Control of equine tapeworms through praziquantel: The hidden impact on the equine microbiome

Boontarikaan Wititkornkul[1,2], Susan E. Girdwood[1], Hilary J. Worgan[1], Russell M. Morphew[1] & Ruth E. Wonfor[1]

1.Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Wales, UK, SY23 3EE
2.Faculty of Veterinary Science, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, THAILAND, 50200

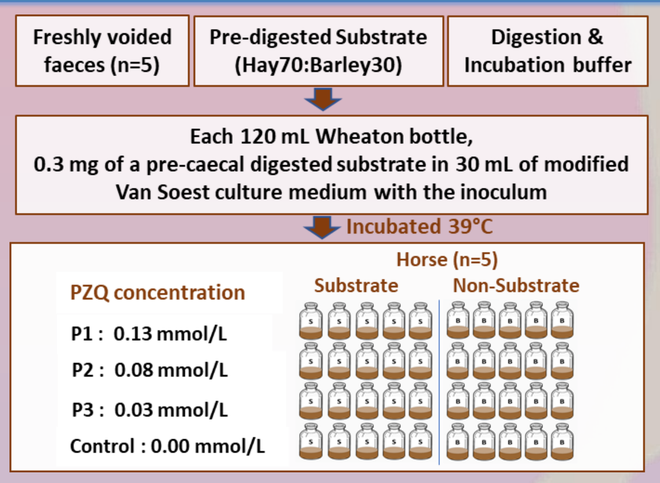
Introduction

Horses are hindgut fermenters and microbes within the hindgut play important roles in enhancing the fermentation processes providing nutrients and energy to the host, as well as maintaining intestinal homeostasis. However, dysbiosis of the equine hindgut microbiota is common and can be influenced by a variety of factors, often associated with metabolic diseases and disorders[1]. The equine tapeworm, *Anoplocephala perfoliata*, infects horses worldwide and are found primarily attached to the caecal wall, adjacent to the ileocaecal valve[2]. Both *A. perfoliata* and praziquantel (PZQ), a commonly used anthelmintic for *A. perfoliata* treatment and control, represent a substantial threat to the fragile equine microbiome. Recent molecular work generating the first *A. perfoliata* transcriptome and characterising the proteome of the secretome has supported our understanding of the host-parasite interaction[3]. However, the interaction between the infection and the exposure of PZQ on the equine gut microbiome has so far been neglected. The current work therefore aimed to initially determine the effect of PZQ on hindgut fermentation kinetics using an *in vitro* hindgut fermentation model.

In vitro hindgut fermentation model

Materials & Methods:

- Substrate were fermented *in vitro* with an equine faecal inoculum (n=5) using **a hindgut fermentation gas production model**[4].
- **Substrate** (Mixed meadow hay & barley in a 70:30 ratio) went through pre-caecal digestion by pepsin and pancreatin treatment[5].
- **Treatments: 4 PZQ** at 0.00, 0.03, 0.08 and 0.13 mmol/L with and without substrate.
- **The kinetics of gas production profile** over a 72 h fermentation period were analysed using the exponential model: $Y = a+b(1-e^{(-ct)})$ [6].
- **Fermentation products, parameters and the metabolome** after 24 hours incubation following exposure to PZQ at various dosage levels were investigated.



Effect of PZQ on hindgut fermentation kinetics

Result:

- No significant different between 4 PZQ treatments ($p>0.05$) on
  - The kinetics of gas production profile over a 72h fermentation period (Figure 1&Table 1).
  - pH, Ammonia and Lactate concentration (Table 2) and metabolome fingerprint after 24h incubation (Figure 2).
- At 24 hours post incubation (Table 2)
  - A low dose of PZQ significantly increase the production of total butyrate level ($p<0.05$).
  - A high dose of PZQ significantly decrease the percentage of acetate levels ($p<0.05$) and leads to a trend in decrease of the production of total VFAs and specifically acetate levels ($p<0.1$).

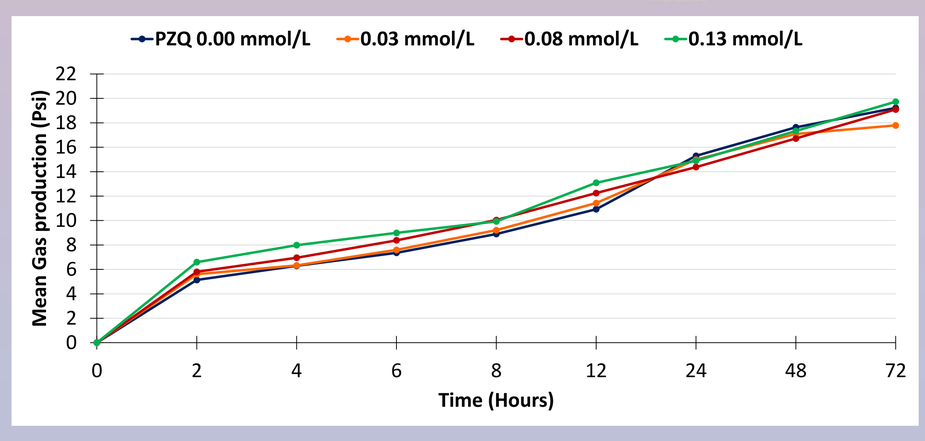


Figure 1. The gas production curve over a 72 h fermentation period of a mixed diet (70meadow hay: 30barlay) in a hindgut model of equine fermentation incubated with PZQ at concentration of 0.00 mmol/L (control), 0.03 mmol/L, 0.08 mmol/L and 0.13 mmol/L.

Table 1: The kinetics of gas production profile over a 72 h fermentation period by the exponential model of a hindgut model of equine fermentation incubated with PZQ at various dosage levels

Fermentation parameters	Praziquantel (mmol/L) 0.00	0.03	0.08	0.13	SED	P value
a+b	13.874	12.242	14.185	15.957	2.735	0.615
c	0.052	0.054	0.043	0.035	0.011	0.358

Table 2: Summary table of fermentation products and pH at 24 h of incubation of a mixed diet (70meadow hay: 30barlay) in a hindgut model of equine fermentation incubated with PZQ at various dosage levels

Fermentation products/parameters	Praziquantel (mmol/L) 0.00	0.03	0.08	0.13	SED	P value
Total VFA (mM)	31.00^{ab}	41.50^{b}	36.30^{ab}	29.20^{a}	4.340	0.058
Acetate (mM)	19.90^{ab}	27.60^{b}	23.60^{ab}	17.90^{a}	3.510	0.073
Propionate (mM)	5.18	6.26	5.59	4.86	0.904	0.471
Total Butyrate (mM)	5.44^{a}	6.95^{b}	6.35^{ab}	5.34^{a}	0.508	0.023
Total Others (mM)	0.48	0.67	0.80	1.11	0.343	0.354
Acetate (%)	65.34^{b}	65.42^{b}	64.63^{ab}	60.51^{a}	1.681	0.038
Propionate (%)	16.01	16.25	15.62	16.66	1.351	0.889
Total-Butyrate (%)	16.99	16.70	17.52	18.79	1.657	0.614
Total-Others (%)	1.66^{a}	1.64^{a}	2.23^{ab}	4.05^{b}	1.163	0.182
Acetate:Propionate Ratio	7.42	7.08	6.61	4.80	1.454	0.321
Ammonia (mM)	1.86	2.36	1.51	2.63	0.813	0.535
D-Lactate (mM)	0.059	0.054	0.053	0.096	0.017	0.157
L-Lactate (mM)	0.157	0.073	0.102	0.212	0.010	0.539
Total-Lactate (mM)	0.234	0.144	0.172	0.313	0.118	0.510
pH Substrate	7.10	7.02	7.04	7.04	0.049	0.389

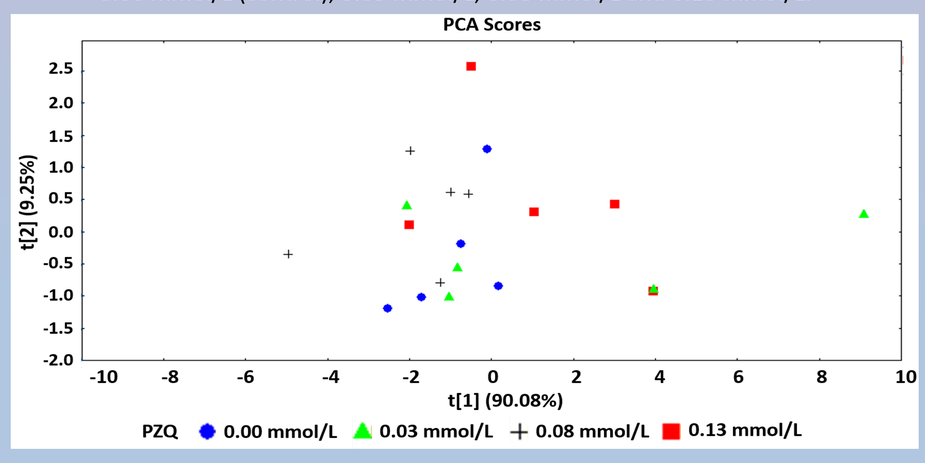


Figure 2. Principal component analysis (PCA) at 24 h of incubation of a mixed diet (70meadow hay: 30barlay) in a hindgut model of equine fermentation incubated with praziquantel at concentration of 0.00 mmol/L (control), 0.03 mmol/L , 0.08 mmol/L and 0.13 mmol/L, coloured symbols in blue, green, black and red, respectively.

Conclusion

Following this first investigation into the impact of PZQ on the equine hindgut microbiome fermentation, we suggest that PZQ treatment may alter the fermentation pathways in the equine hindgut based on microbial activities and fermentation products, which could impact on the nutritional functioning of the caecum. To further characterise PZQ impact, collected microbial samples are undergoing both meta-proteomic and meta-taxonomy analysis for a complete assessment of PZQ induced alterations.

References

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