NEW INNOVATIVE FEEDING STRATEGY FOR REDUCTION OF *SALMONELLA* IN SWINE

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Introduction

Salmonella sp. are a leading cause of gastro-intestinal disease in humans with tens of millions of human cases worldwide every year. Pork is an important food vehicle of Salmonellosis, resulting in fever, abdominal cramps, diarrhea, vomiting, and occasionally death. Reduction of Salmonella in the feed-to-food chain is key to reduce the number of human Salmonellosis cases. Furthermore, it has frequently been demonstrated that the use of antimicrobial agents in food animals favors the development of resistance among foodborne pathogens like Salmonella spp. (WHO, 2012). Therefore, there is an urgent need for new antibacterial strategies to reduce Salmonellosis, since antimicrobial resistance is on the rise. Due to their bacteriostatic and bacteriolytic properties, organic acids are frequently used to control *Salmonella* in the feed production process. Organic acids increase stomach barrier function by reducing stomach pH and destabilize bacterial membrane function resulting in bacterial cell death. New strategies focus on the reduction of *Salmonella* in the intestinal tract by competitive binding of bacteria, and support of intestinal function to reduce colonization. Natural food and feed compounds such as processed grains and fungal extracts are new innovative ingredients that can have high potential in combination with organic acids. Mannobiose obtained by mannanase-hydolysation of copra meal has binding properties to bacteria as well as immunomodulatory properties. Edible mushroom extracts have currently been a hot topic and many prophylactic and therapeutic properties are described, including antimicrobial and immunomodulatory. This research evaluates the ability of hydrolyzed copra meal and rye overgrown with the mycelium of *Agaricus Subrufescens* to reduce *Salmonella* colonization in pigs and compares its efficacy to other currently used products.

Materials and methods

Twenty compounds were evaluated on their ability to bind *Salmonella* typhimurium in an *in vitro* adhesion assay. Besides hydrolyzed copra meal and rye overgrown with mycelium, commercially available compounds were tested including yeast cell wall products, deactivated yeast cells, yeast fermented products, yeast derived products, yeast cell product, gluco-mannans, gluco-oligosaccharides (GOS), mannan-oligosaccharides (MOS), xylo-oligosaccharides (XOS), chitosan-oligosaccharids (COS), 1,3-1,6-beta-D-glucan, and specific dietary fibre abstract.

The compounds were suspended in PBS to a final concentration of 1 % and centrifuged for 5 minutes at 460 g at 20ºC. The supernatant was transferred to a 96 wells plate (MicrolonF) to coat the wells and incubated overnight at 4ºC. The plates were washed with PBS, blocked with 1 % bovine serum albumin and incubated for 1 hour. Subsequently the plates were washed with PBS and *Salmonella typhimurium* culture (OD600 of 0.02) was added to the plates and incubated for 30 minutes and

washed again with PBS. Than growth medium was added to the plate and the plate was incubated at 37ºC. Optical density was read continuously at a wavelength of 600 nm using a microplate reader (Spectramax) and time to onset OD600 0.5 was determined. Well performing components were tested a second time and an average growth time relative to the control group value was determined for all components after two assays.

Efficacy of hydrolyzed copra meal and rye overgrown with mycelium was evaluated in a *Salmonella* typhimurium (ST) challenge pig study. Efficacy was compared to efficacy of the best performing candidate in the *in vitro* assay and fat coated butyrate, a frequently used anti-Salmonella strategy in poultry which alters Salmonella virulence properties and reduces colonization in pigs (Boyen et al., 2008). All four components were combined with a blend of formic and lactic acid and fed from weaning until the end of the study. The acid blend was mixed in the feed at 4 kg/ton; fat coated butyrate at 6 kg/ton; the hydrolyzed copra-meal at 1 kg/ton; the rye overgrown with mycelium at 2 kg/ton; and the mannan-oligosaccharides at 2 kg/ton.

Forty pigs were housed individually directly after weaning and equally divided over five different treatment groups: a control group, a group receiving the acid blend with fat coated butyrate, a group receiving the acid blend with hydrolyzed copra meal, a group receiving the acid blend with rye overgrown with mycelium and a group receiving the acid blend with mannan-oligosaccharides. Pigs of all treatment groups got infected with 10^9 CFU *Salmonella typhimurium* in a feed matrix which was applied from ten days after weaning for seven consecutive days. Body temperature, fecal consistency scores, performance and Salmonella fecal shedding were evaluated.

Results

Three of the best-performing compounds: hydrolyzed copra meal, rye overgrown with mycelium performed and MOS (mannan-oligosaccharides) were further evaluated in the Salmonella infection model. Mannan-oligosaccharides performed better than gluco-mannans or oligosaccharides from other sources. Other well-performing compounds were 1,3-1,6-beta-D-glucan and dietary fibre extract. Binding capacity of yeast products was very variable.

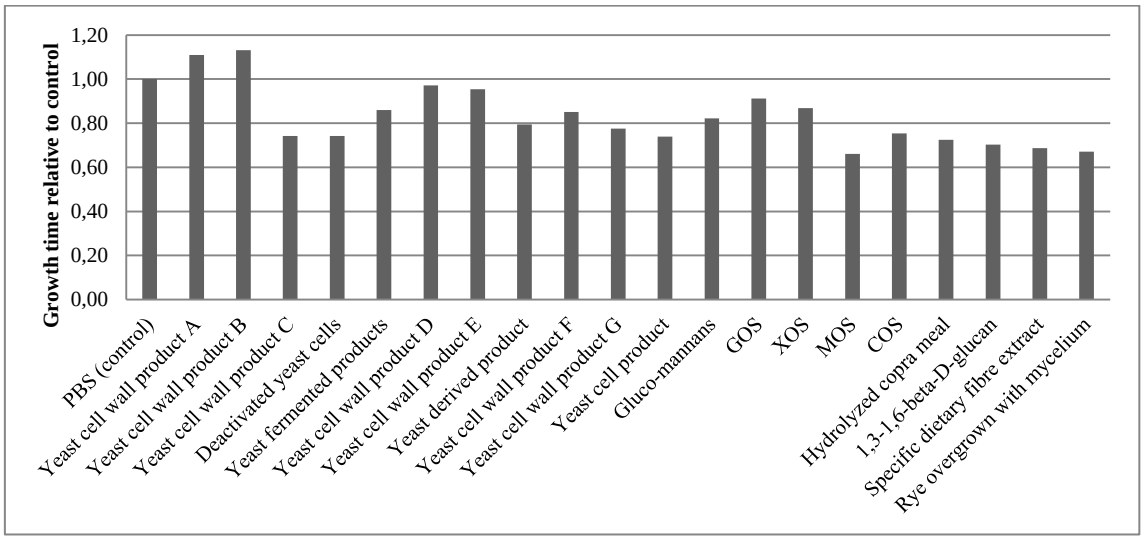

Figure 1. Growth time of bacteria to OD600 after adhesion to substrate (relative to control).

Pigs from the *in vivo* experiment got a slight fever during the first days after infection, which was not influenced by the different treatments. A peak in Salmonella shedding occurred during day 2- 4 after infection. The combination of the acid blend with hydrolyzed copra meal as well as with rye overgrown with mycelium significantly ($p<0.05$) reduced this peak in shedding (figure 2). The acid blend combined with mannan-oligosaccharides showed a numerical reduction; however, this was not significant. The combination of the acid blend with fat coated butyrate did not show a clear effect on Salmonella peak shedding. Pigs receiving rye overgrown with mycelium showed the lowest fecal consistency scores, however these did not significantly differ from the control group (figure 3).

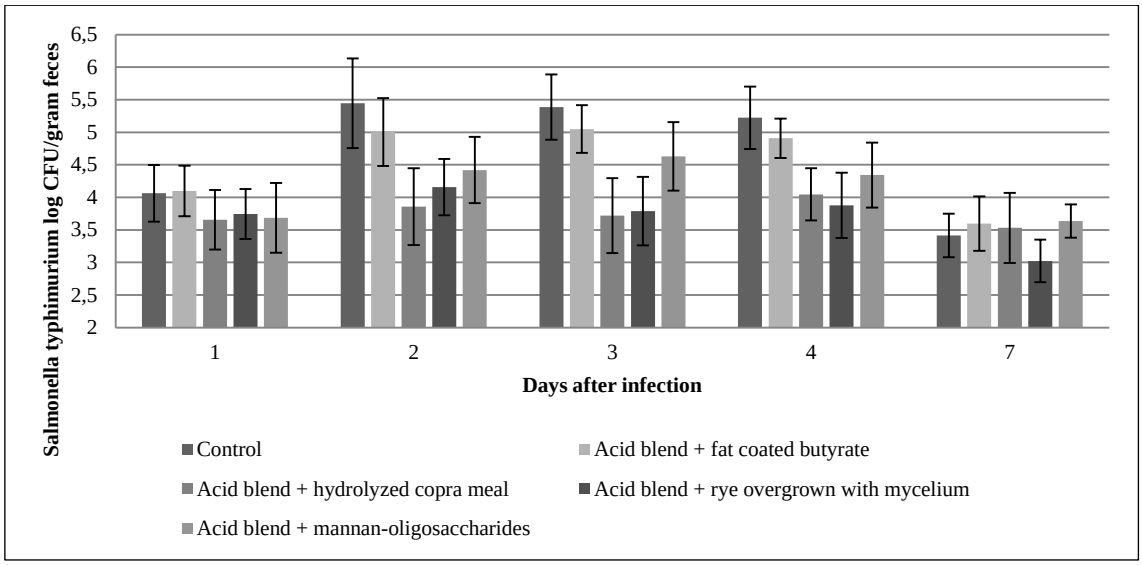

Figure 2. Fecal *Salmonella typhimurium* shedding during infection (n=8).

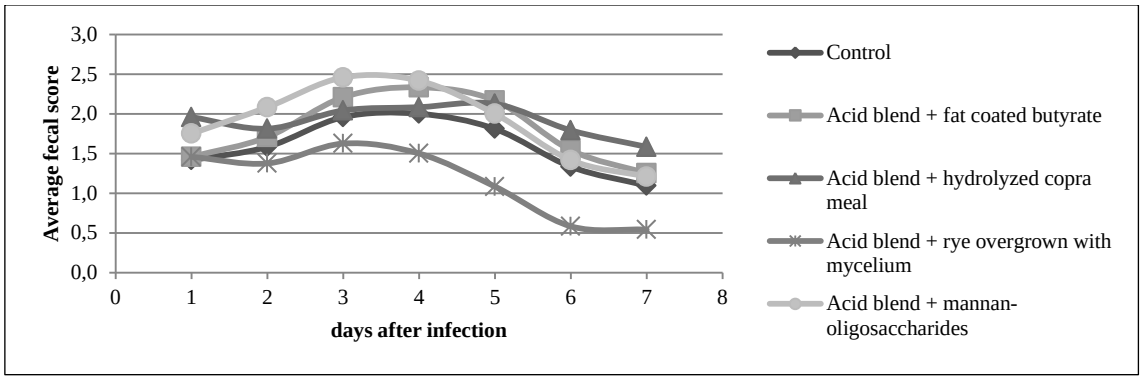

Figure 3. Average fecal consistency scores of piglets during infection (0 = normal feces; 1= flat feces; 2= diarrhea; 3= watery diarrhea)

Average daily gain and feed efficacy tended to improve compared to the control group when feeding rye overgrown with mycelium during the week of infection ($p<0.10$; figure 4).

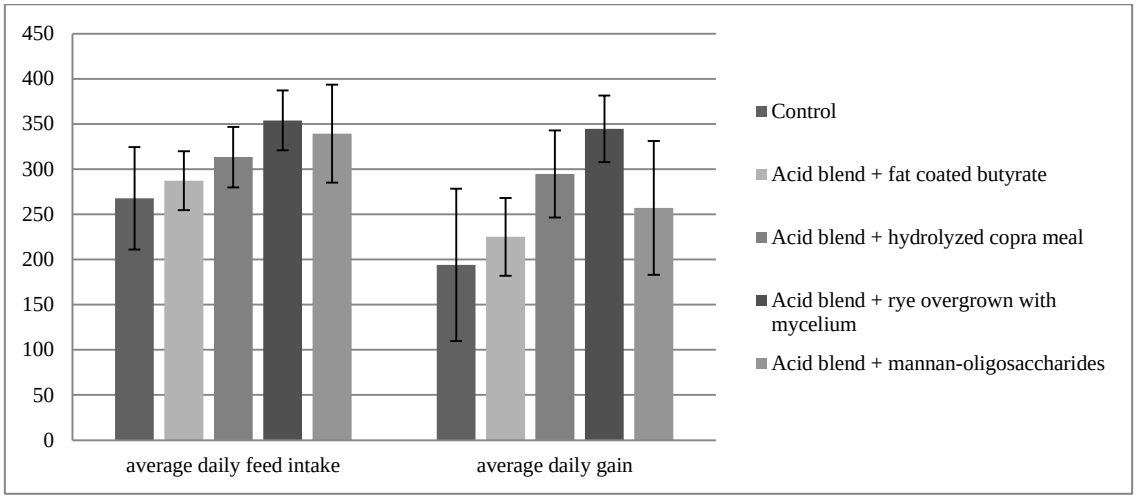

Figure 4. Performance of piglets during infection.

Discussion and conclusion

Both hydrolyzed copra meal and rye overgrown with mycelium performed well in the adhesion assay. Also in the *in vivo* assay, both hydrolyzed copra meal and rye overgrown with mycelium showed a significant reduction in Salmonella shedding in pigs. The variability in binding capacity of yeast products in the adhesion assay may dependent on cell wall structure, strain diversity, structural diversity, structural surroundings, and non-specific interactions (Ganner et al., 2013). Yeast-derived 1,3-1,6-beta-D-glucan and mannan-oligosaccharides which seem to be responsible for the binding of yeast products to *Salmonella typhimurium* indeed strongly did bind the bacterium in the adhesion assay. Mannan-oligosaccharides performed better than gluco-mannans or other oligosaccharides in the adhesion assay and previously proved to be effective in a field study (Andrés-Barranco et al., 2015). However, in this study, infection dose of pigs was higher and number of animals was lower, which may be the reason that the product only numerically reduced Salmonella shedding *in vivo*.

Many previous performed studies have shown a reduction in Salmonella shedding after the use of butyrate (de Ridder et al., 2013; Lynch et al., 2017; Walia et al., 2016). Butyrate did not significantly reduce colonization in this study, possibly because the infection dose of Salmonella used in this study was higher than in previous studies using seeder birds or field data instead of direct infection with a high inoculation dose. Therefore, it seems that both hydrolyzed copra meal and rye overgrown with mycelium have a stronger effect on Salmonella shedding than currently used anti-Salmonella strategies and therefore are promising building blocks of new strategies to control *Salmonella* in the swine production chain.

References

Andrés-Barranco, S., Vico, J.P., Grilló, M.J., Mainar-Jaime, R.C., 2015. Reduction of subclinical Salmonella infection in fattening pigs after dietary supplementation with a ß-galactomannan oligosaccharide. J Appl Microbiol 118, 284-294.

Boyen, F., Haesebrouck, F., Vanparys, A., Volf, J., Mahu, M., Van Immerseel, F., Rychlik, I., Dewulf, J., Ducatelle, R., Pasmans, F, 2008. Coated fatty acids alter virulence properties of Salmonella Typhimurium and decrease intestinal colonization of pigs. Vet Microbiol 132, 319-327.

De Ridder, L., Maes, D., Dewulf, J., Pasmans, F., Boyen, F., Haesebrouck, F., Méroc, E., Butaye, P., Van der Stede, Y., 2013. Evaluation of three intervention strategies to reduce the transmission of Salmonella Typhimurium in pigs. Vet J 197, 613-618.

Ganner, A., Stoiber, C., Uhlik, J.T., Dohnal, I., Schatzmayr, G., 2013. Quantitative evaluation of E. coli F4 and Salmonella Typhimurium binding capacity of yeast derivatives. AMB Express 3, 62.
Lynch, H., Leonard, F.C., Walia, K., Lawlor, P.G., Duffy, G., Fanning, S., Markey, B.K., Brady, C., Gardiner, G.E., Argüello, H., 2017. Investigation of in-feed organic acids as a low cost strategy to combat Salmonella in grower pigs. Prev Vet Med 139, 50-57.

Walia, K., Argüello, H., Lynch, H., Leonard, F.C., Grant, J., Yearsley, D., Kelly, S., Duffy, G., Gardiner, G.E., Lawlor, P.G., 2016. Effect of feeding sodium butyrate in the late finishing period on Salmonella carriage, seroprevalence, and growth of finishing pigs. Prev Vet Med 131, 79-86.

World Health Organization, 2012. The evolving threat of antimicrobial resistance: options for action. ISBN:9789241503181.