Influence of pH and methylation degree on the antibacterial activity of glycine against pig specific enterotoxigenic *Escherichia coli*

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Introduction and Objectives

Enterotoxigenic *Escherichia coli* (ETEC) is worldwide the most common bacterial cause of diarrhoea in humans (1). It is also an important cause of disease in animals, such as postweaning diarrhoea in piglets. The latter is responsible for economic losses due to an increase in mortality and a decrease in growth rate (2). N,N-dimethylglycine (DMG) is known to have an emulsifying effect *in vitro* (3). Therefore, DMG could interact with the plasma membrane lipids of ETEC, resulting in an increase in membrane permeability. It was our objective to determine the susceptibility of a pig specific ETEC to the amino acid glycine and its N-methylated derivatives with one (sarcosine), two (N,N dimethylglycine) or three methyl groups (glycine betaine).The ionization state of the amine group of all these modified amino acids, except for betaine, varies with pH. It was evaluated whether pH influences the potential antibacterial action of these compounds.

Materials and Methods

Overnight grown ETEC were exposed to either saline (control), 50 mM of glycine, sarcosine, DMG or betaine at a pH ranging from 6.5 to 11. Viability of the bacteria was determined by 2-color flow cytometry, which allows to discriminate subpopulations of live, dead and intermediate ("dying") bacteria (4). The latter subpopulation is characterized by an increase in membrane permeability. Culturability was assessed by conventional plate count, based on colony forming unit (CFU) values obtained from a 10-fold serial dilution plated on Trypsone Soy Agar.

Results and Discussion

Flow cytometry and plate count data showed a substantial decrease in viability and culturability when the ETEC were exposed to glycine, sarcosine and DMG at an alkaline pH. This high pH is also described in the working mechanism of alkaline phosphatase and is related to the existence of alkaline surface microclimates in the small intestine (5,6). When incubated with betaine at a high pH, viability and culturability of the ETEC were much less affected. At the lower physiological pig gut pH almost no decrease in viability nor in culturability of the ETEC was observed for all four compounds.

These results suggest that the ionization state of glycine, sarcosine and DMG has a strong influence on their antibacterial potential. It is however also possible that the alkaline stress causes the ETEC to be more susceptible to the antibacterial effect of these ionized amino acid derivatives, causing a decrease in viability and culturability, which was not seen when the bacteria were exposed to the alkaline pH in absence of glycine and its N-methylated analogues.

In conclusion, our results suggest an antibacterial effect of glycine, sarcosine and DMG in an alkaline environment. The mechanism behind this antibacterial effect remains to be elucidated.

References

1. Walker R.I., Steele D., Aguado T., and the ad hoc ETEC Technical Expert Committee (2007). Analysis of strategies to successfully vaccinate infants in developing countries against enterotoxigenic *Escherichia coli* (ETEC) disease. *Vaccine* **25**, 2545-2566.

2. Fairbrother J.M., Nadeau E., Gyles C.L. (2005). *Escherichia coli* in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. *Animal Health Research Reviews* **6**, 17-39.

3.Kalmar I.D., Cools A., Verstegen M.W.A., Huyghebaert G., Buyse J., Roose P., Janssens G.P.J. (2010). Dietary supplementation with dimethylglycine affects broiler performance and plasma metabolites depending on dose and dietary fatty acid profile. *Journal of Animal Physiology and Animal Nutrtion*, DOI: 10.1111/j.1439-0396.2010.01034.x

4. Berney M., Hammes F., Bosshard F., Weilenmann H., Egli T. (2007). Assessment and interpretation of bacterial viability by using the LIVE/DEAD BacLight Kit in combination with flow cytometry. *Applied and Environmental Microbiology*, **73**, 3283-3290.

5. Fan M.Z., Adeola O., Asem E.K. (1999). Characterization of brush border membrane-bound alkaline phosphatase activity in different segments of the porcine small intestine. *Journal of Nutritional Biochemistry,* **10**, 299-305.

6. Mizumori M., Ham M., Gurh p.H., Engel E., Kaunitz J.D., Akiba Y. (2009). Intestinal alkaline phosphatase regulates protective surface microclimate pH in rat duodenum. *Journal of Physology* **587**, 3651-3663.

Theme

Gut microbial metabolism