

## Aberystwyth University

### *Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation*

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## ONLINE SUPPORTING MATERIAL

**SUPPLEMENTAL TABLE 1.** Ingredients and composition of diets (in g/kg DM)

Diets <sup>1</sup>	HP		LP	
	FIB	STA	FIB	STA
Ingredients				
Maize silage	405	405	405	405
Grassland hay	100	100	100	100
Dehydrated lucerne	90	90	90	90
Chopped wheat straw		52		63
Barley		95		119
Wheat		112		141
Maize		36		46
Soybean hulls	224		310	
Dehydrated beet pulp	90		90	
Soybean meal 48	86	108		36
Urea	5.4	1.8	4.5	
Mineral-vitamin premix <sup>2</sup>	0.20	0.20	0.20	0.20
Diet chemical composition				
Organic matter	930	938	933	943
Crude protein	144	142	111	110
Neutral detergent fiber	471	361	507	362
Acid detergent fiber	274	184	307	188
Starch	117	278	117	320
Neutral detergent fiber/Starch	4.03	1.30	4.33	1.13

<sup>1</sup>Diets: HP, high-protein; LP low-protein; FIB, high-fiber; STA, high-starch

<sup>2</sup>Composition in g/kg DM: Ca 200, Na 50, P 45, Mg 45, Mn 3.5, Zn 3.5, Fe 3.0, Cu 1.8, I 0.25, Co 0.15 and Se 0.02. Vitamins in mg/kg DM: retinol 90, cholecalciferol 1.5 and alpha-tocopherol 667.

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**SUPPLEMENTAL TABLE 2.** Primers used for T-RFLP, ARISA and qPCR

Target	Author	Primers		Temperature, °C		Efficiency <sup>1</sup> %	Size <i>bp</i>
		Forward	Reverse	Annealing	Melting		
T-RFLP bacteria	(1)	<sup>2</sup> AGAGTTTGATCCTGGCTCAG	ACGGGCGGTGTGTRC	55			1350
ARISA fungi	(2)	<sup>3</sup> AATCCTTCGGATTGGCT	CGAGAACCAAGAGATCCA	58			330-465
Quantitative PCR							
Total bacteria	(3)	GTGSTGCAYGGYTGTCGTCA	ACGTCRTCCMCACCTTCCTC	61	84.0	1.92	150
Total protozoa	(4)	GCTTTCGWTGGTAGTGATT	CTTGCCCTCYAATCGTWCT	55	84.2	1.93	223
Anaerobic fungi	(5)	GAGGAAGTAAAAGTCGTAACAAGGTTTC	CAAATTCACAAAGGGTAGGATGATT	62	83.8	1.96	120
Methanogens	(6)	TTCGGTGGATCDCARAGRGC	GBARGTCGWAWCCGTAGAATCC	56	80.0	1.88	140
<i>R. albus</i>	(7)	CCCTAAAAGCAGTCTTAGTTCG	CCTCCTTGCGTTAGAACA	62	86.8	1.94	175
<i>R. flavefaciens</i>	(5)	CGAACGGAGATAATTTGAGTTTACTTAGG	CGGTCTCTGTATGTTATGAGGTATTACC	59	83.0	1.97	132
<i>F. succinogenes</i>	(7)	GGTATGGGATGAGCTTGC	GCCTGCCCCTGAACTATC	59	88.4	1.97	446
<i>B. fibrisolvens</i>	(8)	ACACACCGCCCGTACCA	TCCTTACGGTTGGGTACAGA	59	82.8	1.96	63
<i>P. ruminicola</i>	(9)	GGTTATCTTGAGTGAGTT	CTGATGGCAACTAAAGAA	56	88.0	1.75	485
<i>P. bryantii</i>	(9)	ACTGCAGCGGAACTGTCAGA	ACCTTACGGTGGCAGTGTCTC	62	87.6	1.95	540
<i>S. bovis</i>	(10)	TTCCTAGAGATAGGAAGTTTCTTCGG	ATGATGGCAACTAACAATAGGGGT	59	85.2	1.97	127
<i>S. ruminantium</i>	(9)	TGCTAATACCGAATGTTG	TCCTGCACTCAAGAAAGA	59	88.6	1.91	513
<i>M. elsdenii</i>	(11)	GACCGAAACTGCGATGCTAGA	CGCCTCAGCGTCAGTTGTC	62	84.2	1.98	128
<i>A. lipolytica</i>	(9)	TGGGTGTTAGAAATGGATTC	CTCTCCTGCACTCAAGAATT	62	89.0	1.85	597

<sup>1</sup>Efficiency of PCR amplification calculated by serial dilutions of DNA from rumen content.

<sup>2</sup>Labelled with Cyanine 5 at the 5' end.

<sup>3</sup>Labelled with 6 FAM in the 5' end

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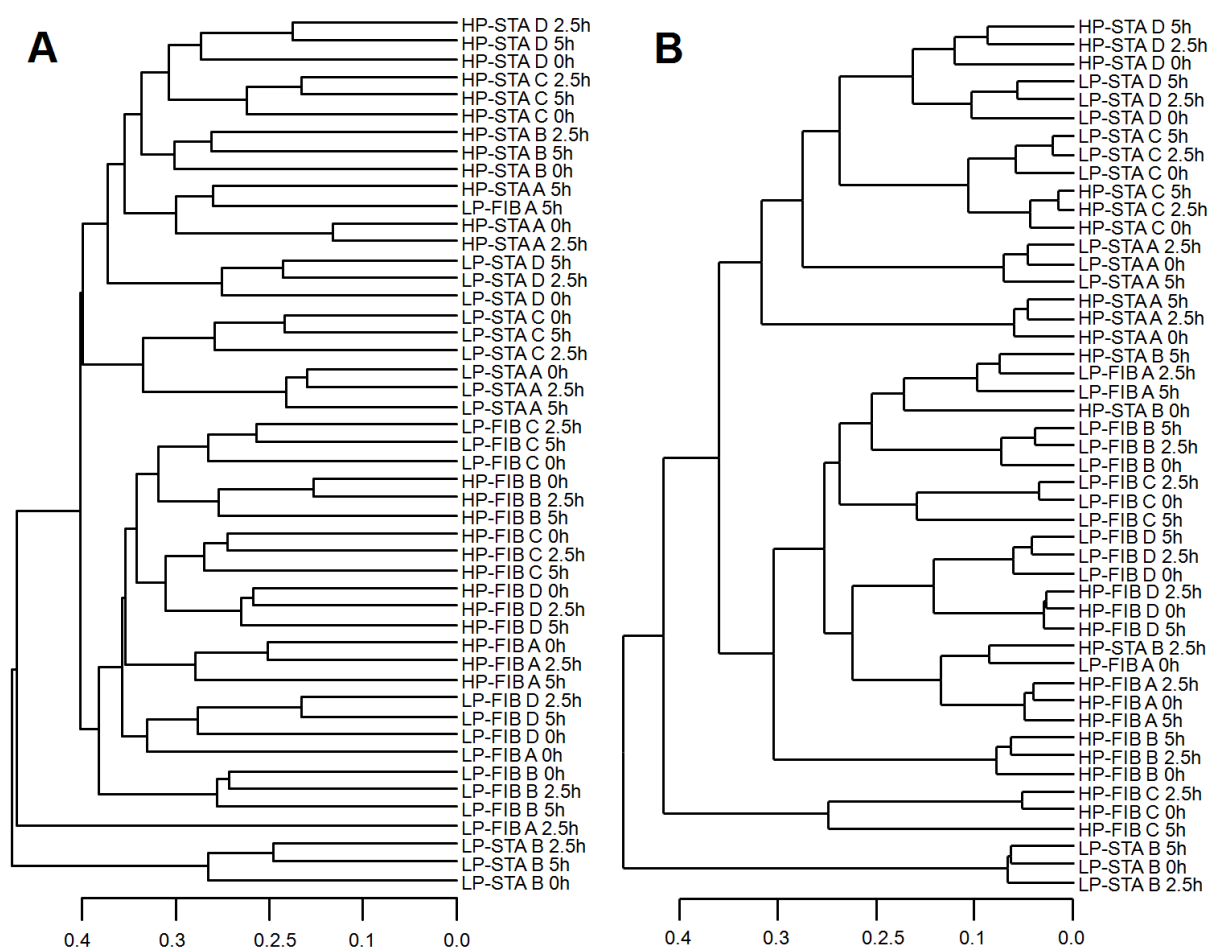
**SUPPLEMENTAL TABLE 3.** Rumen bacterial biodiversity observed in dairy cows fed 4 diets containing 2 levels of protein and 2 types of carbohydrates after 4wk adaptation. Data were analyzed independently for each T-RFLP restriction enzyme.

Diets <sup>1</sup>	HP		LP		SED <sup>2</sup>	P-value		
	FIB	STA	FIB	STA		Protein	Energy	Pr×E
Richness								
HhaI	45.8	43.7	47.1	43.8	2.46	0.70	0.13	0.74
RsaI	41.8 <sup>a</sup>	39.5 <sup>a</sup>	42.2 <sup>a</sup>	33.2 <sup>b</sup>	2.34	0.09	0.002	0.05
HaeIII	33.7	33.7	34.7	35.0	1.42	0.26	0.87	0.87
MspI	25.4 <sup>c</sup>	30.8 <sup>a</sup>	28.2 <sup>b</sup>	26.6 <sup>bc</sup>	1.11	0.38	0.025	<0.001
Shannon-Wiener index								
HhaI	3.18	3.11	3.27	3.18	0.082	0.18	0.18	0.87
RsaI	3.54 <sup>bc</sup>	3.48 <sup>ab</sup>	3.56 <sup>a</sup>	3.27 <sup>c</sup>	0.068	0.05	0.001	0.024
HaeIII	3.33	3.33	3.31	3.35	0.551	0.98	0.57	0.56
MspI	3.03 <sup>b</sup>	3.25 <sup>a</sup>	3.10 <sup>b</sup>	3.09 <sup>b</sup>	0.050	0.19	0.007	0.002
Shannon evenness								
HhaI	0.833	0.825	0.851	0.844	0.0109	0.026	0.36	0.96
RsaI	0.952	0.952	0.952	0.935	0.0072	0.09	0.09	0.10
HaeIII	0.950	0.948	0.934	0.944	0.0088	0.12	0.46	0.33
MspI	0.940	0.950	0.931	0.942	0.0053	0.035	0.008	0.98

<sup>1</sup>Diets: HP, high-protein; LP low-protein; FIB, high-fiber; STA, high-starch. Rumen was sampled at 0, 2.5 and 5h after feeding, but no statistical differences were detected between times. Within a row means without a common superscript differ ( $P<0.05$ ).

<sup>2</sup>SED for the interaction Protein × Energy ( $n=12$ , 4 animals × 3 sampling moments)

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**SUPPLEMENTAL FIGURE 1.** Differences in the rumen bacteria (**Fig.A** based on T-RFLP data) and anaerobic fungal populations (**Fig.B** based on ARISA data) between dairy cows fed 4 different diets. Cows (A, B, C and D) were fed 2 levels of protein (HP, high-protein; LP, low-protein) and 2 types of carbohydrate (FIB, high-fiber; STA, high-starch) and after 4wk adaptation rumen was sampled at 0, 2.5 and 5h after feeding. Dendrograms are presented with Bray Curtis dissimilarity distance after UPGMA clustering.

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