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Looking for natural solutions to reduce methane and ammonia emission potentially from dairy cows without impairing the fermentation characteristics

INTRODUCTION

Ruminants greatly contribute to meet the nutritional challenges of food security because they can convert fibrous plant materials into meat and milk products for human consumption. Meanwhile, rumen protozoa are blamed to contribute to low down feed efficiency, wasting 2–15% of the ingested energy as methane and 75–95% of the ingested feed as NH3-N emission. Therefore, this study aimed at using enriched-bioactives plant to potentially improve feed efficiency in ruminant, through inhibiting rumen protozoa while reducing ammonia and methane emission for a green production.

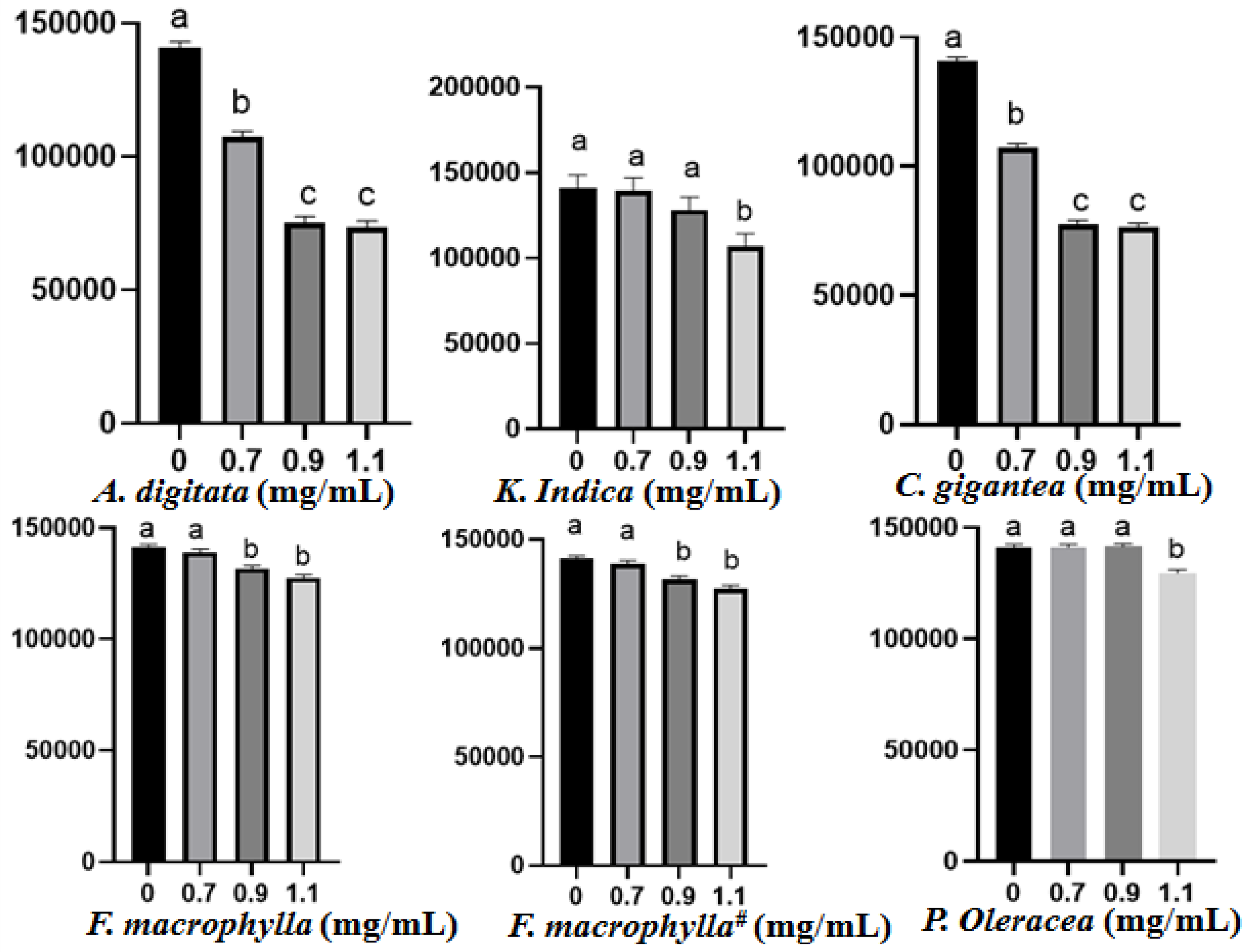

Fig. 1 Inhibitory effect of plants on rumen protozoa $P<0.05$

Table 2: Effect of *Calotropis* leaves on VFAs, CH4 and NH3-N

Fermentation characteristics	Dose					*P*-value		
	0	0.8	1.6	3.2	SEM	Trt*	Linear	Quad
Total VFA, mM	83.9	96.4	107.3	107.3	5.6	0.04	0.028	0.0281
VFA, mol/100 mol								
Acetate	64.6^{a}	60.6^{ab}	60.3^{ab}	53.9^{b}	1.2	0.001	<0.001	0.007
Propionate	19.4^{b}	21.1^{b}	16.3^{b}	23.4^{a}	0.96	0.001	0.001	0.788
Isobutyrate	0.8^{b}	0.9^{b}	0.8^{b}	1.0^{a}	0.04	0.001	<0.001	0.179
Butyrate	10.3^{c}	12.0^{c}	17.8^{a}	15.4^{b}	0.43	0.001	<0.001	<0.001
Isovalerate	1.5^{b}	1.7^{b}	1.6^{b}	2.0^{a}	0.11	0.001	0.002	0.265
Valerate	3.4^{b}	3.6^{b}	3.2^{b}	4.3^{a}	0.18	0.001	<0.001	0.356
A:P ratio	3.3^{a}	2.9^{abc}	3.7^{b}	2.3^{c}	0.18	0.001	<0.002	0.689
NH_3-N, mg/Dl	31.2^{a}	27.7^{ab}	15.4^{d}	20.8^{c}	0.90	<0.001	0.001	<0.001
Methane*, mol/100 mol	27.8^{a}	26.3^{b}	29.8^{a}	24.0^{b}	0.77	<0.001	<.001	0.865
AO, U/Ml	72.0	67.2	61.2	47.3	8.30	0.209	0.045	0.223
ORP, Mv	-354.5	-358.8	-318.3	-314.8	-16.50	0.172	0.061	0.200
Ph	6.68	6.63	6.69	6.57	0.03	0.554	0.425	0.870

MATERIALS AND METHODS

Rumen protozoa were anaerobically cultured and supplemented with six different plant leaves at 4 doses with three replications (0, 0.7, 0.9, and 1.1 mg/mL (Fig.1)) . Indeed, after 24h of protozoa cultivation using Rumen Simulating Technology, the inhibitory effect on rumen was firstly detected using light and scanning electron microscopy. Moreover, methane and ammonia concentrations were evaluated using gas chromatography and colorimetry method respectively. Total bacteria and methanogens were quantified using metagenomic DNA extraction and qPCR. Finally, a phytochemical screening of the tested plants consisted of ultra-sonic extraction then, HPLC identification.

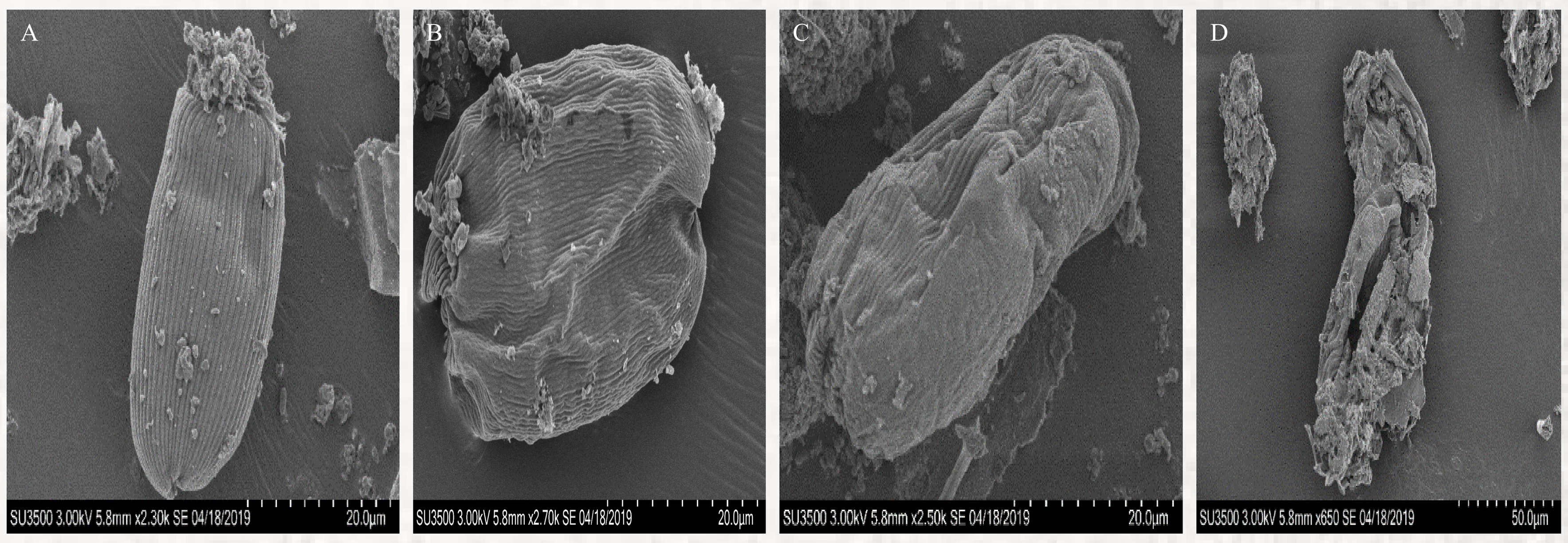

Fig. 2: Damaging of protozoa cell surface. Doses: A< B<C<D

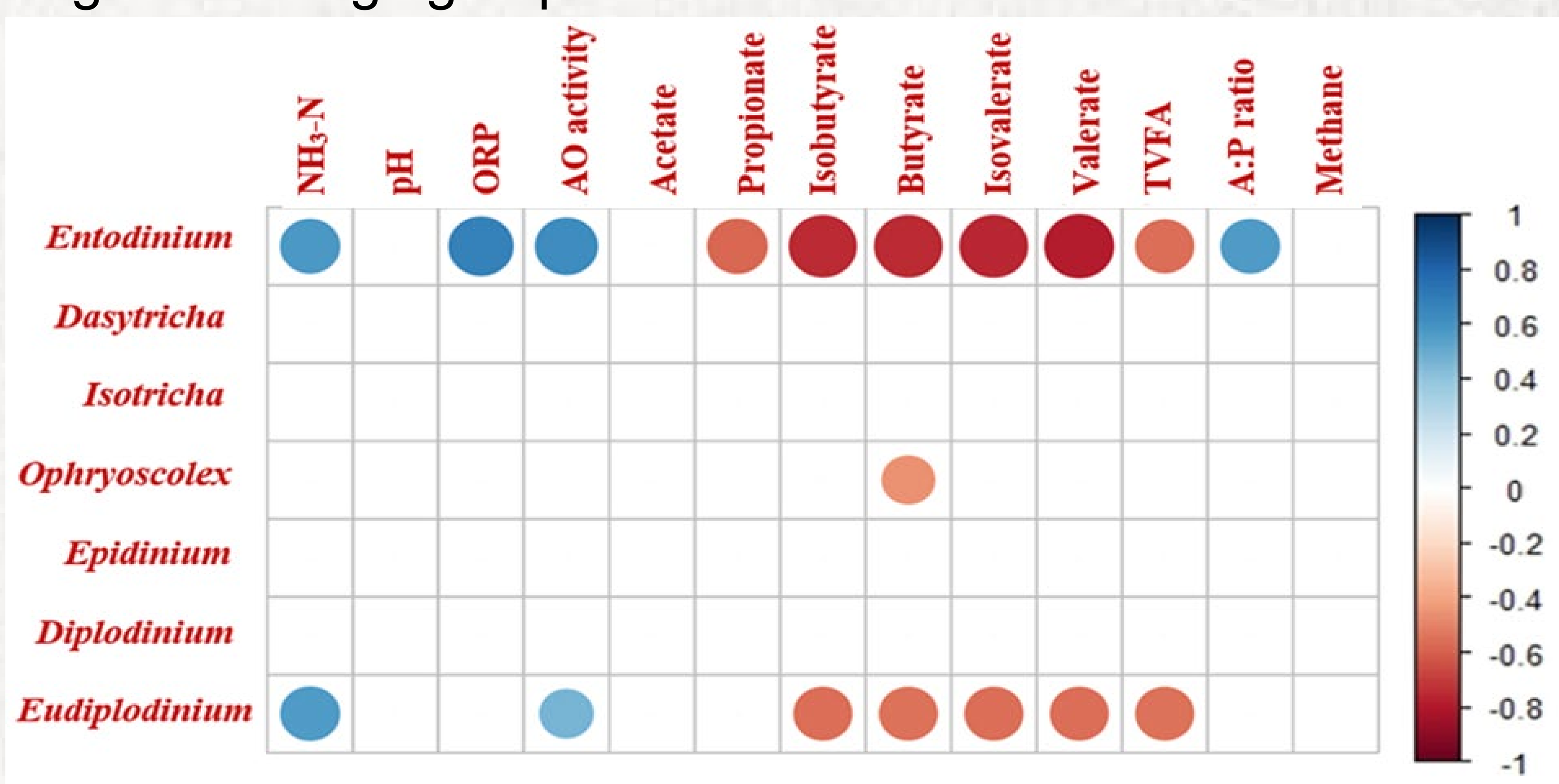

Fig. 3: Correlation between protozoa and fermentation , $P<0.05$

RESULTS

Results showed that the tested plants decreased the protozoa counts (Fig.1) but only *Calotropis* reduced at the same time NH3-N which indicated it potential to enhanced N efficiency while reducing CH4 (Table 2). Therefore, the plant was further analyzed to described its extracellular damaging known as a death metabolic pathway(Fig.2). The inhibition of *Entodinium* led to the decrease of wasteful NH3-N (Fig.3) and increased of VFAs, source of animal energy. Metagenomics analysis revealed that total bacteria and archaea were maintained while HPLC analysis indicated that flavonoids, especially quercetin might be the responsible plant inhibitor of the rumen protozoa.

Overall, this study showed how functional plants can be associated to livestock , replacing the harmful antibiotics and satisfying the increasing animal products demand while reducing the GHG footprint.

FTA-Kunming Scientific Conference 2021

CGIAR Research Program on Forests, Trees and Agroforestry