Effect of supplementation of *Bacillus* spp. on in vitro fermentation, methane production, and nutrient digestibility

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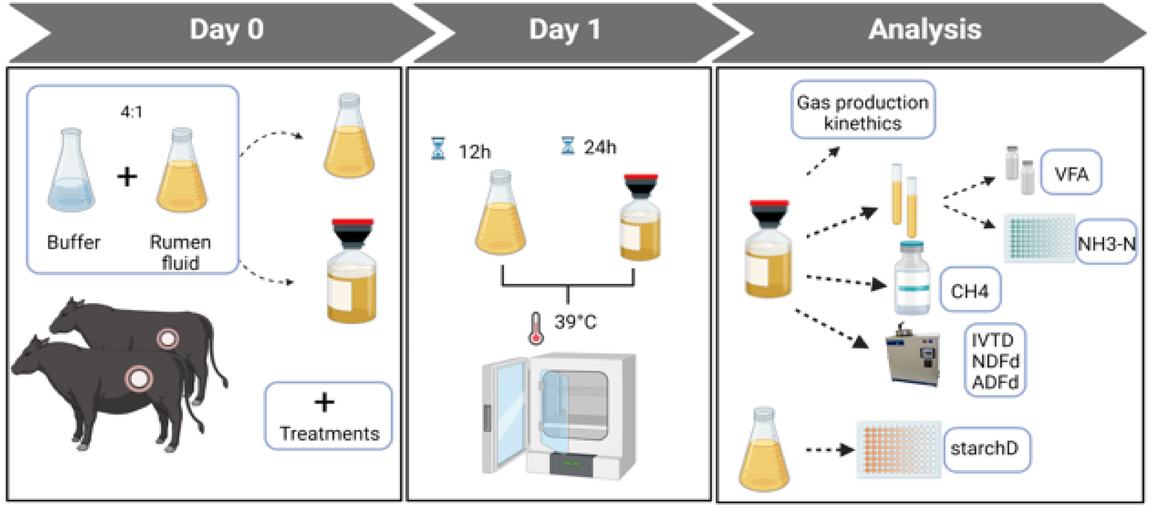
Introduction

- Enteric methane emissions in ruminants account for potential damage to the environment and energy losses to the animal
- Feed management approaches are the most developed methods to mitigate enteric methane emissions
- Probiotics have been used as feed additives to modify ruminal fermentation profile, enhance feed efficiency, promote animal performance, and reduce methane emissions
- *Bacillus* spp. have demonstrated a potential to improve animal production and reduce methane emissions, different strains and their dose-response in ruminal fermentation parameters remain unclear

Objective

To evaluate the effects of increasing doses of a *Bacillus* spp. probiotic on enteric methane production, gas production kinetics, ruminal fermentation profile, and nutrient digestibility in vitro, using a sorghum silage-based substrate.

Materials & Methods



- In vitro incubations were conducted in sixteen separate days (replicates) 4 treatments, and 2 bottles per treatment per incubation day
- Treatments were:
  - Sorghum-silage only (Control)
  - Sorghum silage + one of three doses of *Bacillus* spp. probiotic included at 1, 5, or 10 times the recommended dose (8×10^4 CFU/mL)

Results

Table 1. Effect on gas production kinetics, methane production, and ammonia nitrogen in batch culture incubations with increasing doses of *Bacillus* spp. in the substrate.

	Treatment					Contrast *P*-value		
Item	0	1	5	10	SEM	L	Q	C
M, mL/g of DM incubated	102.50	106.40	113.29	110.95	6.078	0.181	0.262	0.913
K, mL/h	4.86	4.87	5.46	4.76	0.271	0.991	0.002	0.324
Lag, h	1.66	1.39	0.70	0.93	0.429	0.170	0.259	0.979
Kf, h^{-1}	0.05	0.05	0.05	0.04	0.002	0.100	0.278	0.241
Total CH_4, m*M*	4.69	4.39	5.04	4.99	0.206	0.065	0.449	0.138
CH_4 production, mmol/g of DM fermented	5.04	4.74	5.48	5.43	0.251	0.070	0.440	0.192
NH_3-N, m*M*	5.76	5.67	6.47	5.59	0.205	0.994	0.003	0.180

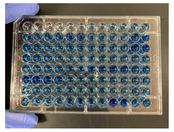
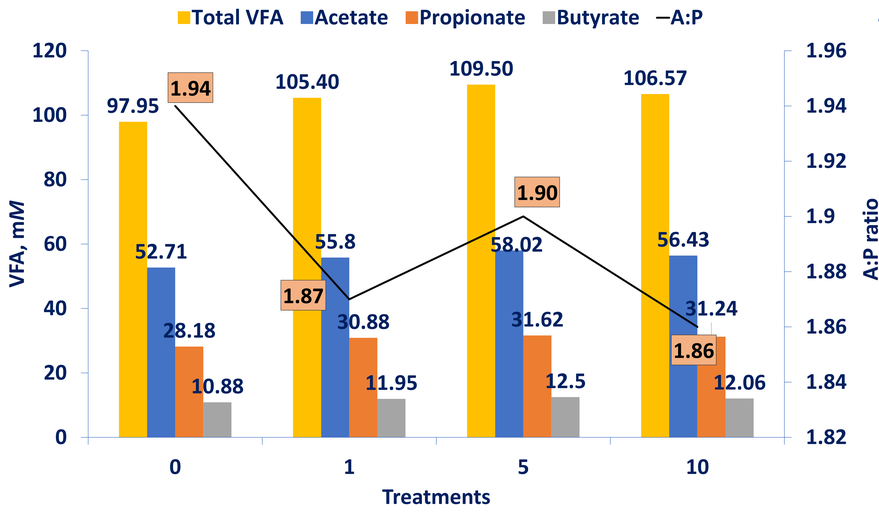


Figure 1. Total fatty acid (VFA), acetate, propionate, butyrate concentrations (m*M*) and acetate to propionate ratio (A:P) in batch culture incubations with increasing doses of *Bacillus* spp. in the substrate.



Total VFA: : quadratic effect *P* = 0.008, SEM = 2.148
Acetate: quadratic effect *P* = 0.022, SEM = 1.202
Propionate: linear effect *P* = 0.004, SEM = 0.582
Butyrate: quadratic effect *P* = 0.002, SEM = 0.261
A:P: cubic effect *P* = 0.008, SEM = 0.017

- Inclusion of *Bacillus* spp. did not affect (*P* > 0.05) nutrients digestibility or pH in vitro

Conclusion

Bacillus spp. exhibited dose dependent effects, with promissory results on fermentation parameters, and more studies should be conducted to assess potential in vivo CH_4 mitigation capabilities.