# Effects of ruminal protozoa on methane emissions in ruminants – a meta-analysis

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## INTRODUCTION

- Complete inhibition of ruminal protozoa (RP) reduces methane production by up to 11% (Newbold et al., 2015).
- Thirty-one percent of 76 *in vivo* experiments have demonstrated a concomitant reduction in RP numbers and methane production (Guyader et al., 2014).
- Changes in RP numbers have a linear relationship with changes in methane production with supplemental saponins ($r$ =0.69), tannins ($r$ = 0.55), and essential oils ($r$ = 0.45; Patra, 2010).

## OBJECTIVES

- To evaluate the relationship between RP numbers and methane production
- To test the effects of dietary chemical composition, ruminal fermentation, total tract digestibility, and milk performance on the relationship between RP numbers and methane production
- To evaluate the predicted model of methane production based on ruminal fermentation, total tract digestibility, and milk production when considering RP numbers

## MATERIALS & METHODS

- 67 published *in vivo* studies, 85 experiments, 256 treatments with 1887 animals (1996-2020)
- Methane production (units): g/kg DMI
- RP (units): log10 cells/mL
- Dataset includes:
  - ✓ Dairy cows: 51%
  - ✓ Beef steers: 22%
  - ✓ Small ruminants: 27%
- 70% of the studies reported a reduction in methane emission
- Treatments:
  - ✓ Defaunation
  - ✓ Phytochemicals (tannins, saponins, or essential oils)
  - ✓ Lipids (MCFA, LCFA)
  - ✓ Probiotics/Prebiotics
  - ✓ Chemicals (iodopropane, nitrate, sulphate)
- Basic model: $CH_4 = \mu + \alpha \times RP + \beta \times RP^2$
- MIXED procedure: (lme4 and lmerTest packages in R)
  - ✓ Random effect of experiment ID
  - ✓ Weight = 1/(pool SEM)$^2$
- Each factor was added to the basic model to evaluate its impact on the relationship
- Predicted models were generated and evaluated based on the basic model

## RESULTS

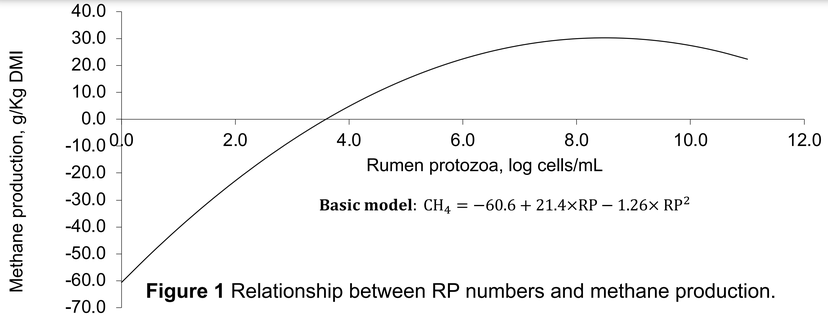


**Figure 1** Relationship between RP numbers and methane production.

**Table 2** Predicted models of methane production

| | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|
| *Predictors* | *Estimates* | *p* | *Estimates* | *p* | *Estimates* | *p* |
| (Intercept) | 49.96 | **<0.001** | -47.86 | **<0.001** | -32.48 | **0.002** |
| RP | 2.38 | 0.072 | 26.06 | **<0.001** | 9.86 | **<0.001** |
| RP$^2$ | -0.15 | 0.187 | -1.77 | **<0.001** | -0.64 | **<0.001** |
| Acetate | -0.36 | **<0.001** | | | | |
| Propionate | -0.77 | **<0.001** | | | | |
| DM digestibility | | | -0.55 | **<0.001** | | |
| NDF digestibility | | | 0.27 | **<0.001** | | |
| Milk yield | | | | | -0.26 | **0.004** |
| Protein, % milk yield | | | | | 7.40 | **0.003** |
| **Random Effects** | | | | | | |
| AICc | 1401 | | 705 | | 489 | |
| RMSE | 2.76 | | 2.50 | | 1.88 | |
| CCC | 0.84 | | 0.88 | | 0.93 | |
| N | 80 ExpN | | 41 ExpN | | 33 ExpN | |

**Figure 2** Comparation of different models

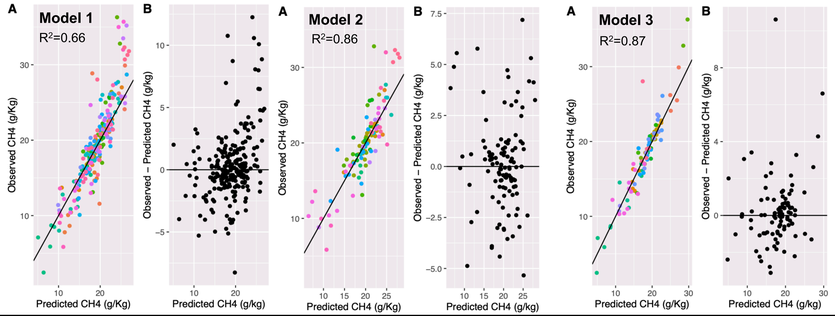


**Table 1** Effects of other factors on the relationship between ruminal protozoa (RP) numbers and methane production

| Items | Intercept | | | RP | | | RP x RP | | | Other factors | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | slope | Std. | *P*-value | slope | Std. | *P*-value | slope | Std. | *P*-Value | slope | Std. | *P*-value |
| **Basic model** | -60.6 | 5.91 | <0.01 | 21.4 | 2.15 | <0.01 | -1.26 | 0.20 | <0.01 | - | - | - |
| **Ruminal fermentation** | | | | | | | | | | | | |
| pH | -20.8 | 7.43 | <0.01 | 4.88 | 1.31 | <0.01 | -0.33 | 0.11 | <0.01 | 3.51 | 1.08 | <0.01 |
| TVFA, m*M* | -6.86 | 4.95 | 0.17 | 3.94 | 1.72 | 0.02 | -0.09 | 0.17 | 0.60 | 0.07 | 0.01 | <0.01 |
| Acetate, % | -22.0 | 6.11 | <0.01 | 5.54 | 1.57 | <0.01 | -0.34 | 0.13 | 0.01 | 0.31 | 0.08 | <001 |
| Propionate, % | 21.3 | 4.72 | <0.01 | 2.62 | 1.36 | 0.05 | -0.17 | 0.12 | 0.14 | -0.57 | 0.05 | <0.01 |
| Butyrate, % | -4.87 | 4.05 | 0.23 | 4.02 | 1.37 | <0.01 | -0.23 | 0.12 | 0.06 | 0.80 | 0.08 | <0.01 |
| Isobutyrate, % | -5.02 | 5.51 | 0.36 | 5.01 | 1.89 | <0.01 | -0.29 | 0.17 | 0.09 | 4.97 | 1.31 | <0.01 |
| Valerate, % | -2.10 | 5.32 | 0.69 | 6.91 | 1.66 | <0.01 | -0.45 | 0.14 | <0.01 | -2.56 | 0.76 | <0.01 |
| Isovalerate , % | -3.72 | 5.84 | 0.52 | 5.46 | 1.98 | <0.01 | -0.33 | 0.17 | 0.05 | 1.38 | 0.76 | 0.07 |
| $NH_3$-N, m*M* | 2.58 | 4.33 | 0.55 | 3.37 | 1.55 | 0.03 | -0.10 | 0.15 | 0.51 | 0.07 | 0.06 | 0.28 |
| **Digestibility, %** | | | | | | | | | | | | |
| DM | -71.6 | 7.00 | <0.01 | 32.9 | 2.16 | <0.01 | -2.23 | 0.20 | <0.01 | -0.36 | 0.03 | <0.01 |
| OM | -95.4 | 7.33 | <0.01 | 36.3 | 2.20 | <0.01 | -2.53 | 0.20 | <0.01 | -0.15 | 0.05 | <0.01 |
| CP | -97.7 | 12.40 | <0.01 | 40.6 | 3.68 | <0.01 | -2.99 | 0.34 | <0.01 | -0.26 | 0.06 | <0.01 |
| NDF | -106 | 5.72 | <0.01 | 35.3 | 2.12 | <0.01 | -2.48 | 0.18 | <0.01 | 0.11 | 0.04 | 0.01 |
| **Production (dairy cows)** | | | | | | | | | | | | |
| Milk yield, kg | -3.87 | 5.17 | 0.45 | 8.63 | 1.51 | <0.01 | -0.55 | 0.14 | <0.01 | -0.27 | 0.09 | <0.01 |
| Milk fat, % | -10.5 | 4.49 | 0.02 | 6.61 | 1.70 | <0.01 | -0.41 | 0.15 | <0.01 | 1.26 | 0.48 | 0.01 |
| Milk protein, % | -41.7 | 10.60 | <0.01 | 9.99 | 1.56 | <0.01 | -0.66 | 0.14 | <0.01 | 0.09 | 7.76 | <0.01 |
| Milk lactose, % | -36.0 | 15.60 | 0.02 | 8.94 | 1.57 | <0.01 | -0.57 | 0.14 | <0.01 | 4.62 | 2.94 | 0.12 |

## CONCLUSIONS

- There is quadratic relationship between methane production and RP numbers:
  $CH_4 = -60.6 + 21.4 \times RP - 1.26 \times RP^2$
- Ruminal fermentation parameters, total tract digestibility, and milk production had significant impacts on the relationship between methane production and RP numbers; however, they only changed the magnitude of intercept and slope of RP and RP$^2$ for the relationship.
- For ruminants, the best-predicted model for methane production would be using total tract digestibility of DM and NDF as predictors when considering RP numbers.
- For dairy cows, the best-predicted model for methane production would be using milk yield and milk protein concentration as predictors when considering RP numbers.

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