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Effect of progressive inoculation of fauna-free sheep with holotrich protozoa and total-fauna on rumen fermentation, microbial diversity and methane emissions

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ONLINE SUPPORTING MATERIAL

1

2 **Supplemental Table 1.** Chemical composition of ryegrass and barley (in g kg⁻¹ DM).

Ingredient	Ryegrass hay	Barley
Dry matter	913	942
Organic matter	887	980
Nitrogen	10.1	12.9
Neutral detergent fibre	616	139
Acid detergent fibre	375	44
Acid detergent lining	54.5	9.08
Starch	93.3	590
Water soluble carbohydrates	133	25.4
Ether extract	14.4	18.4

3 Mineral block: Declared composition in mg kg⁻¹: Mg 1000, Zn 120, Co 100, Mn100, I 50, Na 38 and Se 20.

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5 **Supplemental Table 2.** Primers used for TRFLP and qPCR indicating the annealing temperature and amplicon size.

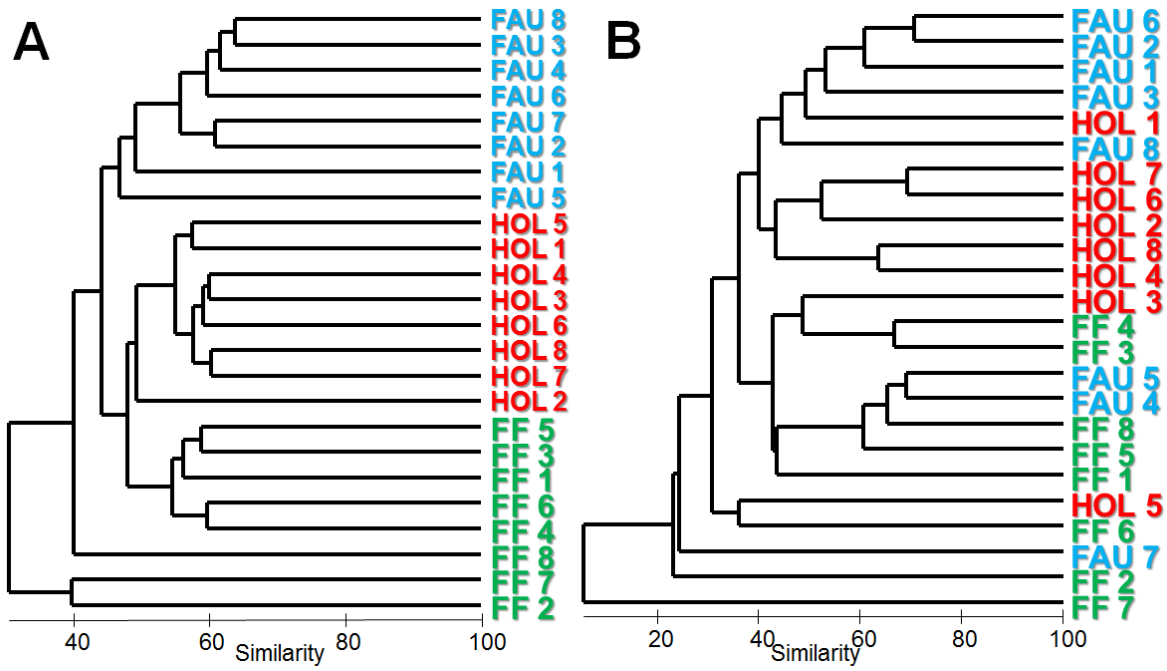
Target	Author	Forward Primer	Reverse Primer	T ^a	bp
TRFLP bacteria	(Hongoh, <i>et al.</i> , 2005)	¹ AGAGTTTGATCCTGGCTCAG	ACGGGCGGTGTGTRC	55	1350
TRFLP archaea	(Wright & Pimm, 2003)	¹ GCTCAGTAACACGTGG	CGGTGTGTGCAAGGAG	55	1254
Quantitative PCR					
Total bacteria	(Maeda, 2003)	GTGSTGCA YGGYTGTCTGTC	ACGTCRTCCMCACCTTCCTC	61	150
Total protozoa	(Sylvester, <i>et al.</i> , 2004)	GCTTTCGWGTGGTAGTGTATT	CTTGCCCTCYAATCGTWCT	55	223
Anaerobic fungi	(Denman & McSweeney, 2006)	GAGGAAGTAAAAGTCGTAACAAGGTTTC	CAAATTCACAAAGGGTAGGATGATT	62	120
Methanogens	(Denman, <i>et al.</i> , 2007)	TTCGGTGGATCDCARAGRGC	GBARGTCGWAWCCGTAGAATCC	56	140
<i>R. albus</i>	(Koike & Kobayashi, 2001)	CCCTAAAAGCAGTCTTAGTTCG	CCTCCTTGCGGTTAGAACA	62	175
<i>R. flavefaciens</i>	(Denman & McSweeney, 2006)	CGAACGGAGATAATTTGAGTTTACTTAGG	CGGTCTCTGTATGTTATGAGGTATTACC	59	132
<i>F. succinogenes</i>	(Koike & Kobayashi, 2001)	GGTATGGGATGAGCTTGC	GCCTGCCCTGAACTATC	59	446
<i>B. fibrisolvens</i>	(Klieve, <i>et al.</i> , 2003)	ACACACCGCCCGTACCA	TCCTTACGGTTGGGTCACAGA	59	63
<i>S. bovis</i>	(Stevenson & Weimer, 2007)	TTCCTAGAGATAGGAAGTTTCTTCGG	ATGATGGCAACTAACAATAGGGGT	59	127
<i>Prevotella spp.</i>	(Matsuki, <i>et al.</i> , 2002)	CACRGTAACGATGGATGCC	GGTCGGGTTGCAGACC	55	528
<i>P. bryantii</i>	(Tajima, <i>et al.</i> , 2001)	ACTGCAGCGCGAACTGTCAGA	ACCTTACGGTGGCAGTGTCTC	62	540
<i>P. albensis</i>	(Tajima, <i>et al.</i> , 2001)	CAGACGGCATCAGACGAGGAG	ATGCAGCACCTTCACAGGAGC	56	861
<i>S. ruminantium</i>	(Tajima, <i>et al.</i> , 2001)	TGCTAATACCGAATGTTG	TCCTGCACTCAAGAAAGA	59	513
<i>M. elsdenii</i>	(Ouwkerk, <i>et al.</i> , 2002)	GACCGAAACTGCGATGCTAGA	CGCCTCAGCGTCAGTTGTC	62	128
<i>Eu. ruminantium</i>	(Tajima, <i>et al.</i> , 2001)	GCTTCTGAAGAATCATTGAAG	TCGTCGCTCAGTGTGAGTGT	57	671
<i>A. lipolytica</i>	(Tajima, <i>et al.</i> , 2001)	TGGGTGTTAGAAATGGATTC	CTCTCCTGCACTCAAGAATT	57	597
<i>Lactobacillus spp.</i>	(Rekha, <i>et al.</i> , 2006)	TGCCTAATACATGCAAGTCGA	GTTTGGGCCGTGTCTCAGT	61	318

6 ¹Labelled with Cyanine 5 at the 5' end.

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8 **Supplemental Table 3.** Effect of the rumen inoculation of fauna-free (FF) sheep with Holotrich (HOL) and
 9 total-fauna (FAU) on the biodiversity indices of rumen bacteria and methanogens using TRFLP and 4
 10 restriction enzymes.

Treatments	FF	HOL	FAU	SED	P-value
Bacterial community					
Richness					
HhaI	34.8 ^b	51.6 ^a	34.6 ^b	3.93	<0.001
MspI	48.4 ^b	72.4 ^a	44.1 ^b	5.39	<0.001
HaeIII	39.5 ^b	53.5 ^a	47.1 ^{ab}	4.25	0.018
RsaI	49.6 ^b	59.0 ^a	40.6 ^c	3.73	<0.001
Shannon index					
HhaI	2.33 ^b	2.98 ^a	2.07 ^b	0.157	<0.001
MspI	2.62 ^b	3.43 ^a	2.28 ^b	0.168	<0.001
HaeIII	2.73 ^b	3.29 ^a	2.77 ^b	0.158	0.005
RsaI	3.02 ^b	3.50 ^a	2.96 ^b	0.166	0.010
Methanogens community					
Richness					
HhaI	21.3	20.0	20.9	2.44	0.87
MspI	23.8	18.3	20.3	2.31	0.088
HaeIII	22.8	20.5	17.8	2.75	0.225
TaqI	19.0	23.6	18.3	3.39	0.262
Shannon index					
HhaI	1.56	1.62	1.64	0.146	0.866
MspI	2.27 ^a	1.89 ^b	1.81 ^b	0.102	0.001
HaeIII	1.93	1.76	1.62	0.138	0.116
TaqI	2.13	2.53	2.23	0.187	0.116



Supplemental Figure 1. Dendrograms based on the UPGMA clustering of the Bray-Curtis distances illustrating the effect of the sheep inoculation with different protozoal species on the rumen bacterial (A) and methanogen communities (B). Treatments: FF, fauna-free; HOL, Holotrich monofaunated; FAU, total-faunated sheep. Animals are indicated with numbers.

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