



EFFECT OF A BLEND OF ESSENTIAL OILS ON RUMEN MICROBIOTA FOR RELIEVING SUB-ACUTE RUMINAL ACIDOSIS

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INTRODUCTION

- Sub-acute ruminal acidosis (SARA) is a nutritional disease affecting high-yielding dairy cattle (Orton et al., 2020)
- This disease is characterised by daily episodes where:
 - Rumen pH drops below 5.6 for a minimum of 3 hours (Gozho et al., 2005)
 - Rumen pH below 5.8 for more than 5-6 hours (Zebeli et al., 2008)
- Negative effects on (Krause and Oetzel, 2006):
 - Feed intake
 - Fiber digestibility
 - Milk production
 - Milk fat depression
 - Health (inflammatory response laminitis, diarrhea)

OBJECTIVE

- Evaluate the impact of a commercial blend of essential oils (EO), containing cinnamaldehyde, eugenol, carvacrol and capsaicin (Activo Premium®), on the rumen microbial compositions under SARA conditions

METHODS

- Donor cows
 - Experimental design: Cross-over design with 21-d of adaptation
 - 4 ruminally fistulated dry Holstein cows
 - Treatment: CONT vs EO (3 g/d; 222 mg/kg DMI)
- In Vitro incubation
 - Gas production (Theodorou method)
 - 24 h incubation
 - Treatment: CONT vs EO (rumen fluid from donor cows)
 - Substrates: C TMR (18% starch on DM basis) vs SARA TMR (46% starch DM basis)
 - Sampling:
 - pH
 - Fermentative profile
 - Lactic acid production
 - Gas production and composition
 - Protozoan count
 - Ruminal microbiome
 - 16S rRNA sequencing data analysed using DADA2 and QIIME2 software
 - SILVA v132 as reference data

RESULTS & DISCUSSION

Table 1 Effects of treatment and substrate on pH, GP, CH₄ and protozoan count

	Treatment				SE	P-value		
	CONT		EO			Trt	Sub	Trt x Sub
	Substrate		Substrate					
	C TMR	SARA TMR	C TMR	SARA TMR				
pH	5.76	5.39	5.84	5.42	0.032	0.06	<0.01	0.4
GP (ml/g DM)	92	103	93.2	104	3.21	0.38	<0.01	0.8
CH ₄ (%)	29.5 ^A	30.3 ^A	26.2 ^B	23.8 ^B	1.8	<0.01	0.2	0.02
Entodinium (10 ³ /ml)	400	410	336	339	7.51	<0.01	0.25	0.48
Holotricha (10 ³ /ml)	6.2	4.6	6.4	3.4	1.1	0.62	0.05	0.5
Protozoa (10 ³ /ml)	406	414	343	342	7.63	<0.01	0.38	0.34

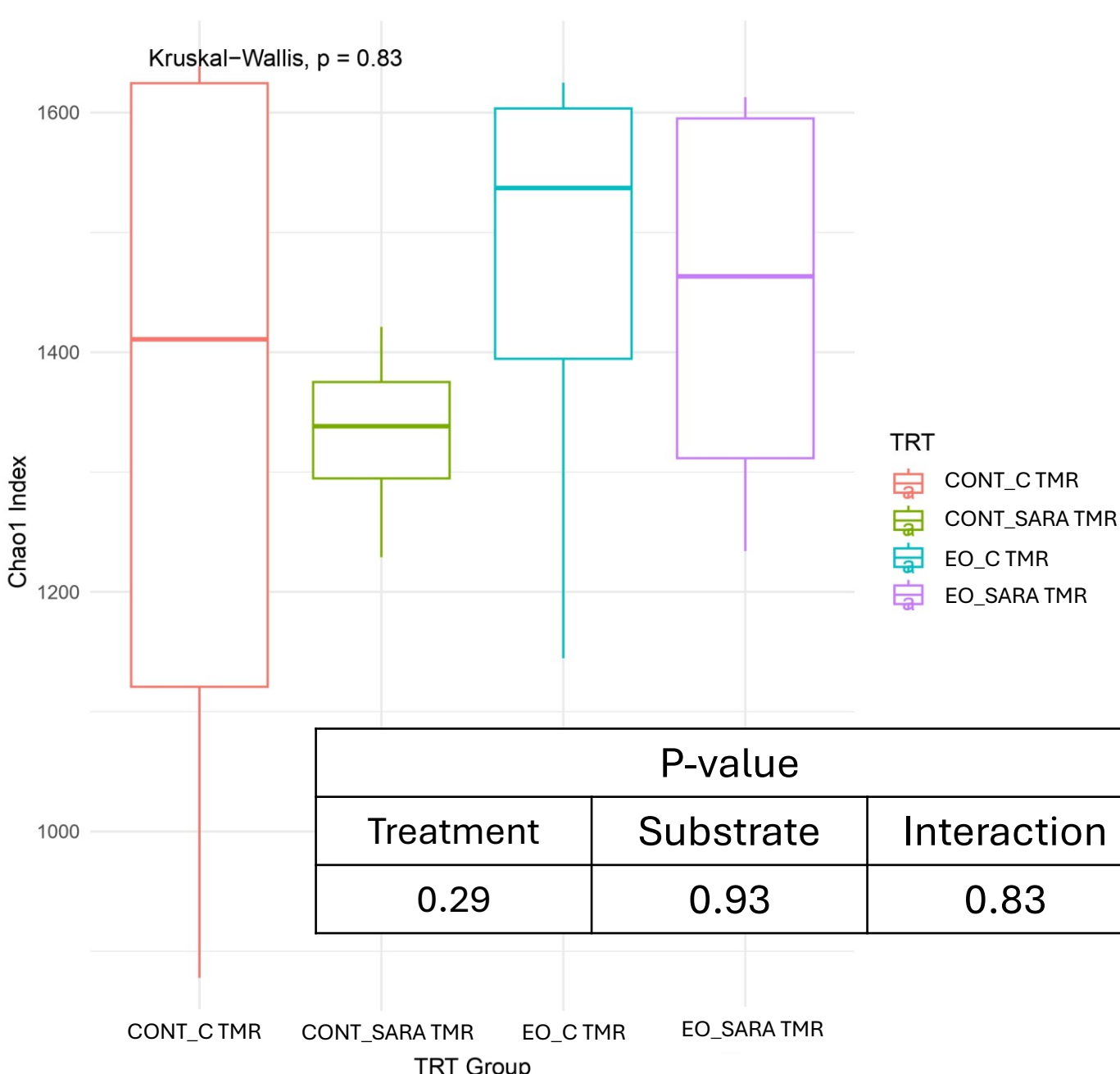


Figure 1 Effects of treatment and substrate on Alpha-diversity

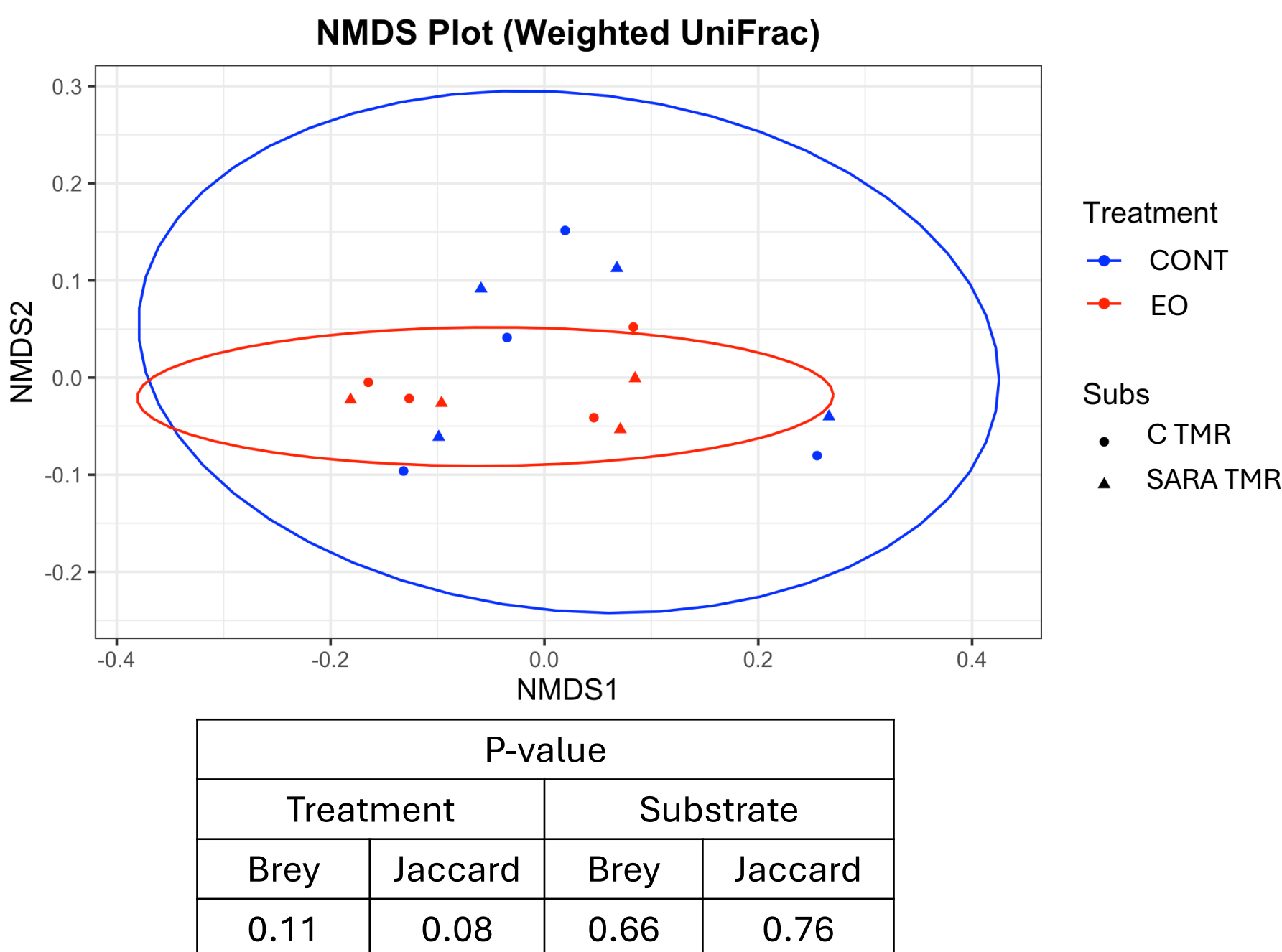


Figure 2 Effects of treatment and substrate on Beta-diversity

Table 2 Effects of treatment and substrate on the relative abundance of the ruminal microbiome

Relative abundance (%)	Treatment				SE	P-value		
	CON		EO			Trt	Sub	Trt x Sub
	Substrate		Substrate					
	C TMR	SARA TMR	C TMR	SARA TMR				
Genus								
Anaerovibrio	0.108	0.084	0.246	0.199	0.039	0.01	0.38	0.77
Eubacterium ventriosum group	0.057	0.134	0.056	0.091	0.019	0.27	0.02	0.30
F082	2.78	1.29	2.51	1.85	0.134	0.53	<0.01	0.08
Methanobrevibacter	0.052	0.035	0.03	0.022	0.002	0.09	0.19	0.14
Prevotellaceae Ga6A1 group	0.332	0.127	0.587	0.493	0.062	<0.01	0.04	0.39
Streptococcus	1.92	5.73	2.09	2.90	1.15	0.28	0.08	0.23
WCHB1_41	3.57	2.36	3.24	2.38	0.279	0.59	<0.01	0.55
Species								
Prevotella bryantii	0.132	0.136	0.604	1.026	0.143	<0.01	0.08	0.09
Treponema ruminis	0.198	0.07	0.213	0.141	0.027	0.15	<0.01	0.33

CONCLUSION

- EO shows a trend of higher pH
- 16 % reduction of CH₄ production and protozoa populations with EO, especially *Entodinium*; in addition, a trend was seen in reducing *Methanobrevibacter*
- Treponema ruminis*, a fiber-utilizing bacteria, was negatively affected by SARA TMR and, although no significant effect was observed, EO showed an increase when incubated under SARA
- With SARA TMR, EO numerically reduced *Streptococcus*, a lactic acid producing bacterium favored by starch-rich environments
- EO increased the abundance of *Prevotella bryantii*, which utilizes starch to produce end products other than lactate, such as acetate, propionate and succinate; also contributes to protein metabolism

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