

Gut microbiota modulation and effects on inflammation of a diet enriched in apple pomace in a DSS-induced colitis model in mice

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Running title: apple pomace modulates microbiota and attenuates severity of a DSS-induced colitis model

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Supplementary Table S1. General physicochemical characterization of the apple pomace used for inclusion in AP-diet (g/100g DM). Abbreviations used: DW (Dry weight), TDF (Total dietary fibre), IDF (insoluble dietary fibre), SDF (soluble dietary fibre) and TPC (total phenolic content). Average of three replicates \pm SD. Methodology employed for the characterization and additional compositional information data were reported in Calvete-Torre et al., 2021¹⁶.

Parameter	Perico variety
Total Carb. (g/100g DW)	82.8 \pm 24.2
%TDF	48.9 \pm 4.3
%IDF	39.5 \pm 0.9
%SDF	9.4 \pm 1.2
Reducing Carb. (g/100g DW)	41.9 \pm 1.1
Protein (%)	3.1 \pm 0.51
Fat (%)	2.4 \pm 0.4
Carbohydrates of low molecular weight (water-soluble)	
Malic acid (mg/g DW)	1.1 \pm 0.1
Fructose (mg/g DW)	126.1 \pm 1.3
Glucose (mg/g DW)	44.3 \pm 1.4
Sucrose (mg/g DW)	27.2 \pm 0.3
TPC (g/kg DW)	6.5 \pm 0.6
% Inhibitory Activity of DPPH	40.9 \pm 0.5
Galacturonic acid (%)	20.9 \pm 0.9
Neutral sugars (%)	20.1

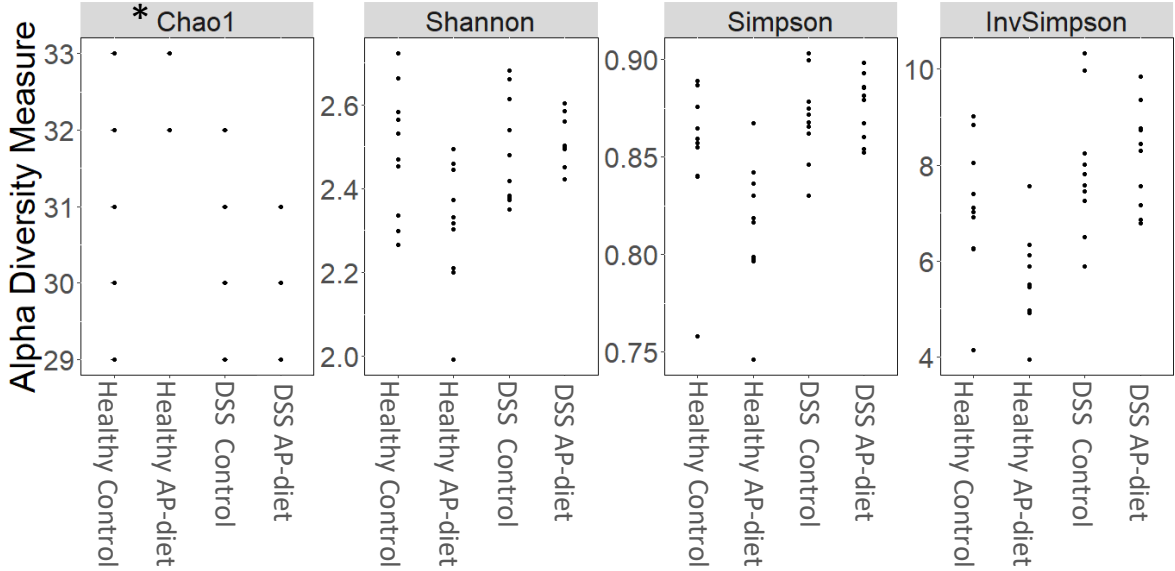
Supplementary Table S2. Composition of the control and AP-diet used for the animal experimentation. Diets were provided by ENVIGO in pelleted form.

	Control diet	AP-diet
Nutritional additives		
Vitamin A (I.U.)	6000	6000
Vitamin D3 (I.U.)	600	600
Fe (mg)	50	50
Mn (mg)	44	44
Zn (mg)	31	31
Cu (mg)	7	7
I (mg)	6.2	6.2
Technological additives		
Sepiolite (mg)	632	632
Analytical constituents		
Crude protein (%)	14.50	14.30
Crude oils and fats (%)	4.00	4.40
Crude fibres (%)	4.50	4.00
Crude ash (%)	4.70	4.50

Supplementary Table S3. Disease activity index (DAI) scoring system used to evaluate the DSS-induced colitis. DAI index was calculated as total score (body weight decrease + stool consistency + bloody stools) divided by 3.

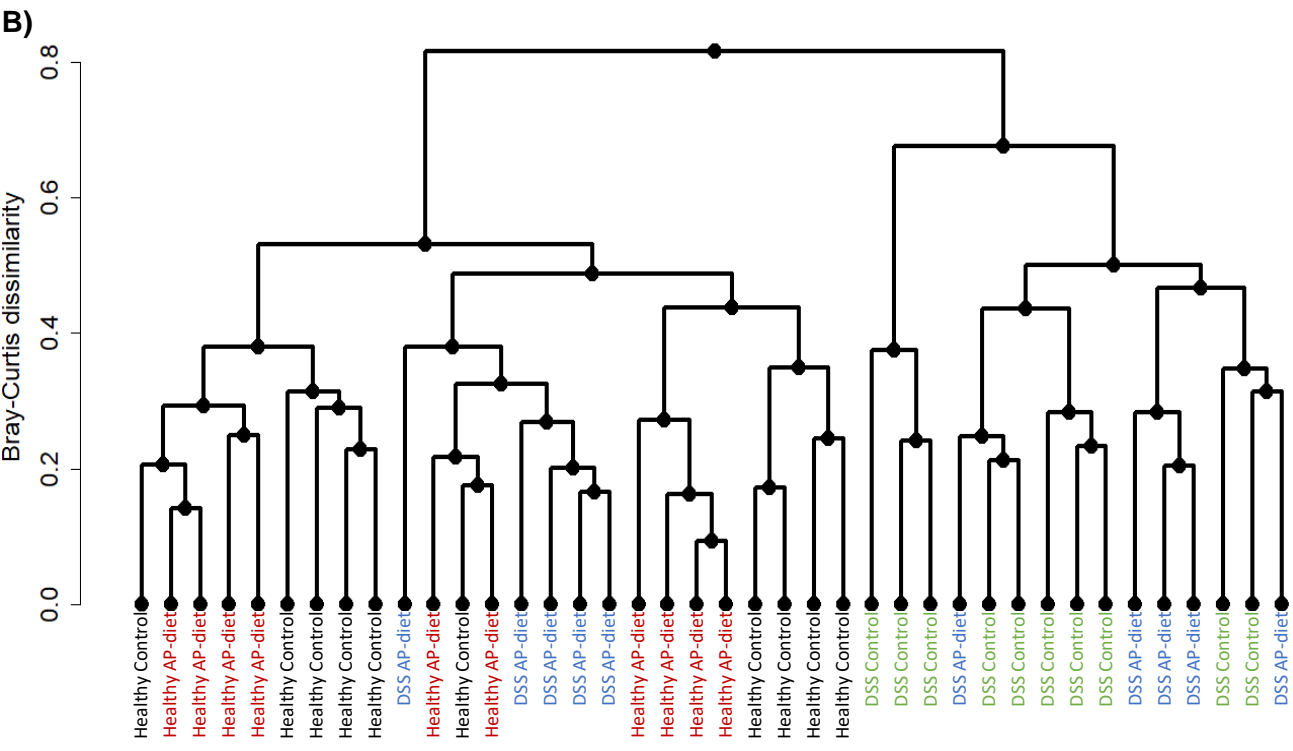
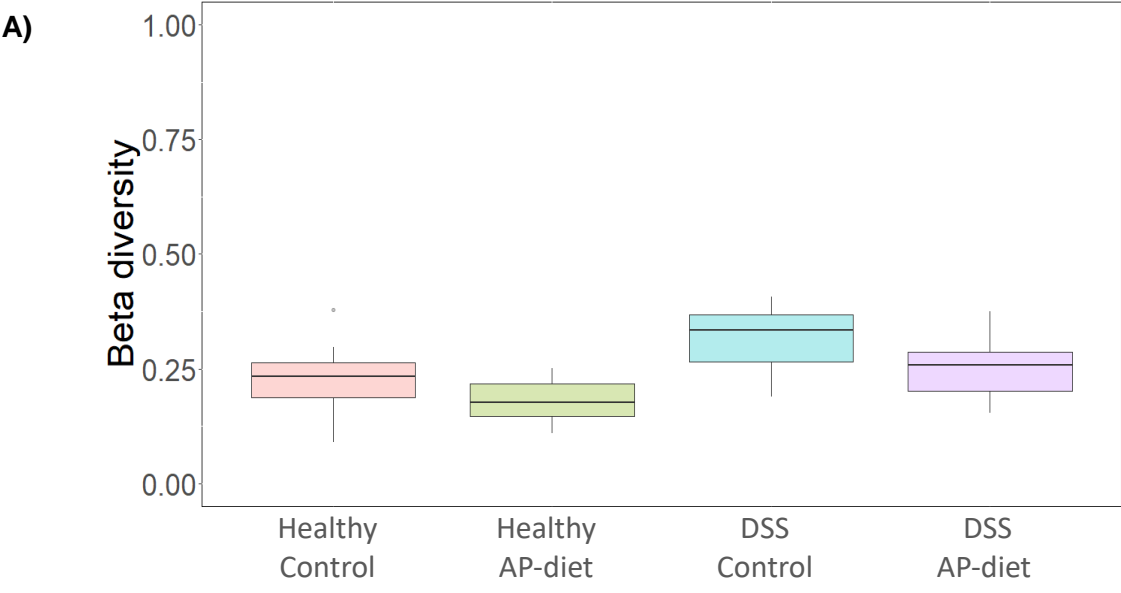
Score	Weight loss	Blood in feces	Feces consistency
0	No weight loss	No blood	Normal
1	< 5 %		
2	< 10 %		Loose stools
3	< 20 %		
4	≥ 20 %	Blood in feces	Diarrhea

Supplementary Figure S1. Comparison of different alpha-diversity indicators (Chao1, Shannon, Simpson and Inverse Simpson) of colonic content samples of mice following control and pomace-enriched diets. These samples correspond to healthy and DSS-induced colitis groups.



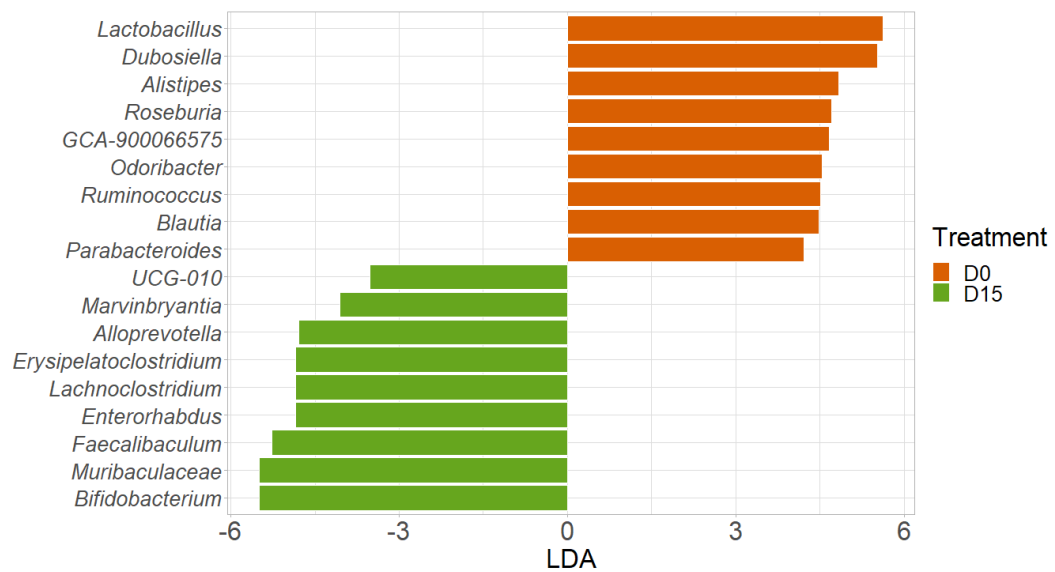
*Several samples from each group showed the same Chao1 values, corresponding to fully overlapped points in the Figure.

Supplementary Figure S2. Beta-diversity (A) and cluster (B) analysis of colonic content samples of mice following control and apple pomace-enriched diets. These samples correspond to healthy and DSS-induced colitis groups. Bray-Curtis dissimilarity method was selected for the calculation

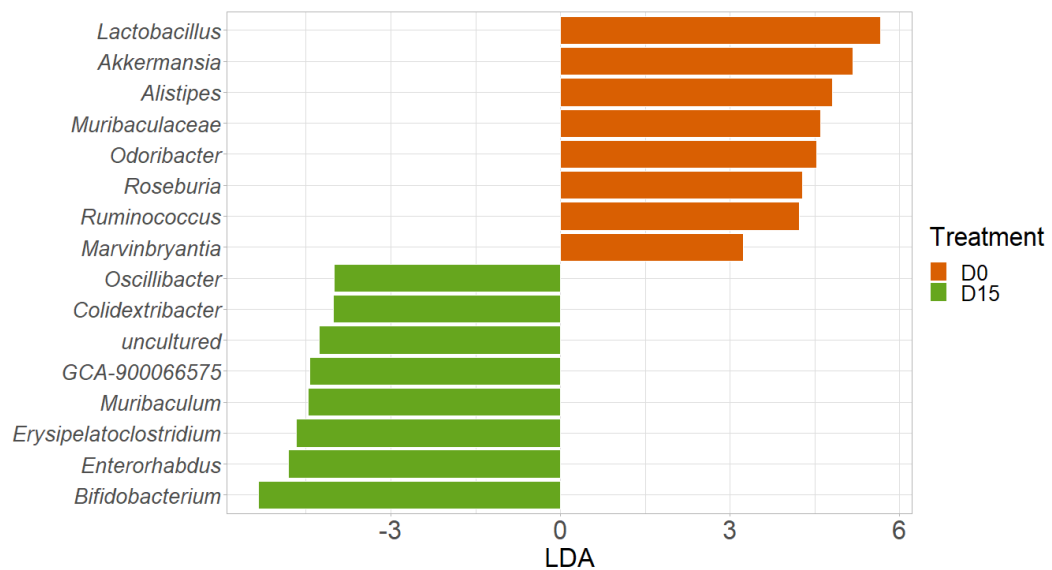


Supplementary Figure S3. Linear Discriminant Analysis (LDA) scores of differentially abundant microbial genera among fecal samples of mice following control (n=20 animals) (A) and pomace-enriched (n=20 animals) (B) diets after 0 and 15 days (D0 and D15) of intervention. Both control diets and diets enriched in apple pomace led to an increase in *Bifidobacterium*, *Muribaculum*, *Enterorhabdus*, *Erysipelatoclostridium* and a reduction in *Lactobacillus*. Linear Discriminant Analysis (LDA) scores of differentially abundant microbial genera among fecal samples of mice following control and pomace-enriched diets after 24 days of intervention. These samples correspond to healthy (A) and DSS-induced colitis (B) groups. Therefore, a total 4 groups (n=10 animals per group) are compared. With regard to the healthy group, diets enriched in apple pomace resulted in higher abundances of *Blautia*, *Alloprevotella* and *Muribaculaceae* compared to those obtained in control diets after 24 days of intervention. On the contrary, in DSS group, diets enriched in apple pomace led to higher abundances of *Alistipes*, *Parabacteroides* and *Oscillibacter* compared to control diets.

A) Control diet adaptation: 0 days vs 15 days

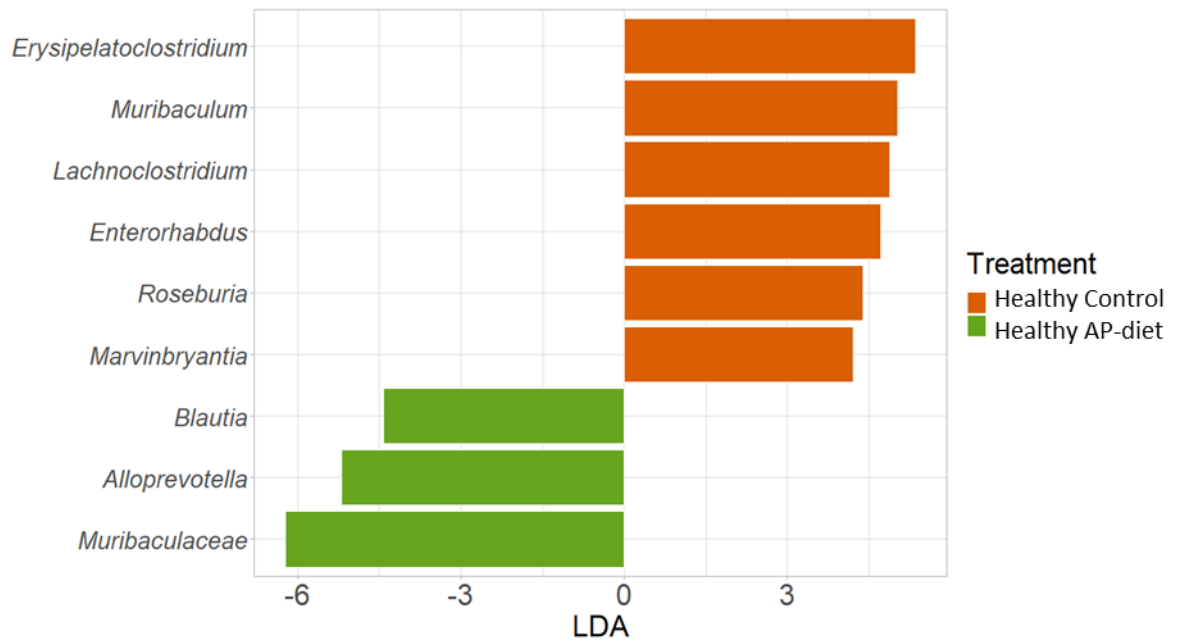


B) AP-diet adaptation: 0 days vs 15 days

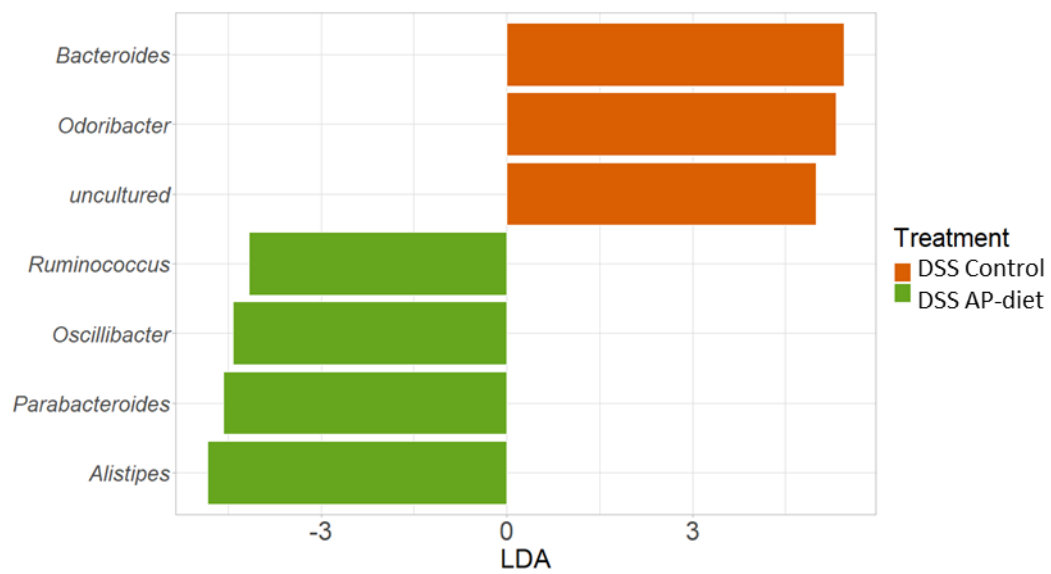


Supplementary Figure S3 cont.

C) Time 24 days – Healthy group: control diet vs pomace-enriched diet



D) Time 24 days – DSS group: control diet vs pomace-enriched diet



Supplementary Figure S4. Major short chain fatty acids (SCFAs) determined in colonic contents of the animals collected from the four groups of animals at the end of the experiment (sacrifice point). Animal groups: Control diet, no DSS; AP-diet (Bagasse), no DSS; Control diet, 2.5% DSS; AP-diet (Bagasse), 2.5% DSS (n=10 mice per group). Diet did not significantly affect the level of the major SCFAs.

