

Article

# Effects of Long-Term Dietary Protein Restriction on Intestinal Morphology, Digestive Enzymes, Gut Hormones, and Colonic Microbiota in Pigs

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Supplementary Materials:

**Table S1.** Primers used for bacterial DNA amplification for microbiota composition analysis.

Target Organisms	Primers <sup>1</sup>	Sequences (5'-3')	Annealing Temperature (°C)
Total bacteria	Total-F	GTGSTGCAYGGYYGTCGTCA	60
	Total-R	ACGTCRTCCMCNCCTTCCTC	
<i>Bacteroidetes</i>	Bac 964-F	GGARCATGTGGTTTAATTCGATGAT	60
	Bac 1060-R	AGCTGACGACAACCATGCAG	
<i>Lactobacillus</i>	Lac-F	AGCAGTAGGGAATCTTCCA	61
	Lac-R	ATTCCACCGCTACACATG	
<i>Enterobacteriaceae</i>	Eco-F	CATGCCGCGTGTATGAAGAA	59
	Eco-R	CGGGTAAACGTCAATGAGCAAA	
<i>Clostridium cluster XIV</i>	Clo14-F	CGGTACCTGACTAAGAAGC	60
	Clo14-R	AGTTTYATTCTTGCGAACG	
<i>Clostridium cluster IV</i>	C. leptum-F	GCACAAGCAGTGGAGT	60
	C. leptum-R	CTTCCTCCGTTTTGTCAA	
<i>Ruminococcus</i>	Rum-F	CGAACGGAGATAATTTGAGTTTACTTAGG	60
	Rum-R	CGGTCTCTGTATGTTATGAGGTATTA CC	

<sup>1</sup> F: forward primer; R: reverse primer.

**Table S2.** Richness and diversity estimator of microbiota in the colonic luminal contents of pigs.

	NP	MP	LP	SEM	<i>p</i> -Value
Ace	423.48	422.91	365.44	34.363	0.633
Chao	427.92	431.93	367.67	25.114	0.673
Shannon	3.102	3.171	2.946	0.191	0.395
Simpson	0.141	0.142	0.171	0.018	0.795
Coverage	0.998	0.998	0.998	<0.001	0.503

NP: normal protein diets according to the National Research Council (NRC, 2012); MP: reduced protein by 3% compared to the NP diet supplemented with Lys, Met, Thr, and Trp; LP: reduced protein by 6% compared to the NP diet supplemented with Lys, Met, Thr, and Trp. SEM: standard error of the mean. <sup>a,b,c</sup> Mean values in the same row differ in significance ( $p < 0.05$ ).