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Effects and mode of action of chitosan and ivy fruit saponins on the microbiome, fermentation and methanogenesis in the rumen simulation technique

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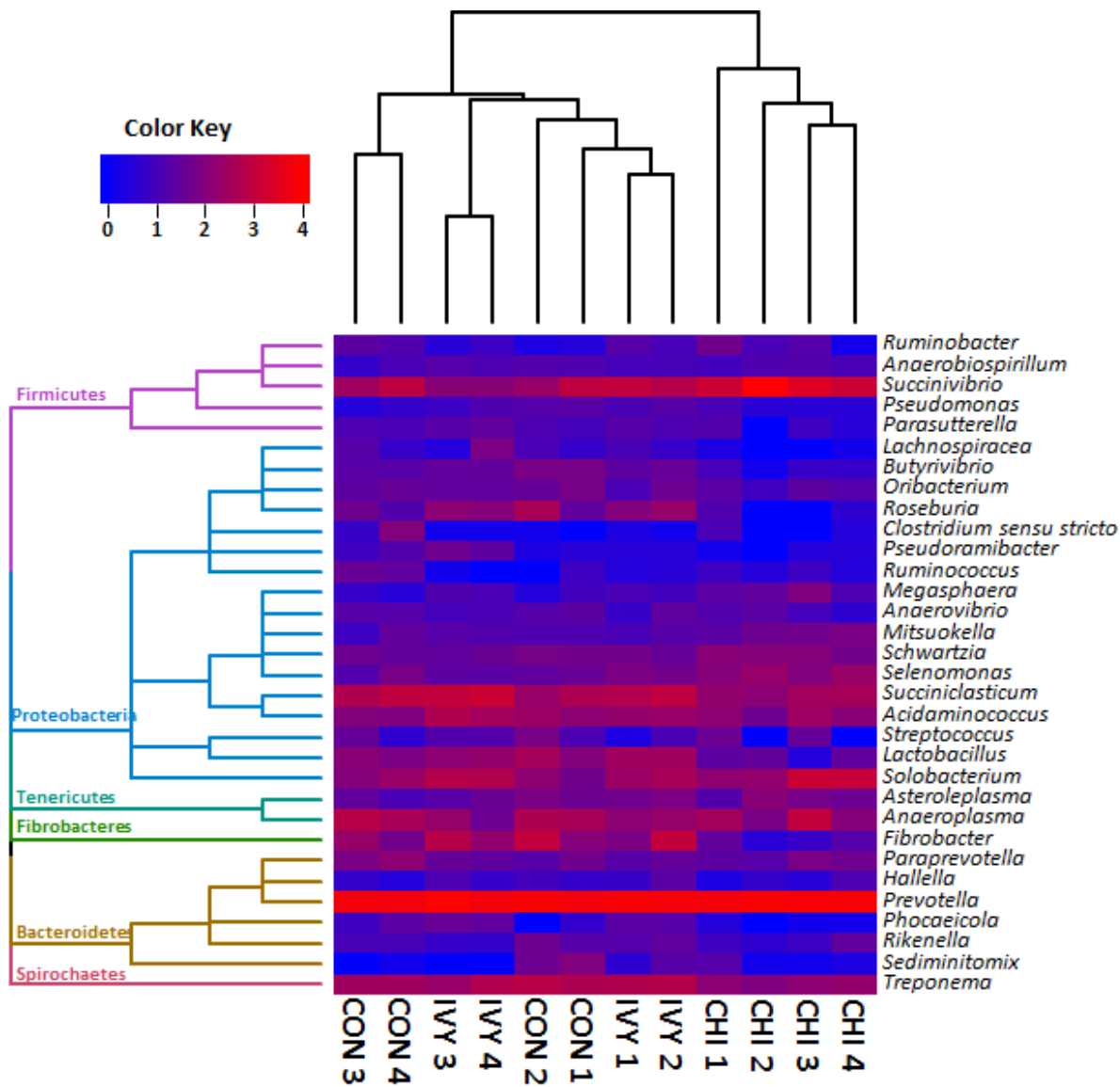
Supplemental Table 1. Ingredients and chemical composition of the experimental diets.

Diets¹	CON	CHI	IVY
Ingredients (g kg⁻¹)			
Alfalfa hay	300	285	285
Grass hay	200	190	190
Barley	300	285	285
Corn	120	114	114
Soya bean meal	77	73	73
Additive	0	50	50
Vitamin premix ¹	3	3	3
Composition (g kg⁻¹)			
Organic matter	949	965	950
Nitrogen	20.5	22.8	19.7
Carbon	405	403	413
Neutral detergent fibre	370	409	342
Acid detergent fibre	211	198	191

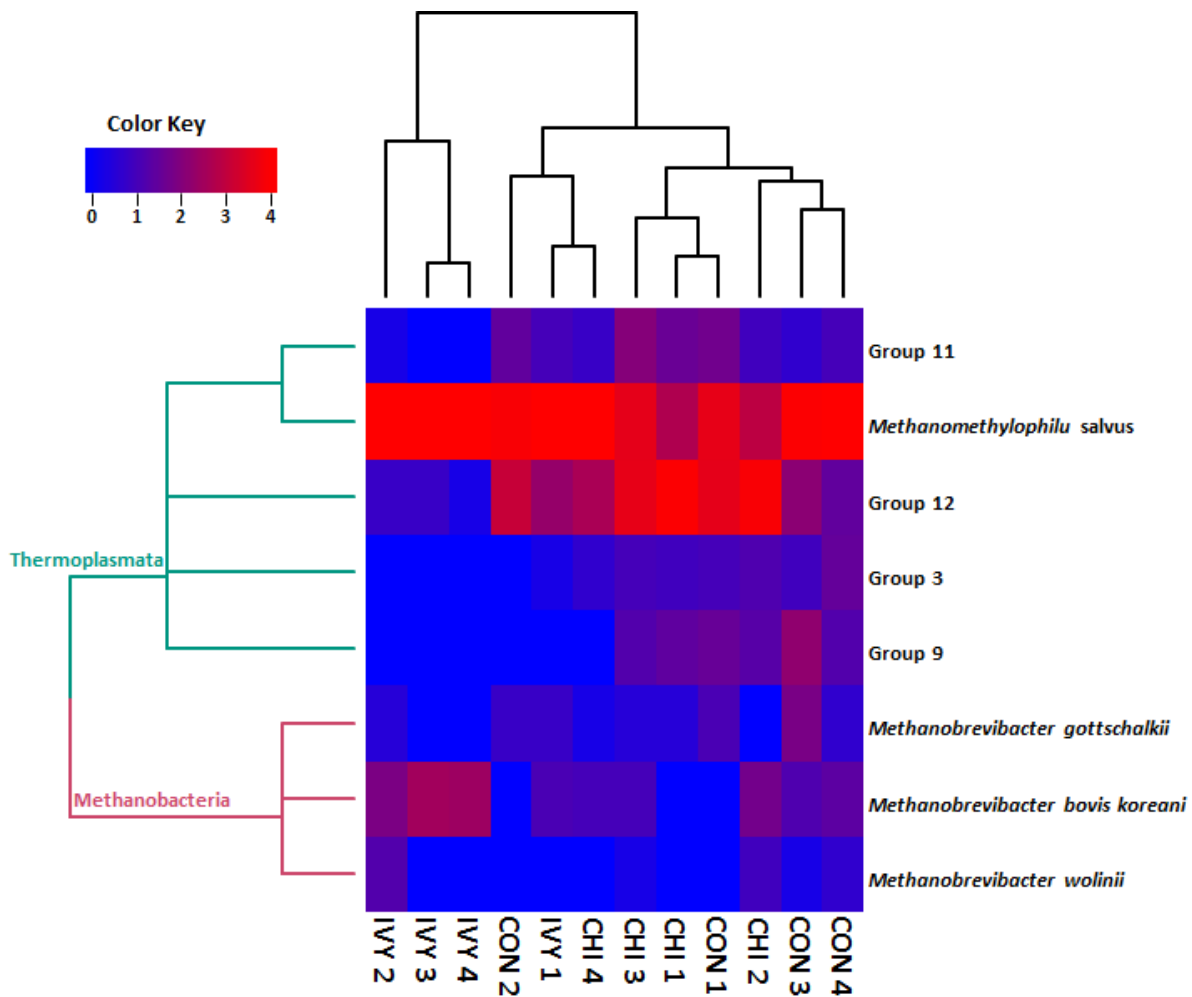
¹ Rumins Cattle GP (Rumeco Ltd, UK.) Declared composition: Ca 240, P 20, Mg 50, Na 80, Se 0.03, Co 0.09, I 0.4, Mn 3, Zn 4 and Cu 1.5 g kg⁻¹, retinol 4×10⁵, cholecalciferol 8×10⁴ and alphotocopherol 10³ IU kg⁻¹

Supplemental Table 2. Primers used for quantitative PCR and Ion-Torrent Next Generation Sequencing.

Target	Author	Forward Primer	Reverse Primer	T ^a	Amplicon (bp)
Quantitative PCR					
Total bacteria	(Maeda <i>et al.</i> , 2003)	GTGSTGCAYGGYTGTCGTCA	ACGTCRTCCMCACCTTCCTC	61	150
Total protozoa	(Sylvester <i>et al.</i> , 2004)	GCTTTCGWTGGTAGTGATT	CTTGCCCTCYAATCGTWCT	55	223
Anaerobic fungi	(Denman & McSweeney, 2007)	GAGGAAGTAAAAGTCGTAACAAGGTTTC	CAAATTCACAAAGGGTAGGATGATT	62	120
Methanogens	(Denman <i>et al.</i> , 2007)	TTCGGTGGATCDCARAGRGC	GBARGTCGWAWCCGTAGAATCC	56	140
Ion Torrent NGS					
Bacterial primers	(Spear <i>et al.</i> , 2008)	AGAGTTTGATCMTGGCTCAG	CTGCTGCCTYCCGTA	58	348
Bacterial Adaptors		CCATCTCATCCCTGCGTGTCTCCGACTCAG	CCTCTCTATGGGCAGTCGGTGAT		
Methanogens primers	(Wright & Pimm, 2003)	GCTCAGTAACACGTGG	GWATTACCGCGGCKGCTG	58	433
Methanogens adaptors		CCATCTCATCCCTGCGTGTCTCCGACTCAG	CCTCTCTATGGGCAGTCGGTGAT		



Supplemental Figure 1. Heat map describing the effect of supplementing a control diet (CON) with chitosan (CHI) or ivy fruit saponins (IVY) on the structure of the rumen bacterial community at the genus level in the Rusitec system. Dendrograms are based on the UPGMA clustering of the Bray-Curtis distances. The total number of reads per sample was log transformed and minor genera were discarded (<1%). Cows used as inoculum are indicated in numbers.



Supplemental Figure 2. Heat map describing the effect of supplementing a control diet (CON) with chitosan (CHI) or ivy fruit saponins (IVY) on the structure of the rumen methanogens community at the species level in the Rusitec system. Dendrograms are based on the UPGMA clustering of the Bray-Curtis distances. The total number of reads per sample was log transformed. Cows used as inoculum are indicated in numbers.

Supplemental Table 3. Effect of supplementing a control diet (CON) with chitosan (CHI) or ivy fruit saponins (IVY) on the relative abundance of the main bacteria at phylum, family and genus level in the Rusitec system.

Phylum	Family	Genus	CON	CHI	IVY	SED	Adjusted P-value	
<i>Actinobacteria</i>	<i>Bifidobacteriaceae</i>	<i>Bifidobacterium</i>	0.94	1.03	1.21	0.134	0.198	
		<i>Coriobacteriaceae</i>	0.46	0.58	0.78	0.272	0.558	
	<i>Eggerthella</i>		0.79	0.89	1.05	0.091	0.123	
		<i>Olsenella</i>	0.40	0.00	0.76	0.207	0.058	
<i>Bacteroidetes</i>	<i>Bacteroidales</i>	<i>Phocaeicola</i>	0.54	0.88	0.74	0.120	0.121	
		<i>Flammeovirgaceae</i>	4.10 ^b	4.24 ^a	4.15 ^{ab}	0.043	0.048	
		<i>Sediminitomix</i>	0.85 ^{ab}	0.30 ^b	1.55 ^a	0.238	0.023	
	<i>Porphyromonadaceae</i>		2.72	1.21	1.94	0.398	0.069	
		<i>Prevotellaceae</i>	1.05	0.62	0.56	0.376	0.474	
	<i>Hallella</i>		0.58	0.45	0.35	0.182	0.527	
		<i>Paraprevotella</i>	4.02	4.07	4.09	0.043	0.437	
		<i>Prevotella</i>	0.87	0.82	1.16	0.204	0.341	
		<i>Others Prevotellaceae</i>	1.89	1.69	1.59	0.146	0.272	
		<i>Rikenellaceae</i>	4.00	4.06	4.07	0.044	0.399	
	<i>Others</i>	<i>Rikenella</i>	2.62 ^{ab}	2.43 ^b	2.69 ^a	0.062	0.036	
			1.41	1.09	1.18	0.254	0.527	
<i>Fibrobacteres</i>	<i>Fibrobacteraceae</i>	<i>Fibrobacter</i>	3.14 ^b	3.71 ^a	3.18 ^b	0.121	0.023	
<i>Firmicutes</i>	<i>Acidaminococcaceae</i>		2.44 ^a	1.16 ^b	2.65 ^a	0.397	0.048	
		<i>Acidaminococcus</i>	3.86 ^a	3.53 ^b	3.84 ^a	0.098	0.048	
	<i>Clostridiaceae</i>	<i>Succiniclasticum</i>	2.96 ^b	2.71 ^c	3.24 ^a	0.085	0.023	
		<i>Sensu stricto</i>	2.26	2.24	2.60	0.182	0.238	
	<i>Erysipelotrichaceae</i>		2.82 ^b	2.52 ^c	3.12 ^a	0.092	0.023	
		<i>Erysipelotrichaceae incertae</i>	1.35	0.86	0.76	0.363	0.288	
	<i>Eubacteriaceae</i>	<i>Solobacterium</i>	0.86	0.46	0.35	0.482	0.570	
		<i>Eubacterium</i>	2.26	2.84	2.79	0.172	0.069	
	<i>Lachnospiraceae</i>		1.08	0.34	0.19	0.254	0.055	
		<i>Pseudoramibacter</i>	2.20	2.80	2.78	0.175	0.052	
		<i>Butyrivibrio</i>	0.94 ^a	0.40 ^b	1.23 ^a	0.148	0.023	
		<i>Catonella</i>	0.27	0.00	0.48	0.164	0.117	
		<i>Coprococcus</i>	0.90 ^a	0.40 ^b	1.19 ^a	0.143	0.029	
		<i>Lachnospiraceae incertae</i>	2.72	2.50	2.78	0.134	0.226	
		<i>Oribacterium</i>	1.71 ^a	0.81 ^b	1.63 ^a	0.218	0.034	
		<i>Pseudobutyrvibrio</i>	0.66	0.78	0.98	0.314	0.664	
		<i>Roseburia</i>	0.60 ^a	0.00 ^b	0.68 ^a	0.142	0.029	
		<i>Syntrophococcus</i>	1.12	0.19	1.19	0.316	0.064	
	<i>Lactobacillaceae</i>	<i>Lactobacillus</i>	1.72	1.36	1.55	0.173	0.272	
	<i>Ruminococcaceae</i>		0.59 ^a	0.12 ^b	0.83 ^a	0.082	0.003	
		<i>Ruminococcus</i>	1.90 ^a	0.52 ^b	2.29 ^a	0.425	0.036	
	<i>Streptococcaceae</i>	<i>Streptococcus</i>	0.68 ^b	0.47 ^b	1.28 ^a	0.165	0.030	
		<i>Veillonellaceae</i>	2.28 ^a	1.35 ^b	2.49 ^a	0.202	0.023	
	<i>Others</i>		1.85	1.67	1.43	0.156	0.133	
		<i>Anaerovibrio</i>	1.09	0.81	0.40	0.382	0.341	
		<i>Megasphaera</i>	1.43	0.87	1.12	0.521	0.588	
		<i>Mitsuokella</i>	2.26 ^b	2.72 ^a	2.25 ^b	0.063	0.002	
		<i>Schwartzia</i>	1.45	1.19	1.22	0.158	0.341	
		<i>Selenomonas</i>	0.82 ^b	1.62 ^a	1.22 ^{ab}	0.146	0.029	
			1.33 ^b	1.79 ^a	1.36 ^b	0.121	0.036	
	<i>Others</i>	1.84 ^b	2.13 ^a	1.74 ^b	0.078	0.029		
	<i>Proteobacteria</i>		1.65 ^b	2.31 ^a	1.74 ^b	0.149	0.034	
		<i>Desulfovibrionaceae</i>	3.69	2.85	3.49	0.282	0.109	
		<i>Pseudomonadaceae</i>	2.91 ^b	3.62 ^a	2.89 ^b	0.255	0.048	
		<i>Succinivibrionaceae</i>	<i>Pseudomonas</i>	1.17	0.88	1.32	0.144	0.111
			<i>Anaerobiospirillum</i>	1.09	0.84	1.25	0.169	0.174
		<i>Sutterellaceae</i>	<i>Ruminobacter</i>	2.84	3.61	2.68	0.283	0.081
<i>Succinivibrio</i>			1.17	1.25	1.25	0.139	0.830	
<i>Parasutterella</i>			0.99	1.20	1.09	0.417	0.882	
<i>Others</i>			2.81	3.60	2.64	0.295	0.064	
			1.24	0.78	1.44	0.239	0.123	
<i>Spirochaetes</i>			1.77	0.94	1.78	0.465	0.253	
<i>Synergistetes</i>	<i>Spirochaetaceae</i>	<i>Treponema</i>	2.76 ^a	2.26 ^b	2.81 ^a	0.125	0.048	
			2.76 ^a	2.26 ^b	2.81 ^a	0.125	0.030	
<i>Tenericutes</i>	<i>Anaeroplasmataceae</i>		0.73	1.16	0.58	0.211	0.087	
		<i>Jonquetella</i>	0.73 ^a	0.08 ^b	0.42 ^{ab}	0.139	0.034	
		<i>Pyramidobacter</i>	0.00 ^b	1.14 ^a	0.27 ^b	0.182	0.023	
<i>Unclassified</i>			2.86 ^a	2.68 ^{ab}	2.38 ^b	0.137	0.048	
			2.82	2.52	2.23	0.206	0.121	
			1.67	1.84	1.77	0.197	0.766	
			3.83 ^a	3.14 ^b	3.75 ^a	0.215	0.048	

¹The total number of reads per sample was log-transformed and minor genera were discarded. Within a raw means without a common superscript differ ($P < 0.05$).

Supplemental Table 4. Effect of supplementing a control diet (CON) with chitosan (CHI) or ivy fruit saponins (IVY) on the relative abundance of the main archaea at family, genus and species level in the Rusitec system.

Family	Genus	Species	CON	CHI	IVY	SED	Adjusted P-value
<i>Methanomassiliicoccaceae</i>			3.06	3.06	3.05	0.005	0.060
	<i>Group 11</i>		2.95	2.55	3.04	0.157	0.066
		<i>Methanomethylophilus alvus</i>	2.94	2.52	3.04	0.162	0.041
		<i>Others</i>	1.00	1.09	0.29	0.242	0.062
	<i>Group 12</i>		1.98 ^b	2.69 ^a	0.87 ^c	0.198	0.001
	<i>Group 3a</i>		1.03	0.86	0.15	0.295	0.052
	<i>Group 9</i>		0.99	0.80	0.00	0.368	0.077
<i>Methanobacteriaceae</i>			1.12	0.96	1.63	0.230	0.060
	<i>Methanobrevibacter</i>		1.12	0.96	1.63	0.230	0.060
		<i>M bovis koreani</i>	0.52	0.77	1.55	0.272	0.062
		<i>M gottschalkii</i>	0.92	0.31	0.29	0.230	0.071
		<i>M wolinii</i>	0.23	0.27	0.25	0.266	0.986

¹The total number of reads per sample was log-transformed. Within a row means without a common superscript differ ($P < 0.05$).