Fermentation of endogenous protein in the colon of pigs determined by a gas production technique

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Background

Protein fermentation in the gastrointestinal tract of monogastric animals, like pigs, can yield several biologically active and often deleterious metabolites (Gilbert et al. 2018). One of the strategies to reduce the negative impact is to limit protein intake. However, not only dietary protein but also endogenous protein may contribute to fermentation as it enters the hindgut. As dietary protein digestibility increases, the relative contribution of EP to the ileal digesta protein content increases and protein fermentation will depend more and more on them as well as the degree of hydrolysis of the proteins. Therefore, the fermentation potential of porcine endogenous protein was investigated.

Objectives

Ileal digesta samples from pigs fed nitrogen (N)-free diets in 5 different studies, colonic mucus and whey protein isolate (WPI) were tested in an in vitro gas production system with porcine faecal inoculum. Nitrogen limiting microbial activity using a N - free buffer and an excess of fermentable carbohydrates. Original samples were obtained in five protein digestibility studies conducted in China (P. Li et al. 2015; Z. Li et al. 2015; Liu et al. 2015; Ma et al. 2019; Zhang et al. 2019), hereafter named study A through E.

Methods

Sealed bottles of 250 ml containing 60 ml 2% buffer-faecal solution with added test substrates containing 10 mg N and same buffer-faecal mixture (Blank) were allocated in 39°C water baths. Gas production was recorded continuously for 48 h. Lag time (h), maximum gas production rate (R_{max}, ml/h), time when maximum rate occurred (T_{Rmax}, h) and cumulative 48 h gas production (GP_{48}, ml) were calculated and compared by the MIXED model procedure in SAS.

Based on N content and solubility, samples were dissolved in same N-free buffer to prepare the supernatant for size exclusion analysis (SEC). The molecular weight distribution was analyzed by an Akta pure 25 system with Superdex 75 column. The chromatograms obtained were separated into different molecular weight ranges by calculating the eluent volumes based on the calibration curve. Pearson correlation analysis were conducted for SEC results and fermentation parameters.

Results

Compared to most of the digesta samples, mucus had a greater R_{max}, 25.0 ± 2.0 vs 18.5 ± 4.0) and shorter T_{Rmax} (5.1 ± 0.6 vs 7.2 ± 2.8) while WPI as intact protein showed the lowest R_{max} (12.2 ± 2.3) and highest T_{Rmax} (12.9 ± 5.0). Differences in R_{max} and T_{Rmax} were also found between different digesta samples ($p < 0.05$).

SEC results showed that endogenous proteins are comprised of much smaller molecules compared to WPI. However, negative correlation was found between solubility and R_{max} in ileal digesta samples. Also, higher percentage of small particles with molecular weight between 0-5 k Dalton is linked to a lower R_{max}.

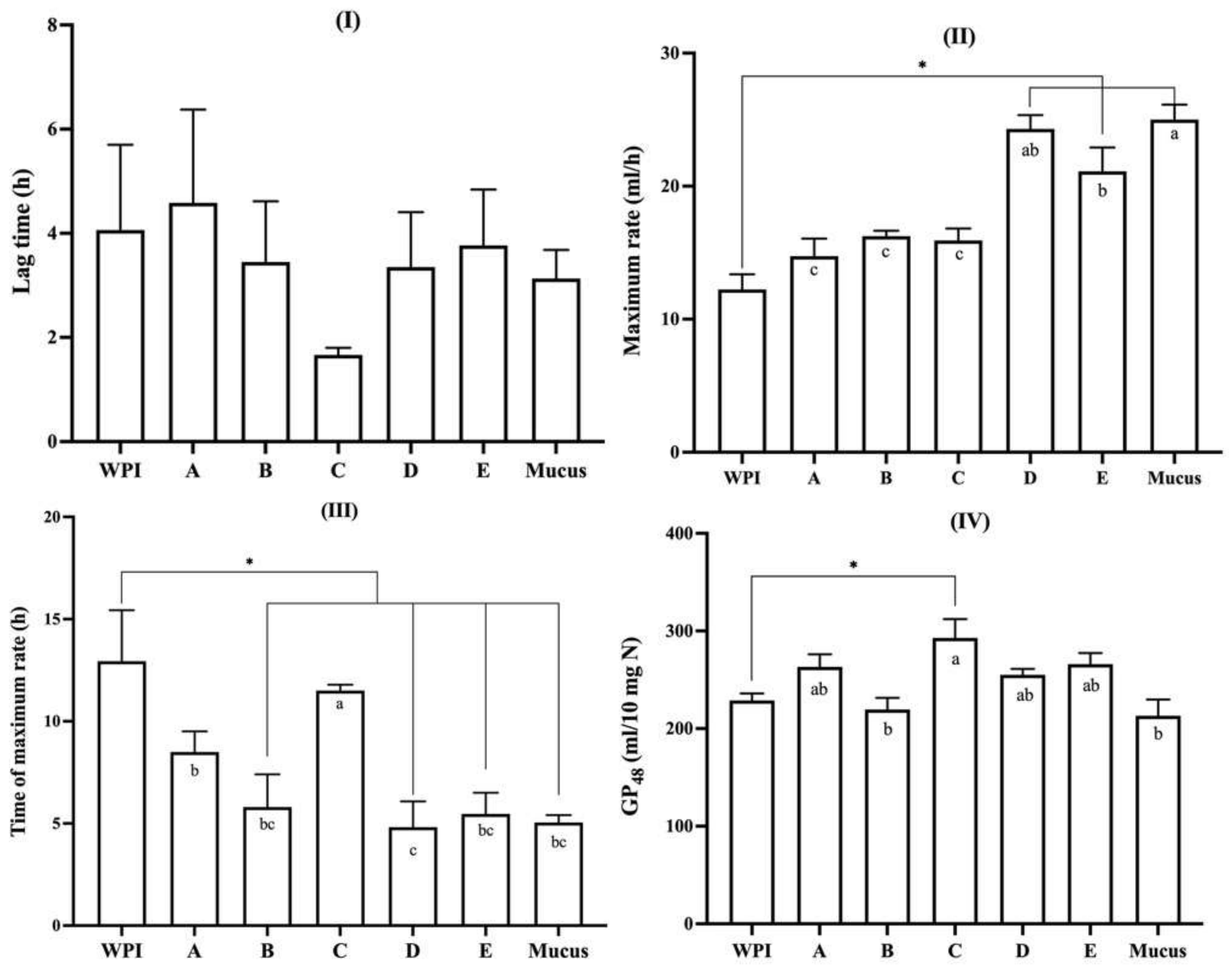

Figure 1. Lag time (I), maximum gas production rate (II), time when maximum rate occurred (III) and cumulative 48 h *in vitro* gas production (GP_{48}, IV) of whey protein isolate (WPI, n=4), colonic mucus (n=3) and five ileal digesta samples (n=3). Ileal digesta samples were obtained from pigs fed N-free diets in five separate digestibility studies. Bars with asterisk show a significant difference between WPI group and different endogenous protein groups ($p < 0.05$). Values are means ± SEMs. Bars with different subscripts within panel show significant differences between endogenous protein groups ($p < 0.05$).

Conclusions

Compared to WPI, endogenous proteins are more accessible for microbiota to utilize as due to smaller molecular weight. Although the potential fermentation of endogenous protein can vary between different animal studies. Underlying reasons still need further investigation.

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