

## Aberystwyth University

### *A metagenomics approach to evaluate the impact of dietary supplementation with *Ascophyllum nodosum* or *Laminaria digitata* on rumen function in *Rusitec* fermenters*

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ONLINE SUPPORTING MATERIAL

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

Diets <sup>1</sup>	CON	ASC	LAM
<b>Ingredients (g/kg)</b>			
Alfalfa hay	300	285	285
Grass hay	200	190	190
Barley	300	285	285
Corn	120	114	114
Soya bean meal	77	73	73
Brown seaweed	0	50	50
Vitamin premix <sup>1</sup>	3	3	3
<b>Composition (g/kg)</b>			
Organic matter	949	940	937
Nitrogen	20.5	20.4	20.8
Neutral detergent fibre	370	366	371
Acid detergent fibre	211	212	214

<sup>1</sup> Ruminants 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, retinol  $4 \times 10^5$ , cholecalciferol  $8 \times 10^4$  and  $\alpha$ -tocopherol  $10^3$  IU/kg

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

Target	Author	Forward Primer	Reverse Primer	T <sup>a</sup>	Amplicon (bp)
Quantitative PCR					
Total bacteria	(Maeda, <i>et al.</i> , 2003)	GTGSTGCAYGGYTGTCGTCA	ACGTCRTCCMCACCTTCCTC	61	150
Total protozoa	(Sylvester, <i>et al.</i> , 2004)	GCTTTCGWTGGTAGTGTATT	CTTGCCCTCYAATCGTWCT	55	223
Anaerobic fungi	(Denman & McSweeney, 2006)	GAGGAAGTAAAAGTCGTAACAAGGTTTC	CAAATTCACAAAGGGTAGGATGAT	62	120
Methanogens	(Denman, <i>et al.</i> , 2007)	TTCGGTGGATCDCARAGRGC	GBARGTCGWA <sup>~</sup> CCGTAGAATCC	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 Table 3.** Effect of supplementing a control diet (CON) with *Ascophyllum nodosum* (ASC) and *Laminaria digitata* (LAM) on the relative abundance of the main bacteria at phylum, family and genus level in a Rusitec system. The total number of reads per sample was log-transformed and minor genera were discarded. Within a row means without a common superscript differ ( $P < 0.05$ ).

Phylum	Family	Genus	CON	ASC	LAM	SED <sup>1</sup>	P-value
<i>Bacteroidetes</i>			4.02	4.07	4.04	0.052	0.664
	<i>Flammeovirgaceae</i>		2.64 <sup>a</sup>	2.59 <sup>a</sup>	1.76 <sup>b</sup>	0.195	0.007
		<i>Sediminitomix</i>	1.01	1.52	1.01	0.381	0.364
	<i>Prevotellaceae</i>		3.94	3.95	3.96	0.081	0.984
		<i>Paraprevotella</i>	1.81	1.73	1.81	0.163	0.849
		<i>Prevotella</i>	3.92	3.93	3.94	0.085	0.981
	<i>Rikenellaceae</i>	<i>Rikenella</i>	1.33	1.24	1.22	0.071	0.319
<i>Fibrobacteres</i>	<i>Fibrobacteraceae</i>	<i>Fibrobacter</i>	2.36	2.34	2.30	0.294	0.976
<i>Firmicutes</i>			3.78	3.70	3.69	0.045	0.182
	<i>Acidaminococcaceae</i>		2.87	2.70	2.76	0.175	0.636
		<i>Acidaminococcus</i>	2.18	2.12	2.33	0.172	0.489
		<i>Succiniclasticum</i>	2.74	2.56	2.51	0.227	0.590
	<i>Clostridiaceae</i>	<i>Clostridium sensu stricto</i>	0.83	1.41	1.27	0.324	0.250
		<i>Clostridiales_Incertae Sedis XIII</i>	0.97	1.51	0.89	0.234	0.073
	<i>Erysipelotrichaceae</i>		2.18	2.21	2.39	0.251	0.691
		<i>Solobacterium</i>	2.12	1.90	2.01	0.261	0.703
	<i>Lachnospiraceae</i>		2.64	2.74	2.77	0.124	0.599
		<i>Butyrivibrio</i>	1.64	1.73	1.61	0.150	0.735
		<i>Oribacterium</i>	1.64	1.50	1.49	0.141	0.518
		<i>Pseudobutyrvibrio</i>	0.48 <sup>ab</sup>	0.74 <sup>a</sup>	0.42 <sup>b</sup>	0.103	0.045
		<i>Roseburia</i>	1.82	1.69	1.87	0.325	0.851
	<i>Lactobacillaceae</i>	<i>Lactobacillus</i>	2.20	2.35	2.45	0.252	0.636
	<i>Ruminococcaceae</i>		1.81	2.11	1.89	0.156	0.212
		<i>Ruminococcus</i>	1.03	1.30	1.39	0.269	0.426
	<i>Streptococcaceae</i>	<i>Streptococcus</i>	1.36 <sup>b</sup>	2.01 <sup>ab</sup>	2.61 <sup>a</sup>	0.353	0.034
	<i>Veillonellaceae</i>		2.18	2.26	2.19	0.064	0.449
		<i>Anaerovibrio</i>	1.38	1.37	1.42	0.132	0.909
		<i>Megasphaera</i>	0.73	0.60	0.92	0.201	0.357
		<i>Mitsuokella</i>	1.25	1.35	1.32	0.209	0.885
		<i>Schwartzia</i>	1.76	1.89	1.80	0.047	0.067
		<i>Selenomonas</i>	1.56	1.58	1.41	0.115	0.349
<i>Proteobacteria</i>			2.83	3.02	3.14	0.101	0.061
	<i>Succinivibrionaceae</i>		2.76	2.94	3.09	0.107	0.058
		<i>Ruminobacter</i>	0.95 <sup>b</sup>	2.36 <sup>a</sup>	2.15 <sup>a</sup>	0.373	0.019
		<i>Succinivibrio</i>	2.73	2.74	2.92	0.110	0.249
	<i>Sutterellaceae</i>	<i>Parasutterella</i>	1.16	1.30	1.29	0.160	0.632
<i>Spirochaetes</i>	<i>Spirochaetaceae</i>	<i>Treponema</i>	2.67	2.81	2.65	0.093	0.272
<i>Tenericutes</i>			2.78	2.86	2.84	0.097	0.701
	<i>Anaeroplasmataceae</i>		2.78	2.86	2.84	0.097	0.703
		<i>Anaeroplasma</i>	2.74	2.82	2.75	0.142	0.844
		<i>Asteroleplasma</i>	1.60	1.66	1.76	0.260	0.824
<i>Unclassified</i>			3.75	3.63	3.73	0.107	0.506

**Supplemental Table 4.** Effect of supplementing a control diet (CON) with *Ascophyllum nodosum* (ASC) and *Laminaria digitata* (LAM) on the relative abundance of the main archaea at family, genus and species level in the Rusitec system. The total number of reads per sample was log-transformed.

Family	Genus	Species	CON	ASC	LAM	SED <sup>1</sup>	P-value
		<i>Methanomassiliicoccaceae</i>	2.99	2.99	2.99	0.006	0.465
	Group 11		2.88	2.45	2.74	0.232	0.245
		<i>Methanomethylophilus alvus</i>	2.88	2.37	2.72	0.284	0.264
		<i>Others</i>	0.95	1.12	1.00	0.236	0.753
	Group 12		1.91	2.64	2.37	0.418	0.290
	Group 3a		0.99	1.16	0.95	0.248	0.670
	Group 9		0.95	1.15	1.05	0.358	0.856
		<i>Methanobacteriaceae</i>	1.06	1.09	1.11	0.239	0.971
		<i>Methanobrevibacter</i>	1.06	1.09	1.11	0.239	0.971
		<i>M bovis koreani</i>	0.49	0.31	0.50	0.398	0.872
		<i>M gottschalkii</i>	0.85	1.04	0.98	0.264	0.787
		<i>M wolinii</i>	0.12	0.00	0.25	0.240	0.608