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Effect of *Propionibacterium acidipropionici* P169 on the rumen and faecal microbiomes of beef cattle fed a maize-based finishing diet

E. Azad, N. Narvaez, H. Derakhshani, A.Y. Allazeh, Y. Wang, T.A. McAllister and E. Khafipour

Table S1. **Effect of *Propionibacterium acidipropionici P169* supplementation and site of sampling (liquid and solid fragments of rumen digesta) on the relative abundances of bacterial taxa.**

Taxa present		Percentage of sequences in:					*P*-value		
phylum	Genus[1]	Liquid		Solid		SED[2]	Treatment	Site	Trt*Site
		Control	Probiotic	Control	Probiotic				
Above 1% of population									
Bacteroidetes	Unclass. *Bacteroidales* (o)	2.485	5.167	1.598	2.942	1.09	0.121*	0.107*	0.917*
Firmicutes	*Bulleidia*	1.601	2.583	2.179	2.401	0.710	0.590*	0.074*	0.181*
Firmicutes	*Butyrivibrio*	7.947	6.893	9.029	5.878	1.715	0.203*	0.636*	0.226*
Firmicutes	Unclass. *Clostridia* (c)	1.289	1.209	1.879	1.757	0.293	0.587*	0.003*	0.452*
Firmicutes	Unclass. *Clostridiaceae* (f)	0.418	1.507	0.487	1.756	0.520	0.006*	0.415*	0.506*
Firmicutes	Unclass. *Clostridiales* (o)	0.712	1.173	0.937	1.326	0.172	0.005*	0.155*	0.359*
Firmicutes	Unclass. *Coriobacteriaceae* (f)	2.163	1.981	2.676	1.913	0.697	0.473*	0.577*	0.468*
Proteobacteria	*Desulfovibrio*	1.528	1.558	1.309	1.242	0.298	0.623**	0.365**	0.850**
Firmicutes	*Dialister*	9.038	5.551	8.104	3.962	2.128	0.097**	0.354**	0.919**
Firmicutes	Unclass. *Lachnospiraceae* (f)	3.956	7.608	5.573	11.075	1.994	0.001*	0.013*	0.995*
Firmicutes	*Phascolarctobacterium*	0.471	1.345	1.053	1.361	0.304	0.016*	0.275*	0.397*
Bacteroidetes	*Prevotella*	21.069	15.283	17.946	13.673	2.890	0.027*	0.990*	0.834*
Tenericutes	Unclass. RF39 (o)	1.554	1.848	1.789	2.062	0.779	0.391*	0.416*	0.986*
Firmicutes	Unclass. Ruminococcaceae (f)	8.386	13.534	8.474	10.830	1.954	0.071	0.259	0.229
Firmicutes	*Ruminococcus*	2.730	2.983	2.284	5.258	2.622	0.837*	0.309*	0.557*
Bacteroidetes	Unclass. S24-7 (f)	3.384	3.596	2.502	4.012	1.153	0.759*	0.959*	0.418*
Firmicutes	*Sharpea*	4.128	1.788	3.543	2.364	1.38	0.167*	0.362*	0.511*
Firmicutes	*Shuttleworthia*	5.810	3.505	7.012	4.482	1.838	0.546**	0.744**	0.932**
Cyanobacteria	Unclass. *Streptophyta* (o)	0.182	0.417	2.549	1.640	0.449	0.887*	>0.001*	0.018*
Proteobacteria	Unclass. *Succinivibrionaceae* (f)	7.168	7.939	6.007	8.151	4.873	0.521*	0.663*	0.543*
Firmicutes	Unclass. *Veillonellaceae* (f)	4.078	1.739	3.399	1.606	0.654	0.005*	0.988*	0.532*
Between 0.1% and 1% of population									
Firmicutes	*Acidaminococcus*	0.664	0.454	1.318	0.556	0.138	<.0001	0.005	0.041
Actinobacteria	Unclass. *Bifidobacteriaceae* (f)	0.698	0.252	0.872	0.472	0.671	0.470*	0.724*	0.499*
Actinobacteria	*Bifidobacterium*	0.512	0.286	0.317	0.279	0.215	0.496*	0.781*	0.826*
Bacteroidetes	CF231	0.116	0.239	0.057	0.109	0.071	0.541*	0.285*	0.567*
Firmicutes	Unclass. *Christensenellaceae* (f)	0.021	0.196	0.050	0.301	0.134	0.088*	0.626*	0.856*
Firmicutes	*Coprococcus*	0.125	0.149	0.337	0.244	0.100	0.998*	>0.001*	0.342*
Firmicutes	Unclass. *Erysipelotrichaceae* (f)	0.147	0.168	0.222	0.151	0.072	0.381*	0.231*	0.121*
Firmicutes	*Firmicutes* (p)	0.436	0.339	0.407	0.427	0.074	0.557*	0.437*	0.251*
Firmicutes	*Lachnobacterium*	0.317	0.016	0.156	0.007	0.184	0.215*	0.465*	0.516*
Firmicutes	*Megasphaera*	0.458	0.407	0.842	0.526	0.350	0.314*	0.446*	0.055*
Proteobacteria	Unclass. Mitochondria (f)	0.019	0.027	0.467	0.365	0.135	0.671*	0.001*	0.580*
Firmicutes	*Mitsuokella*	0.340	0.261	0.378	0.304	0.078	0428*	0.505*	0.968*
Firmicutes	*Moryella*	0.065	0.169	0.073	0.197	0.101	0.438*	0.469*	0.758*
Firmicutes	*Oscillospira*	0.221	0.454	0.136	0.332	0.186	0.277*	0.324*	0.868*
Firmicutes	*Pseudoramibacter*	0.216	0.339	0.347	0.403	0.093	0.648*	0.008*	0.330*
Firmicutes	*Succiniclasticum*	0.452	0.385	0.446	0.713	0.340	0.130*	0.451*	0.449*
Proteobacteria	*Succinivibrio*	0.850	0.314	0.345	0.332	0.397	0.019*	0.093*	0.816*
Spirochaetes	*Treponema*	0.661	0.273	0.166	0.386	0.337	0.666*	0.419*	0.291*
Bacteroidetes	YRC22	0.229	0.211	0.201	0.113	0.096	0.048*	0.068*	0.651*

* Statistical analyses were conducted on $\log_{10}$-transformed data following MIXED procedure of SAS.

** *P*-value obtained following GLIMMIX procedure of SAS.

[1] Some sequences could only be affiliated to phylum (p), class (c), order (o), or family (f) levels.

[2] SED = standard error of difference between least square means of treatments.

Table S2. Effect of *Propionibacterium acidipropionici P169* supplementation on the relative abundances of bacterial genera in faecal microbial communities.

Taxonomic classification		Percentage of sequences in:		SED[1]	P-value
Phylum	Genus[2]	P169	control		
Above 1% of population					
Actinobacteria	*Bifidobacterium*	1.041	0.958	0.908	0.241*
Bacteroidetes	Unclass. *Bacteroidales* (o)	1.452	0.704	0.650	0.967*
Bacteroidetes	Unclass. *Bacteroidetes* (p)	4.546	3.671	1.532	0.574
Firmicutes	*Blautia*	1.464	0.897	0.517	0.286
Firmicutes	Unclass. *Clostridia* (c)	2.297	2.082	0.384	0.579
Firmicutes	Unclass. *Clostridiaceae* (f)	9.046	12.010	1.923	0.140
Firmicutes	Unclass. *Clostridiales* (o)	2.339	2.876	0.596	0.378
Firmicutes	*Clostridium*	1.254	1.332	0.346	0.824
Actinobacteria	Unclass. *Coriobacteriaceae* (f)	2.161	3.366	0.694	0.098
Firmicutes	Unclass. *Erysipelotrichales* (f)	1.788	0.870	0.503	0.519*
Firmicutes	*Faecalibacterium*	1.581	0.789	0.781	0.829*
Firmicutes	Unclass. *Lachnospiraceae* (f)	6.807	5.718	1.872	0.625*
Firmicutes	Unclass. *Peptostreptococcaceae* (f)	11.636	8.951	1.673	0.124
Bacteroidetes	*Prevotella*	1.979	1.245	0.838	0.831*
Proteobacteria	Unclass. RF32 (o)	1.698	1.728	0.714	0.967
Firmicutes	*Roseburia*	2.537	2.700	1.266	0.774*
Firmicutes	Unclass. *Ruminococcaceae* (f)	9.784	9.083	2.414	0.775
Firmicutes	*Ruminococcus*	10.759	12.943	1.144	0.071
Bacteroidetes	Unclass. S24-7 (f)	3.619	6.525	1.431	0.056
Firmicutes	*Turicibacter*	5.397	7.089	1.136	0.152
Between 1% and 0.1% of population					
Tenericutes	Unclass. *Anaeroplasmatales* (o)	0.334	0.023	0.152	0.089*
Firmicutes	*Anaerostipes*	0.327	0.045	0.120	
Firmicutes	*Anaerovibrio*	0.067	0.157	0.065	0.030*
Bacteroidetes	*Bacteroides*	0.793	0.474	0.222	0.873*
Firmicutes	*Bulleidia*	0.454	0.518	0.170	0.974*
Firmicutes	*Butyrivibrio*	0.4267	0.476	0.129	0.904*
Bacteroidetes	CF231	0.867	0.927	0.350	0.904
Firmicutes	Unclass. *Christensenellaceae* (f)	0.542	0.322	0.271	0.596*
Firmicutes	*Coprococcus*	0.548	0.585	0.070	0.714
Firmicutes	*Dorea*	0.659	0.410	0.168	0.481*
Firmicutes	Unclass. *Erysipelotrichaceae* (o)	0.436	0.492	0.115	0.733
Firmicutes	Unclass. *Firmicutes* (p)	0.225	0.247	0.023	0.501
Firmicutes	*Lachnobacterium*	0.215	0.298	0.166	0.937*
Firmicutes	*Olsenella*	0.123	0.195	0.063	0.528*
Firmicutes	*Oscillospira*	0.828	0.508	0.166	0.242*
Bacteroidetes	*Paludibacter*	0.309	0.025	0.257	0.458*
Bacteroidetes	*Parabacteroides*	0.567	0.536	0.106	0.801*
Firmicutes	*Phascolarctobacterium*	0.350	0.307	0.088	0.731
Firmicutes	Rc4-4	0.568	0.615	0.098	0.744
Bacteroidetes	Unclass. *Rikenellaceae* (f)	0.624	0.616	0.288	0.984
Firmicutes	*Sarcina*	0.489	0.041	0.150	0.084*
Spirochaetes	Unclass. *Spirochaetaceae* (f)	0.151	0.689	0.417	0.419*
Proteobacteria	*Succinivibrio*	1.476	0.286	0.537	0.128*
Proteobacteria	*Sutterella*	0.193	0.445	0.121	0.160
Spirochaetes	*Treponema*	1.327	0.243	0.557	0.036*
Bacteroidetes	5-7N15	0.282	0.172	0.193	0.736*

*Statistical analyses were conducted on $\log_{10}$-transformed data.

[1] SED = standard error of difference between treatments.

[2] Some sequences could only be affiliated to phylum (p), class (c), order (o), or family (f) levels.

Table S3. Ruminal metabolites of cannulated feedlot steers fed a corn-based diet supplemented without (Control) or with *Propionibacterium acidipropionici* (P169).

	Control	**P 169**	**SEM**	**P value**
Total VFA (mM)	144.8	152.5	4.402	0.225
Molar%				
Acetate	48.9	45.7	0.989	0.027
Propionate	36.8	35.3	1.266	0.412
Butyrate	9.6	12.3	0.644	0.004
BCVFA	4.2	5.8	0.307	0.001
Succinate	0.3	0.2	0.026	0.025
A:P	1.50	1.36	0.092	0.289
Lactate (mM)	0.184	0.376	0.156	0.386
NH3_N (mM)	1.05	4.44	0.597	0.001

BCVFA; branched-chain VFA; A:P, acetate:propionate ratio; NH_3-N, Ammonia-N
Adapted from Narvaez *et al*. (2014)

References

Narvaez, N., Alazzeh, A.Y., Wang, Y. and McAllister, T.A., 2014. Effect of *Propionibacterium acidipropionici* P169 on growth performance and rumen metabolism of beef cattle fed a corn- and corn dried distillers' grains with solubles-based finishing diet. Canadian Journal of Animal Science 94: 363-369.

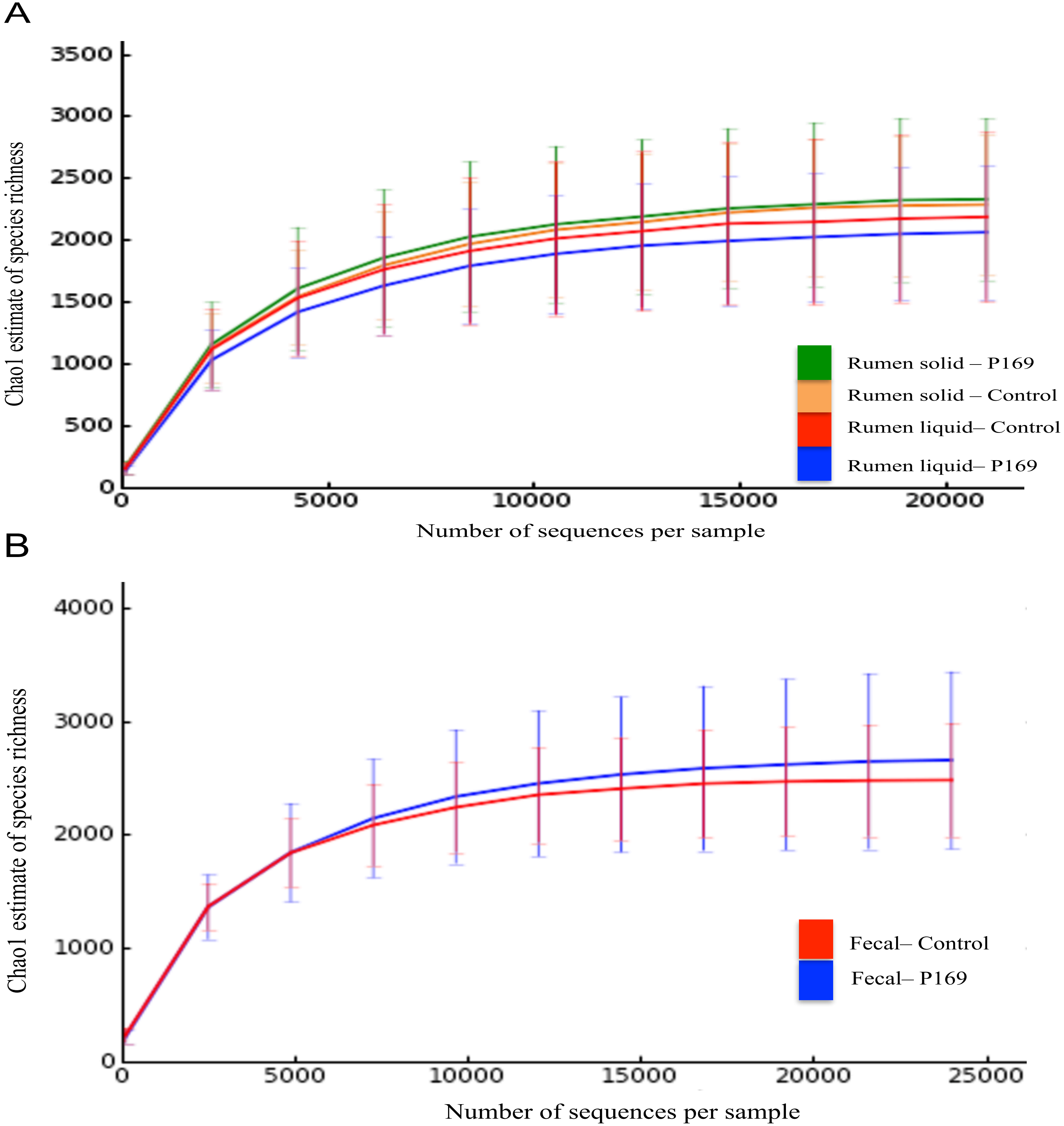
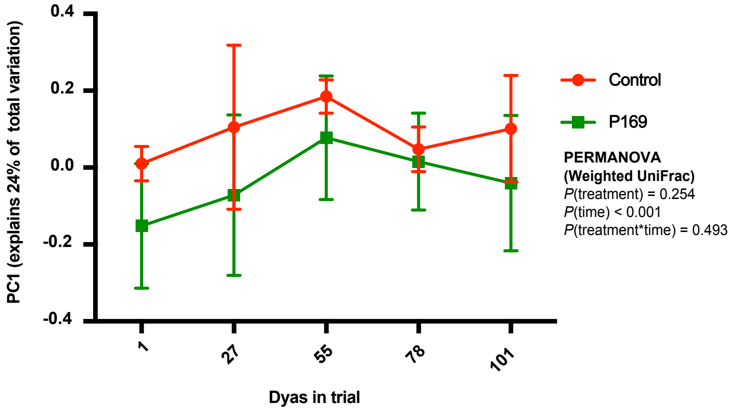
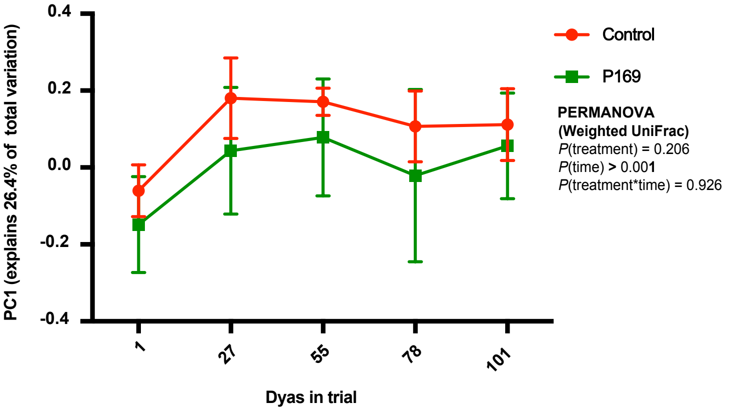


Figure S1. Rarefaction analysis based on Chao1 estimator of species richness. (A) Effect of probiotic supplementation (P169) on species richness of liquid and solid rumen fractions (rarefied at an even depth of 21,000 sequences per sample), (B) Effect of probiotic supplementation (P169) on the species richness of fecal microbial communities rarefied at an even depth of 24,000 sequences per sample. Error bars indicate the 95% confidence intervals.

a) Rumen liquid microbiota



b) Rumen solid microbiota



c) Fecal microbiota

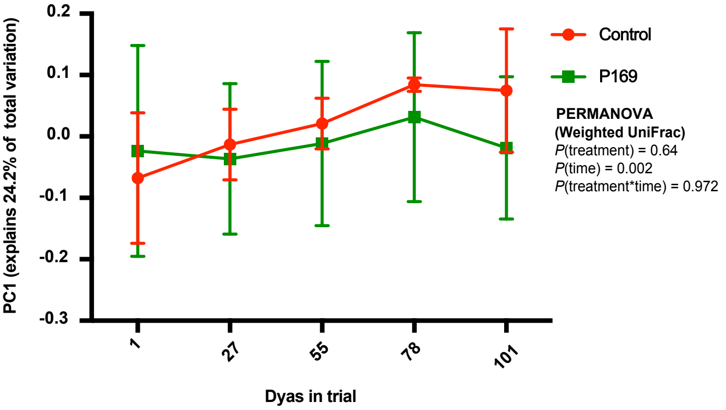


Figure S2. Compositional dynamics of rumen and hindgut microbial communities overtime. Principal coordinate analyses (PCoA) of weighted UniFrac distances of (a) rumen liquid, (b) rumen solid, and (c) faecal microbial communities have been projected over sampling time points. The Y-axis shows the differences between microbial communities as explained by first component (PC1) of the PCoA analysis. The X-axis indicates the sampling time points relative to the starting date of the experiment. Color codes have been used to differentiate between control (red) and P169 (green) groups. Error bars indicate the 95% confidence interval. *P*-value for each comparison was obtained from PERMANOVA and considered significant at $P < 0.05$