

## Supplementary Material

### **Supplementation of live yeast based feed additive in early life promotes rumen microbial colonization and fibrolytic potential in lambs**

Chaucheyras-Durand Frédérique<sup>1,2</sup>, Ameilbonne Aurélie<sup>1,2</sup>, Auffret Pauline<sup>2#</sup>, Bernard Mickael<sup>3</sup>, Mialon Marie-Madeleine<sup>4</sup>, Dunière Lysiane<sup>1,2</sup>, Forano Evelyne<sup>2</sup>

<sup>1</sup>Lallemand SAS, 31702 Blagnac, France

<sup>2</sup>Université Clermont Auvergne, INRA, UMR 454 MEDIS, F-63000 Clermont-Ferrand, France

<sup>3</sup>UE 1414 Herbipôle, INRA Auvergne Rhône Alpes, F-63122 Saint-Genès Champanelle, France

<sup>4</sup>Université Clermont Auvergne, INRA, VetAgro Sup, UMR 1213 Herbivores, F-63000 Clermont-Ferrand, France

# present address: Ifremer, UMR 241 EIO, Tahiti, French Polynesia

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Table S2: Alpha diversity measures in the rumen of the two lamb groups across time (averages of 5 individuals for each group). ns= not significant ( $P>0.05$ ).

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Table S4: Differential OTU analysis for Bacteria in the rumen and the feces of Control and Supplemented lambs. (Excel file)

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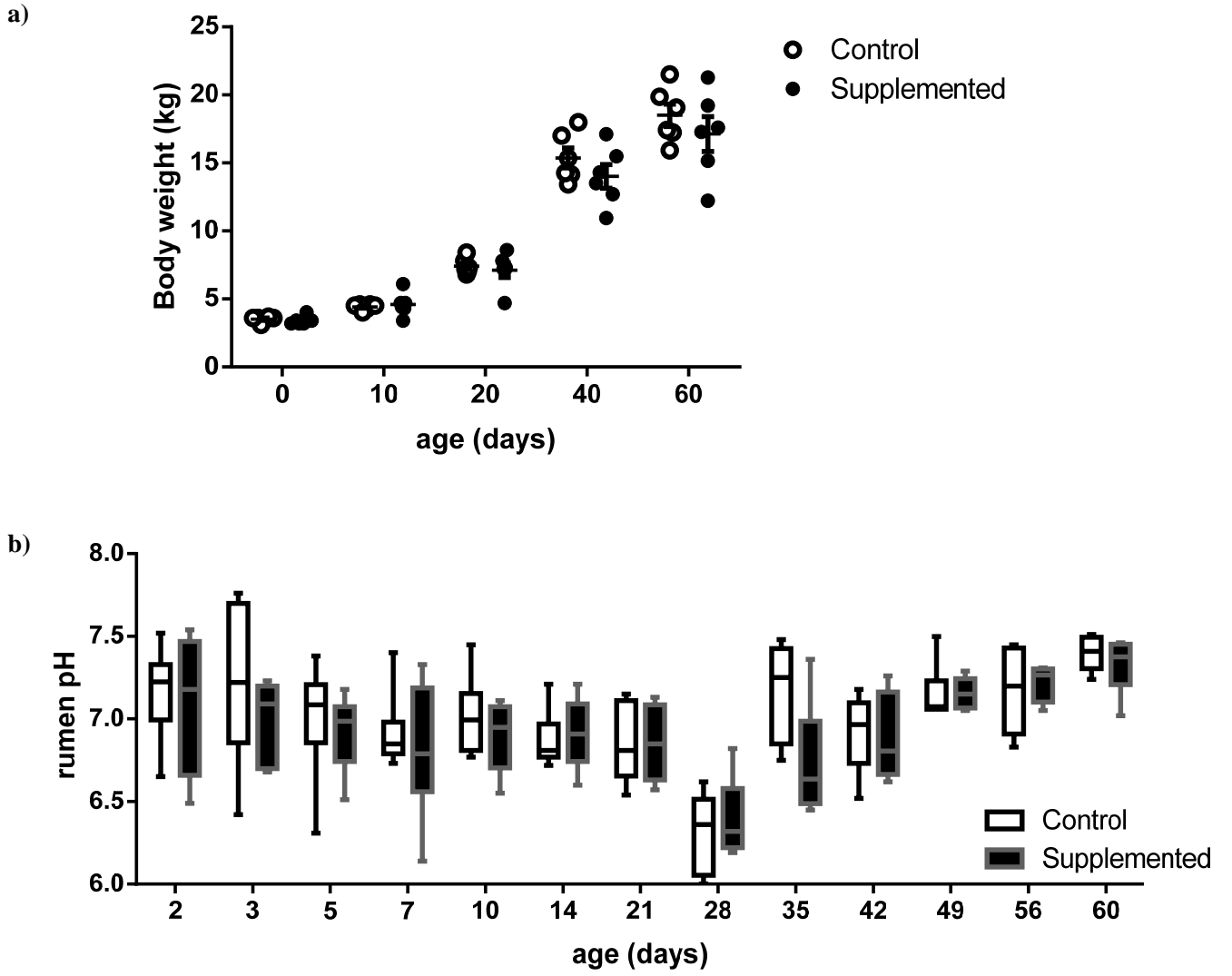


Figure S1

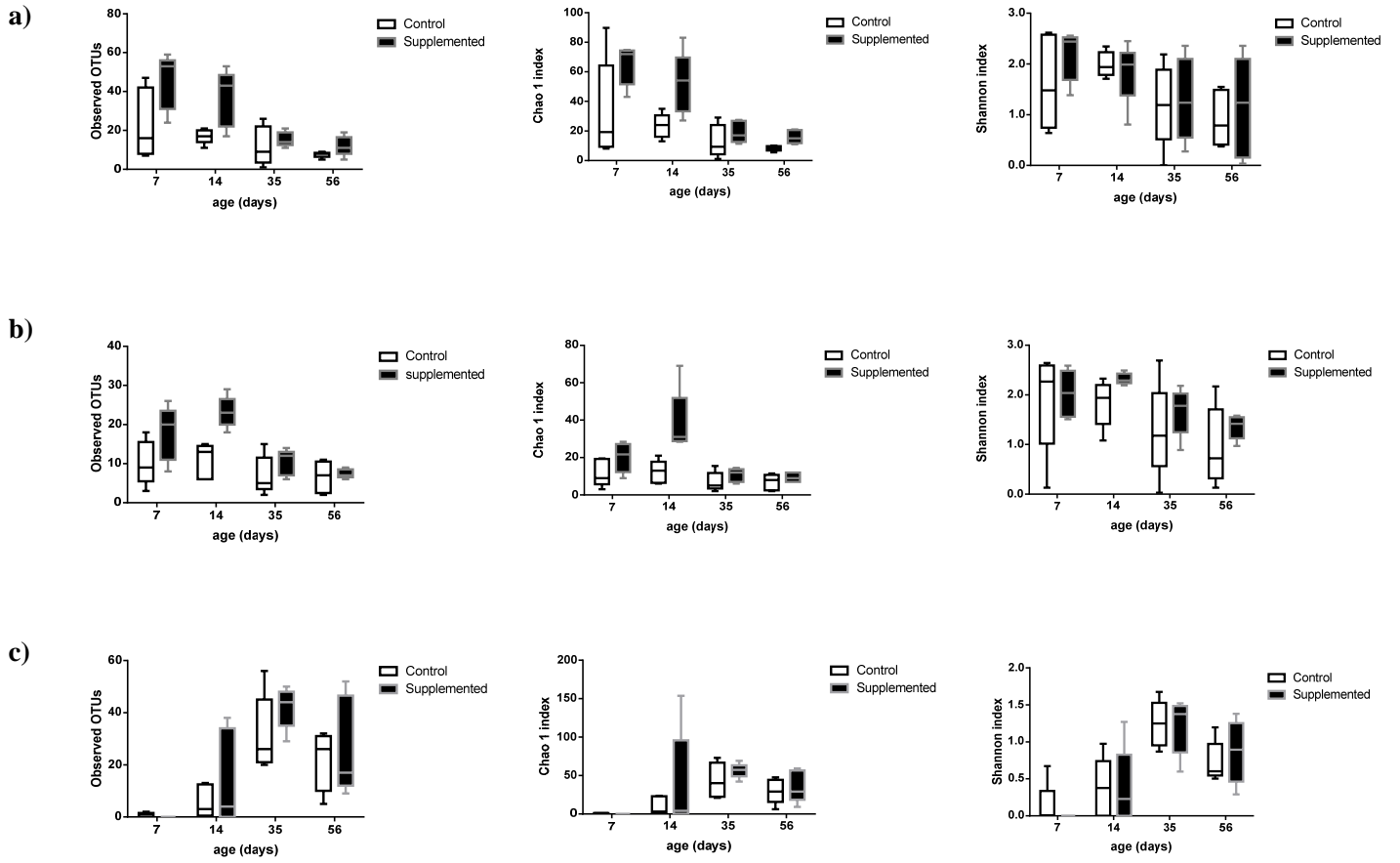


Figure S2

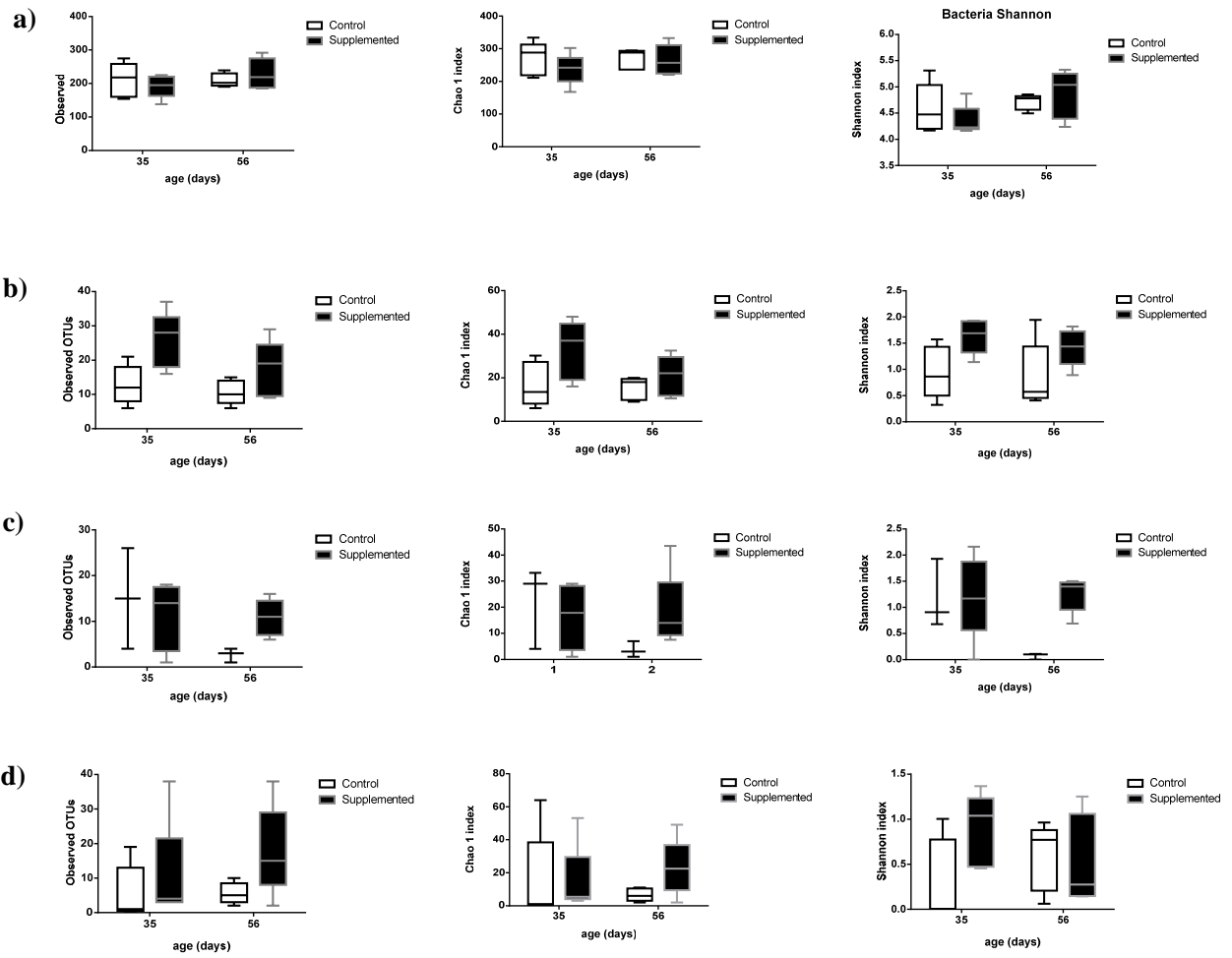


Figure S3

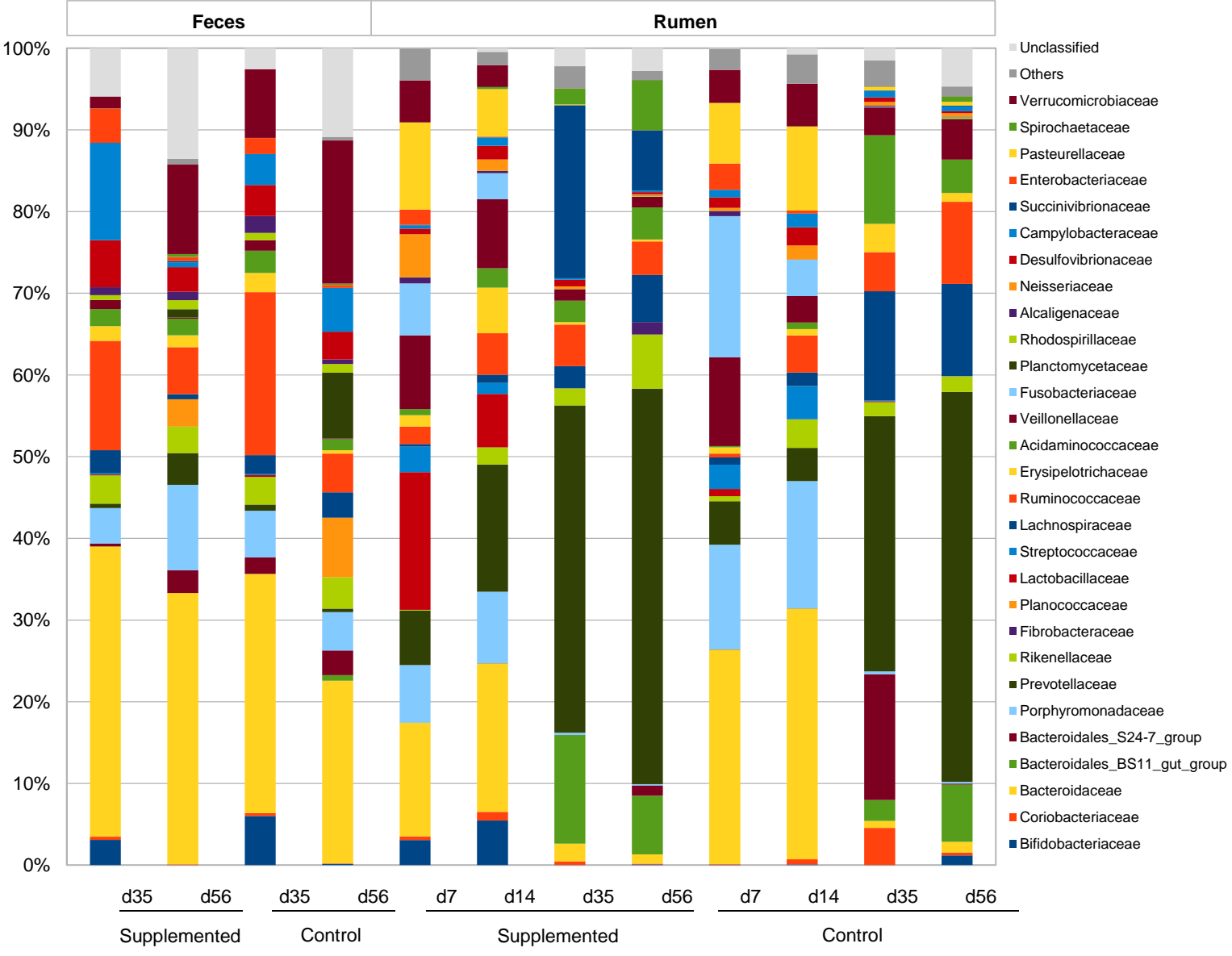


Figure S4

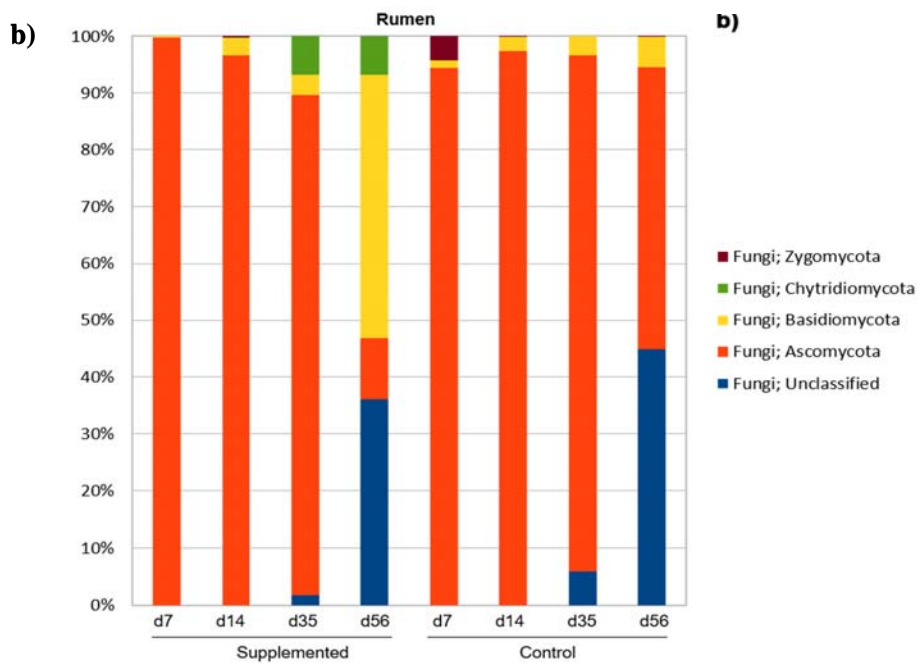
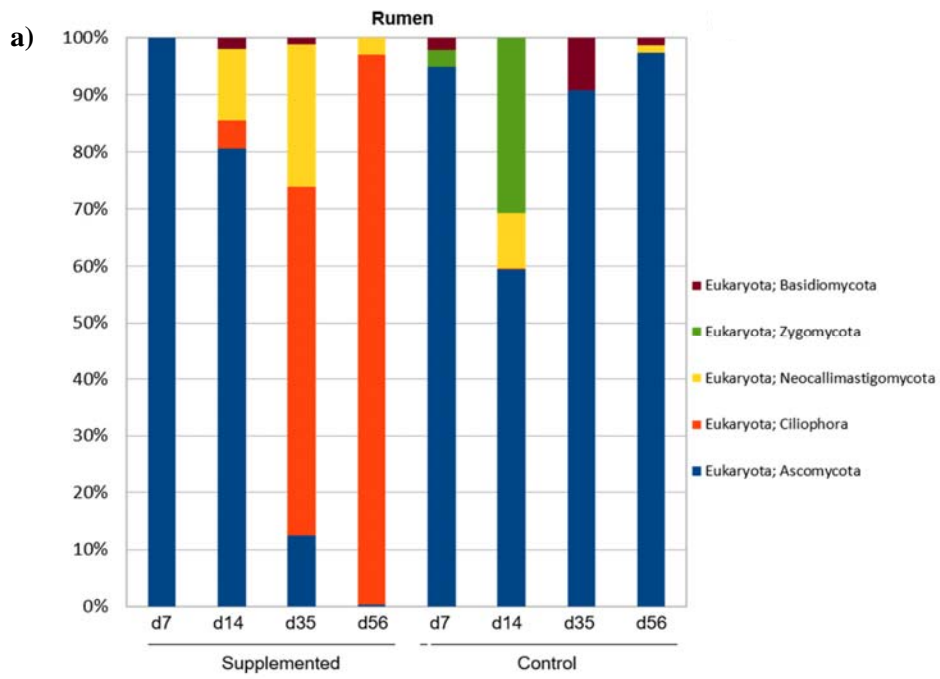


Figure S5



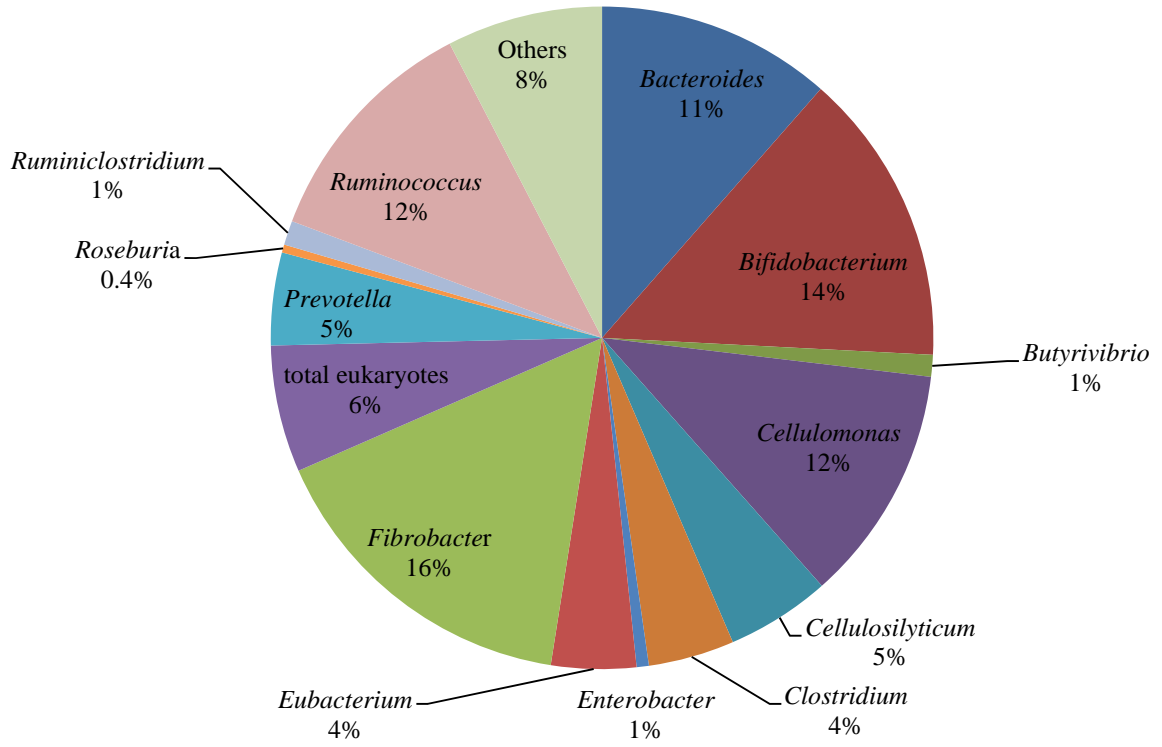


Figure S6

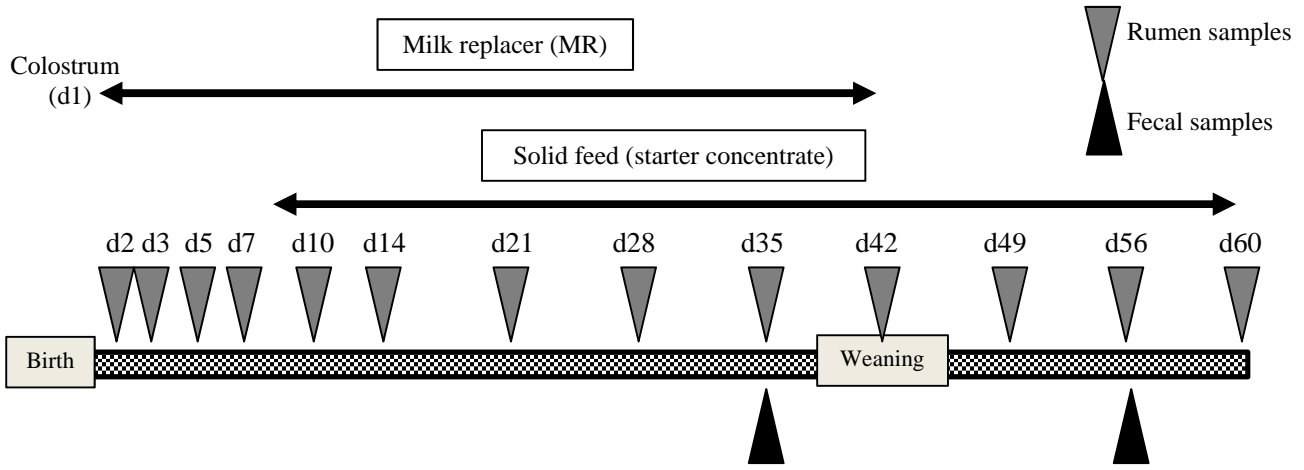


Figure S7

a)	Age (days)	Total bacteria		Archaea		Protozoa		Fungi	
		Control	Supplemented	Control	Supplemented	Control	Supplemented	Control	Supplemented
	2	8.61±0.18	8.22±1.02	5.05±2.48	5.78±0.65	nd	nd	nd	nd
	3	8.71±0.51	8.56±0.57	5.96±0.24	6.27±0.72	nd	nd	nd	nd
	5	7.40±0.61	8.03±0.79	na	3.65±3.36	nd	nd	nd	nd
	7	8.83±0.33	8.45±0.60	6.25±0.32	4.49±3.82	nd	nd	nd	nd
	10	8.89±0.28	8.39±0.55	5.38±1.41	4.28±2.40	nd	nd	nd	nd
	14	8.56±0.10	8.46±0.16	4.61±2.40	5.17±2.69	nd	nd	nd	nd
	21	8.56±0.17	8.41±0.83	6.57±0.54	6.79±0.82	nd	1.95±3.22	1.92±2.18	3.83±2.16
	28	8.11±0.56	8.31±0.10	6.52±0.70	7.31±0.31	1.08±1.76	3.83±2.76	1.86±2.05	2.80±1.78
	35	9.10±0.50	9.39±0.20	5.98±0.70	6.52±0.49	2.73±2.25	6.36±1.90	0.87±1.35	2.39±2.13
	42	9.31±0.26	9.27±0.27	5.91±0.30	6.36±0.42	1.84±2.02	5.32±3.01	0.73±1.78	1.96±1.59
	49	9.72±0.47	9.22±0.88	6.08±0.46	5.67±0.91	2.55±2.10	6.67±1.45	nd	1.42±1.57
	56	9.55±0.46	9.81±0.32	6.01±0.29	6.29±0.63	0.49±1.20	7.53±1.99	nd	1.57±2.45

b)	Age (days)	<i>Prevotella sp.</i>		<i>Fibrobacter succinogenes</i>		<i>Ruminococcus albus</i>		<i>Ruminococcus flavefaciens</i>	
		Control	Supplemented	Control	Supplemented	Control	Supplemented	Control	Supplemented
	2	8.00±1.44	7.36±1.69	2.07±0.33	1.66±0.93	1.50 ±1.41	nd	2.07 ±0.33	1.65±0.92
	3	8.54±0.90	8.60±1.14	1.96±1.16	1.60±0.25	0.74±0.93	0.39±0.48	2.11 ±1.72	1.45±1.63
	5	7.28±1.38	8.26±0.98	1.64±1.54	1.89±0.51	nd	nd	1.67 ±1.07	0.91±1.37
	7	8.29±0.28	8.35±1.55	2.00±0.25	1.88±0.34	nd	0.23±0.56	3.55 ±0.58	2.95±0.29
	10	8.34±0.33	8.56±1.02	2.36±0.32	2.09±0.46	1.46 ±1.62	2.73±0.60	3.51 ±0.76	4.79±2.17
	14	8.14±1.20	8.05±0.40	2.02±0.32	2.38±0.34	2.08±0.22	2.90±1.82	5.84 ±1.81	6.20±1.86
	21	8.09±0.14	8.40±0.25	1.00±1.00	0.79±1.10	5.11 ±1.64	5.33 ±1.93	7.72 ±1.73	7.64±0.98
	28	8.14±1.72	8.36±0.11	1.22±1.34	2.21±0.40	5.50 ±1.29	6.29 ±0.32	6.72 ±1.06	6.29±1.10
	35	8.32±0.58	8.09±0.32	1.62±0.79	2.01±0.38	5.37 ±1.53	5.59 ±1.11	7.26 ±1.01	5.84±1.48
	42	8.67±0.20	8.65±0.31	2.19±0.69	4.16±2.54	5.08 ±0.89	5.91 ±0.66	6.86 ±1.44	6.41±2.44
	49	8.78±0.31	8.82±0.61	2.94±0.56	6.95±1.06	6.24 ±0.81	6.23 ±1.10	7.74 ±0.96	7.57±1.44
	56	8.48±0.44	9.06±0.42	2.55±0.81	7.66±0.70	5.84 ±1.18	6.48 ±0.52	7.72 ±1.09	8.04±1.56

Table S1

<b>Bacteria</b>				<b>Supplemented</b>		
		Control		Observed OTUs	Chao	Shannon
Lamb age (days)	Observed OTUs	Chao	Shannon			
7	152	185	3.87	140	171	3.77
14	185	238	4.25	165	215	4.24
35	159	192	4.33	167	192	4.11
56	195	234	4.50	192	222	4.21
Age effect	P<0.01	P<0.05	ns			
SC effect	ns	ns	ns			
Interaction	ns	ns	ns			

<b>Eukaryota</b>				<b>Supplemented</b>		
		Control		Observed OTUs	Chao	Shannon
Lamb age (days)	Observed OTUs	Chao	Shannon			
7	23	33	1.63	45	64	2.17
14	17	23	1.99	36	52	1.84
35	12	13	1.20	15	19	1.31
56	7	8	0.92	12	15	1.15
Age effect	P<0.0001	P<0.0001	P<0.05			
SC effect	P<0.001	P<0.001	ns			
Interaction	ns	ns	ns			

<b>Fungi</b>				<b>Supplemented</b>		
		Control		Observed OTUs	Chao	Shannon
Lamb age (days)	Observed OTUs	Chao	Shannon			
7	10	11	1.90	17	20	2.03
14	10	12	1.83	23	38	2.32
35	7	7	1.28	10	10	1.66
56	6	6	0.95	7	9	1.35
Age effect	P<0.0001	P<0.0001	P<0.05			
SC effect	P<0.001	P<0.001	P<0.10			
Interaction	P<0.05	P<0.01	ns			

<b>Archaea</b>				<b>Supplemented</b>		
		Control		Observed OTUs	Chao	Shannon
Lamb age (days)	Observed OTUs	Chao	Shannon			
7	0	0	0.14	0	0	0
14	5	10	0.37	14	39	0.38
35	31	43	1.24	42	56	1.21
56	21	29	0.73	26	35	0.87
Age effect	P<0.0001	P<0.001	P<0.0001			
SC effect	ns	ns	ns			
Interaction	ns	ns	ns			

Table S2

<b>Bacteria</b>				<b>Supplemented</b>		
		Control		Observed OTUs	Chao	Shannon
Lamb age (days)	Observed OTUs	Chao	Shannon	Observed OTUs	Chao	Shannon
35	211	270	4.59	192	237	4.36
56	210	269	4.71	228	265	4.87
SC effect	ns	ns	ns			
<b>Eukaryota</b>				<b>Supplemented</b>		
		Control		Observed OTUs	Chao	Shannon
Lamb age (days)	Observed OTUs	Chao	Shannon	Observed OTUs	Chao	Shannon
35	12	16	0.95	25	33	1.64
56	10	15	0.87	17	20	1.42
SC effect	P<0.01	P<0.05	P<0.01			
<b>Fungi</b>				<b>Supplemented</b>		
		Control		Observed OTUs	Chao	Shannon
Lamb age (days)	Observed OTUs	Chao	Shannon	Observed OTUs	Chao	Shannon
35	15	22	1.17	11	16	1.21
56	2	3	0.07	10	18	1.25
SC effect	ns	ns	ns (P=0.059)			
<b>Archaea</b>				<b>Supplemented</b>		
		Control		Observed OTUs	Chao	Shannon
Lamb age (days)	Observed OTUs	Chao	Shannon	Observed OTUs	Chao	Shannon
35	5	15	0.31	10	14	0.89
56	5	6	0.59	17	23	0.54
SC effect	ns	ns	ns			

Table S3

<b>Ingredient</b>	<b>%</b>
Barley	21.5
Soyabean meal	18.0
Corn	16.0
Wheat	16.0
Wheat bran	10.0
alfalfa	6.5
Beet pulp	5.0
Sugar cane molasses	3.5
Sodium Carbonate	3.0
Mineral supplement	0.5

<b>Chemical composition</b>	<b>g/kg of DM</b>
Starch	389
Sugars	66
NDF	209
ADF	87
ADL	17
CP	188

Table S6

Microbial group/genus/species	Target gene	Primers	Reference
Total bacteria	16S rDNA	5'-AGCAGCCGCGGTAAT-3' 5'-CAGGGTATCTAATCCTGTT-3'	Bayat et al.
Fungi	ITS1	5'-GAGGAAGTAAAAGTCGTAACAAGGTTTC-3' 5'-CAAATTCACAAAGGGTAGGATGATT-3'	Bayat et al.
Archaea	16S rDNA	5'-GAGGAAGGAGTGGACGACGGTA-3' 5'-ACGGGCGGTGTGTGCAAG-3'	Bayat et al.
Protozoa	18S rDNA	5'-GCTTTCGWTGGTAGTGTATT-3' 5'-CTTGCCCTCYAATCGTWCT-3'	Bayat et al.
<i>Prevotella sp.</i>	16S rDNA	5'-GGTTCTGAGAGGAAGGTCCCC-3' 5'-TCCTGCACGCTACTTGGCTG-3'	Stevenson and Weimer 2007
<i>Fibrobacter succinogenes</i>	16S rDNA	5'-GTTCGGAATTACTGGGCGTAAA-3' 5'-CGCCTGCCCCTGAACTATC-3'	Bayat et al.
<i>Ruminococcus albus</i>	16S rDNA	5'-CCCTAAAAGCAGTCTTAGTTCG-3' 5'-CCTCCTTGCGGTTAGAACA-3'	Mosoni et al.
<i>Ruminococcus flavefaciens</i>	16S rDNA	5'-CGAACGGAGATAATTTGAGTTTACTTAGG-3' 5'-CGGTCTCTGTATGTTATGAGGTATTACC-3'	Bayat et al.
<i>Saccharomyces cerevisiae</i>	26S rDNA	5'- AGGAGTGCGGTTCTTTG -3' 5'- TACTTACCGAGGCAAGCTACA -3'	Chang et al 2007

Table S7

References for Table S7:

Bayat, A. R. *et al.* Effect of camelina oil or live yeasts (*Saccharomyces cerevisiae*) on ruminal methane production, rumen fermentation, and milk fatty acid composition in lactating cows fed grass silage diets. *J. Dairy Sci.* **98**, 3166–3181 (2015).

Chang, H.W. *et al.* Quantitative real time PCR assays for the enumeration of *Saccharomyces cerevisiae* and the *Saccharomyces sensu stricto* complex in human feces. *J. Microbiol. Methods.* **71**, 191–201 (2007).

Chaucheyras-Durand, F. *et al.* Live yeasts enhance fibre degradation in the cow rumen through an increase in plant substrate colonization by fibrolytic bacteria and fungi. *J. Appl. Microbiol.* **120**, 560–570 (2016).

Mosoni, P., Martin, C., Forano, E. & Morgavi, D. P. Long-term defaunation increases the abundance of cellulolytic ruminococci and methanogens but does not affect the bacterial and methanogen diversity in the rumen of sheep. *J. Anim. Sci.* **89**, 783–791 (2011).

Stevenson, D.M. & Weimer, P.J. Dominance of *Prevotella* and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. *Appl. Microbiol. Biotechnol.* **75**, 165–174 (2007).

<b>Microbial group</b>	<b>Targeted region</b>	<b>Primers</b>
Bacteria	V4 region of the 16S rDNA	515f-806r
Archaea	Region of the 16S rDNA	349f-806r
Eukaryota	Region of the 18S rDNA	566f-1200r
Fungi	ITS3-ITS4	ITS3-ITS4

Table S8