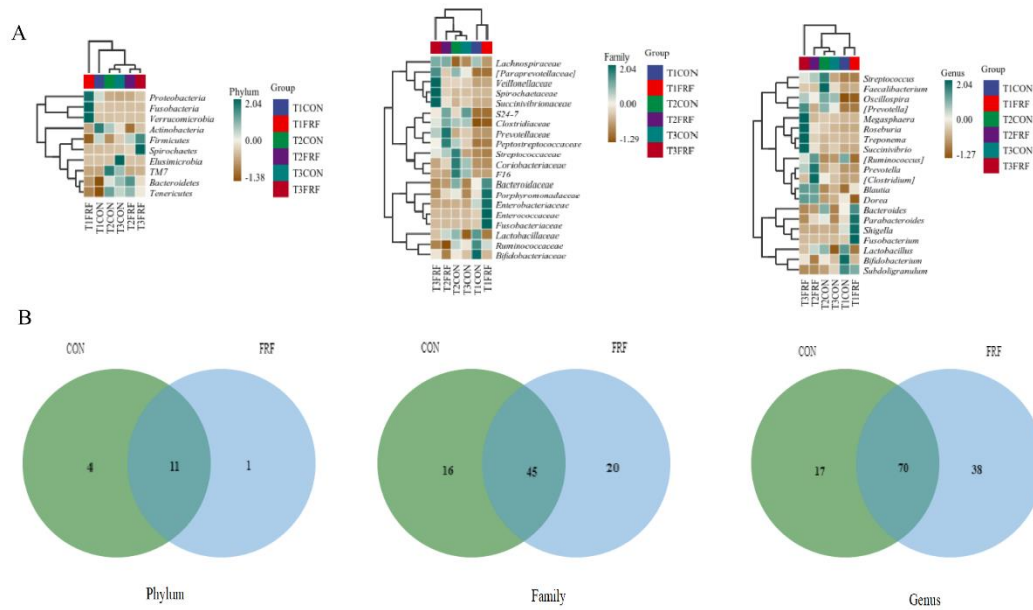


Figure S4. Effect of maternal rumen microbiota transplantation on the fecal microbial taxa of sika deer calves based on heatmaps and Venn diagrams. Heatmaps displaying the microbial taxa at the phylum, family, and genus levels (A). Venn diagrams displaying the numbers of shared microbial phyla, families, and genera within the sample categories at experiment day 35 (B). T1, T2, and T3 represent days 1, 28, and 35 of the experiment, respectively; CON (n = 5), control group sika deer calves inoculated without fresh rumen fluid and were grown separately from mother deer over the trial; FRF (n = 6), fresh rumen fluid group sika deer calves inoculated with fresh rumen fluid and were grown separately from mother deer over the trial; n = the number of sika deer calves.

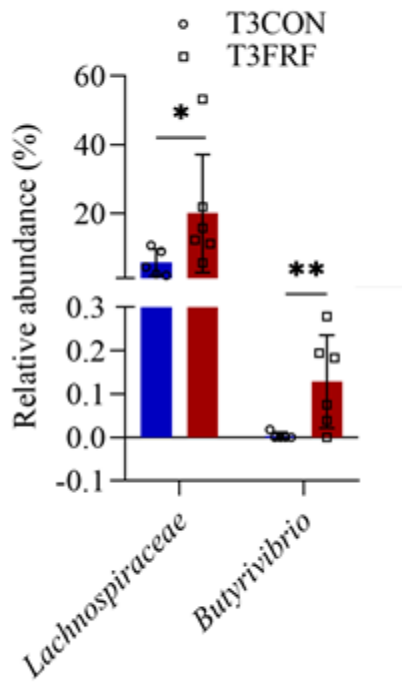


Fig. S5. The relative abundances of fecal microbiota on experimental day 35. T3 represents day 35 of the experiment; CON (n = 5), control group sika deer calves inoculated without fresh rumen fluid and were grown separately from mother deer over the trial; FRF (n = 6), fresh rumen fluid group sika deer calves inoculated with fresh rumen fluid and were grown separately from mother deer over the trial; n = the number of sika deer calves; * $P < 0.05$, ** $P < 0.01$.

References

1. Zhang S, Wang H, Zhu MJ. A sensitive GC/MS detection method for analyzing microbial metabolites short chain fatty acids in fecal and serum samples. *Talanta*. 2019;196:249-54. Epub 2019/01/27. doi: 10.1016/j.talanta.2018.12.049. PubMed PMID: 30683360.
2. Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. *J Clin Pathol*. 1960;13(2):156-9. Epub 1960/03/01. doi: 10.1136/jcp.13.2.156. PubMed PMID: 13821779; PubMed Central PMCID: PMC480024.