PROBEEF Appendix 1

Table 1: Chemical composition of experimental diets.

	Straw/concentrate mix	Fresh grass
Dry Matter (g kg^{-1})	948	912
Total nitrogen	22.6	22.8
Water soluble carbohydrate (WSC)	80.6	196
Neutral detergent fiber (NDF)	489	369
Acid detergent fiber (ADF)	309	199
Oil	10.3	25.1
Fatty acid composition		
12:0	0.03	0.05
14:0	0.18	0.14
16:0	3.12	4.92
16:1*n*-7	0.15	0.04
18:0	0.34	0.46
18:1*n*-9	4.63	0.37
18:2*n*-6	6.70	3.82
18:3*n*-3	1.19	24.6
Total fatty acids	18.5	36.5

[1] Values are means; n = 2 for both diets.

Table 2. Rumen protozoal density and protozoal chloroplast, nitrogen (N), DNA and fatty acid content following feeding with either straw:concentrate (S : C) or fresh perennial ryegrass (PRG)[1].

	Diet		SED	*P*
	S : C	PRG		
Protozoal Density (10^3 cells mL^{-1})				
Total	903	121	0.18[2]	**<0.01**
Holotrich	ND	ND	NA	NA
Entodiniomorphid	903	121	0.18[2]	**<0.01**
Entodinium spp. (%)	86.0	79.4		
Diplodinium spp. (%)	10.8	14.2		
Eudiplodinium spp. (%)	0.0	0.2		
Polyplastron spp. (%)	2.8	4.8		
Ermplastron spp. (%)	0.0	0.2		
Metadinium spp. (%)	0.0	1.8		
% protozoa containing intracellular chloroplasts				
Total	26.0	27.5	4.43	NS
Holotrich	ND	ND	NA	NA
Entodiniomorphid	26.0	27.5	4.43	NS
% protozoa saturated with intracellular chloroplasts (>10 cell^{-1})				
Total	0.00	5.00	1.60	**<0.05**
Holotrich	ND	ND	NA	NA
Entodiniomorphid	0.00	5.00	1.60	**<0.05**
Rumen protozoal standard data				
Protozoal N content (mg/g)	44.0	40.8	9.57	NS
DNA : N (µg/mg)	3.37	6.29	1.42	NS
Protozoal fatty acid content (µg/mg N)				
14:0	7.78	8.65	1.50	NS
15:0	8.90	12.4	2.85	NS
16:0	132	101	38.4	NS
17:0	3.24	4.03	3.98	**0.012**
18:0	201	288	167	NS
18:1 *trans*-11	36.6	87.6	43.0	**<0.001**
18:2*n*-6	33.5	8.80	3.54	**<0.001**
18:3*n*-3	3.30	18.1	5.14	**<0.001**
cis-9, *trans*-11 CLA	3.82	0.67	0.84	**<0.001**
trans-10, *cis*-12 CLA	0.17	0.00	0.07	**<0.001**
Total	608	653	287	NS

[1] Mean values, n=4. [2] SED values are in log scale

NS = Not significant (*P*>0.10); ND = not detected; NA = Not applicable.

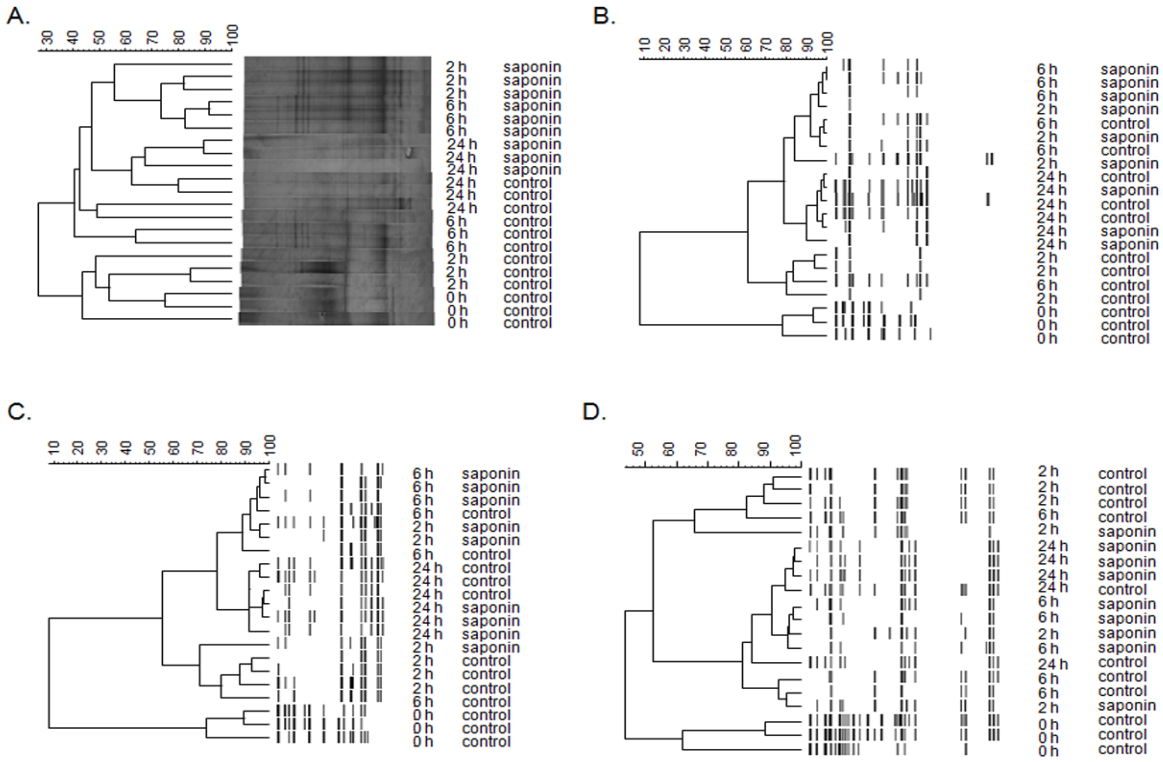

Figure. 1. Unweighted pair group method with arithmetic mean dendogram showing the effect of saponin (Deodorase®) on the rumen microbiota using 16S rRNA (16S cDNA) **A.)** Denaturing gradient gel electrophoresis **B.)** HaeIII based terminal –restriction fragment length polymorphism (T-RFLP) **C.)** MSP1 based T-RFLP or **D.)** Alu1 based T-RFLP compared to control incubations in the absence of any treatment. Scale relates to per cent similarity.

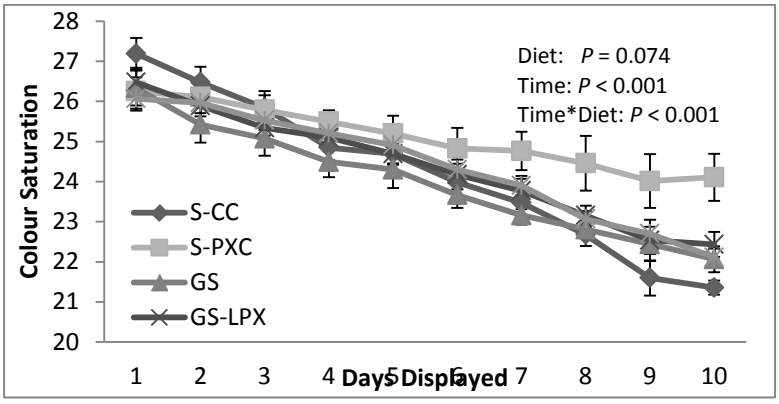

Figure 2. The colour saturation (mean ± sem) over 10 days storage of *M. longissimus* from Belgium Blue cattle fed different diets (P<0.05).

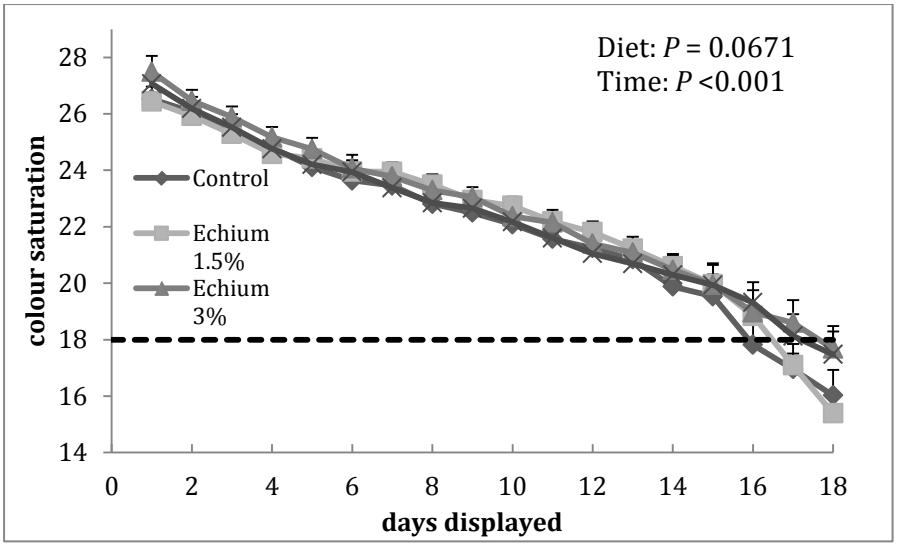

Figure 3. The colour saturation (mean ± sem) over 10 days storage of *M. longissimus* from Belgium Blue cattle fed different diets. (--) Threshold for acceptable colour saturation, where >18 is acceptable.

Table 3. The concentration (mg/100 g muscle) of CLA isomers in the *M. longissimus* from Charolais cross steers fed different diets.

	Diet					
	Control	Echium 1.5%	Echium 3%	Linseed 3%	S.E.D	*P*
CLAtr12,tr14	0.62	1.01	1.03	1.26	0.235	0.077
CLAtr11,tr13	0.84^{a}	1.46^{ab}	1.59^{ab}	1.75^{b}	0.317	**0.039**
CLAtr10,tr12	0.11^{a}	0.20^{ab}	0.25^{b}	0.17^{ab}	0.040	**0.011**
CLAtr9,tr11	0.44	0.62	0.77	0.63	0.123	0.080
CLAtr8,tr10	0.08^{a}	0.18^{ab}	0.26^{b}	0.10^{a}	0.044	**<0.001**
CLAtr7,tr9	0.19^{a}	0.36^{b}	0.35^{ab}	0.21^{ab}	0.061	**0.012**
CLAc12,tr14	0.61	1.03	0.70	1.02	0.211	0.123
CLAtr11,c13	2.43^{a}	4.26^{ab}	5.83^{b}	4.30^{ab}	0.846	**0.005**
CLAc11,tr13	0.28	0.40	0.36	0.36	0.067	0.330
CLAtr10,c12	0.07	0.11	0.12	0.14	0.034	0.261
CLAc9,tr11	10.73^{a}	16.32^{ab}	21.55^{b}	14.89^{ab}	2.739	**0.005**
CLAtr8,c10	0.61^{a}	1.03^{ab}	1.81^{b}	1.25^{ab}	0.339	**0.012**
CLAtr7,c9	1.18^{a}	1.91^{ab}	2.79^{b}	2.04^{ab}	0.372	**0.002**
CLAc11,c13	ND	ND	ND	ND	-	-
CLAc10,c12	ND	ND	ND	ND	-	-
CLAc9,c11	ND	ND	ND	ND	-	-
CLAc8,c10	ND	ND	ND	ND	-	-

^{ab} Means with different superscript differ significantly, within row. ND, non detectable.