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Cranberry extract inhibits *in vitro* and *in vivo* adhesion of F4 and F18$^+$ *E. coli* to pig intestinal epithelium and reduces excretion in experimentally infected pigs

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Introduction

Enterotoxigenic or verotoxigenic *Escherichia coli* (*E. coli*) expressing F4 or F18 fimbriae are an important cause of these diseases, leading to considerable economic losses due to mortality, decreased growth rate and cost of medication. Antibiotics are routinely used to combat these infections, but due to the alarming emergence of microbes resistant to these antibiotics, there is an urgent need to develop alternatives.

Cranberry (Vaccinium macrocarpon Ait.) has been shown to be effective in prevention of urinary tract infections (UTIs) in humans caused by type 1- or P-fimbriated *Escherichia coli*.

In the present study, we aimed to assess whether cranberry can also act against other types of fimbriated *E. coli*, namely F4+ *E. coli* and F18+ *E. coli.*

Materials and Methods

The inhibitory capacity of cranberry on F4+ and F18+ *E. coli* adherence was assessed via *in vitro* villous adhesion tests, *in vivo* intestinal loop experiments and an F18+ *E. coli* challenge experiment in pigs.

For the challenge experiment with F18+ *E. coli*, 6 F18-seronegative and F18R positive conventionally bred pigs were selected from 1 litter. They were divided into two groups: (i) a control group receiving standard feed (n = 3), (ii) the cranberry group which received 1% cranberry powder (= 10 g/kg feed) in the feed and 0.1% cranberry powder (= 1 g/L water) in the drinking water (n = 3). The piglets received the supplemented feed/water five days before challenge and they were not deprived from food and drinking water prior to the challenge infection.

Results

Cranberry extract was found to inhibit *in vitro* F4+ and F18+ *E. coli* adherence to pig intestinal villi (Figure 1A). This effect was not due to antimicrobial activity (Figure 1B).

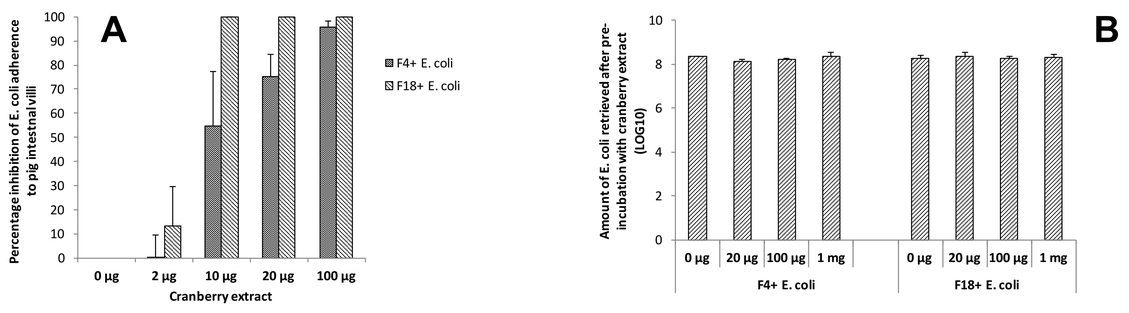

Figure 1: A. Effect of cranberry extract on F4$^+$ *E. coli* and F18$^+$ *E. coli* adherence *in vitro*. Different amounts of cranberry extract (2 µg, 10 µg, 20 µg, 100 µg) were pre-incubated with F4$^+$ *E. coli* or F18$^+$ *E. coli* (4*10^8) during 1 h. Subsequently, these mixtures were tested for adherence to pig intestinal villi using light microscopy. **B**. Effect of cranberry extract on viability of F4+ *E. coli* and F18+ *E. coli*. Different amounts of cranberry extract (0 µg, 20 µg, 100 µg and 1 mg) were pre-incubated with F4+ *E. coli* or F18+ *E. coli* (4*10^8) during 1 h. Subsequently, these mixtures were plated on agar plates to determine the number of surviving bacteria.

Furthermore, cranberry extract was found to inhibit in vivo adherence of F4 and F18 fimbriae to pig gut epithelium (Figure 2).

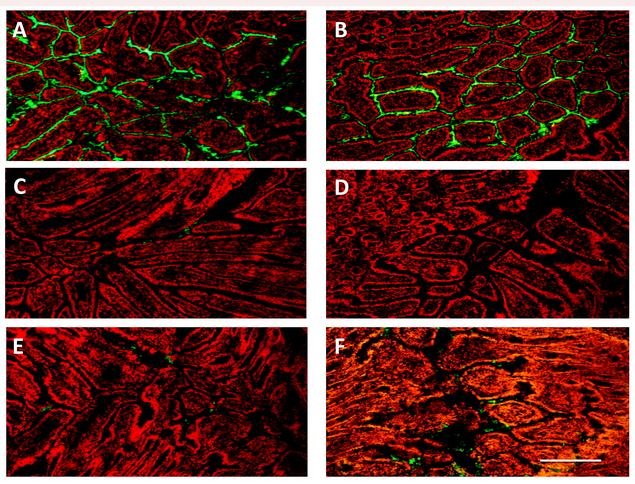

Figure 2: Effect of cranberry extract on adhesion of F4 and F18 fimbriae in ligated intestinal loops. Immunohistochemical detection of F4 or F18 fimbriae (green fluorescence) with a specific mAb (IMM01 and IMM02, respectively) on frozen sections of porcine jejunum. Nuclei of pig gut cells are stained with propidiumiodide (red fluoresence). Loops were incubated with (A) F4 fimbriae, (B) F18 fimbriae, (C) F4 fimbriae + 10 mg cranberry extract, (D) F18 fimbriae + 10 mg cranberry extract, (E) F4 fimbriae + 100 mg cranberry extract and (F) F18 fimbriae + 100 mg cranberry extract. Bar, 100 µm

Next, an F18+ *E. coli* infection experiment was performed and it was found that F18+ *E. coli* excretion tended to be reduced in pigs that received cranberry extract both in feed and drinking water (Figure 3A). Also, diarrhea score was observed to be reduced in the cranberry treated group (Figure 3B).

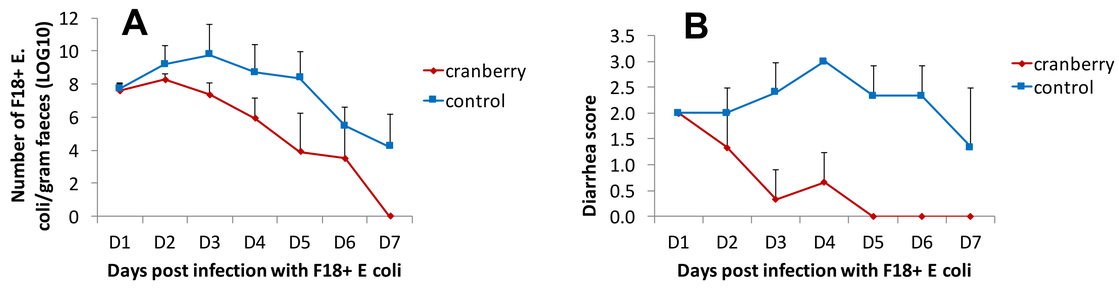

Figure 3: **A.** Mean faecal excretion of F18+ E. coli (log10) per gram faeces (+/- S.D.). (i) Pigs fed with 1% cranberry extract in feed and 0.1% cranberry extract in drinking water (cranberry group; n = 3) and (ii) pigs fed with standard feed (control group; n = 3). **B.** Diarrhea score of faeces from pigs infected with F18+ E. coli (cranberry versus control group). Score 'o' reflects no diarrhea, score '1' stands for pasty diarrhea, score '2' = semi-liquid diarrhea and score '3' stands for watery diarrhea.

Higher antibody levels were observed in the control group compared to the cranberry group (Figure 4), which is consistent with a lower exposure to the pathogen of the pigs from the cranberry group.

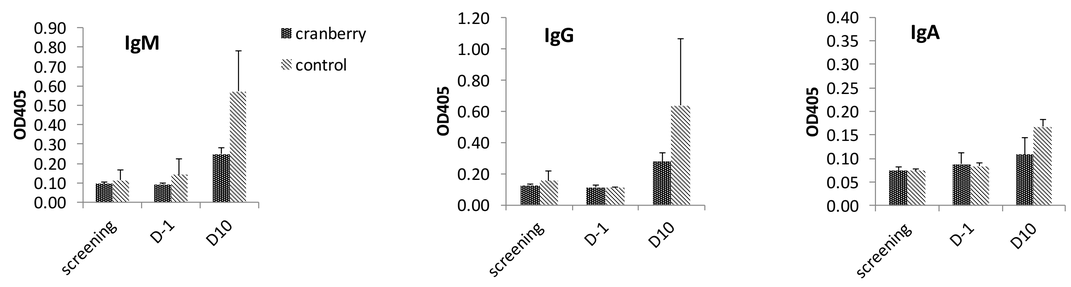

Figure 4: Detection of F18-specific serum antibodies upon F18+ *E. coli* challenge infection by ELISA. F18-coated microtiter wells are incubated with pig serum, and isotype-specific antibodies are used to detect the F18-specific IgM, IgG and IgA responses. Serum collected at three time points is compared (i) the day of screening (D-14 before challenge), (ii) the day before challenge (D-1) and (iii) 10 days after challenge (D10).

Conclusion

In conclusion, we showed that cranberry extract, which has been proven to be a safe and natural product against a variety of pathogens, leads to inhibition of *in vitro* F4+ and F18+ *E. coli* adherence to pig intestinal epithelium. Moreover, a pilot challenge experiment with F18+ *E. coli* revealed that cranberry extract added to both feed and drinking water tended to reduce excretion of the pathogen and the duration of shedding. For this reason, cranberry could be considered as alternative for antibiotics to combat *E. coli* infections in piggeries.

Further studies need to be conducted to determine the optimal dose and route of administration.

Also, the effects of cranberry on pig health and production parameters and the economic feasibility need to be assessed in future experiments.