SWINE PLASMA IMMUNOGLOBULINS FOR PREVENTION AND TREATMENT OF POST-WEANING DIARRHEA: OPTIMIZING STABILITY TOWARDS GUT CONDITIONS

Chris Juul Hedegaard¹, Anne-Sofie Ballegaard¹, Nanna Røjel¹, Marie Bendix Hansen², Bodil Kjær Lindved³, Kirsten Bisgaard Frantzen⁴, Lars E. Larsen¹, Allan Lihme², and Peter M.H. Heegaard¹*

1. National Veterinary Institute, Technical University of Denmark, Frederiksborg, Denmark. 2. Upfront Chromatography A/S, Copenhagen, Denmark. 3. KiBiF ApS. 4. Multimerics ApS. *contact: PMHH@vet.dtu.dk

Background
Post-weaning diarrhea (PWD) is a common condition in intensive swine production, resulting in reduced welfare of weaners and economic losses for the farmer as a result of illness, death, treatment costs, e.g. high consumption of antibiotics and zinc oxide.

Aim
1. Developing feed additives for oral provision for protection against PWD based on natural antibodies (immunoglobulins) derived directly from inexpensive raw materials.
2. To increase stability (reducing gut proteolysis) by cross-linking the immunoglobulins (Igs).

Conclusions
• The optimal conditions for Igs-multimerisation were observed to at pH 9 using 5-10 mM NaIO4, which confers to increased reactivity towards Salmonella Dianzorae after pepsin digestion.
• These results suggest that cross-linked Igs could be used for prevention/treatment of PWD and reduce antibiotic consumption.

Materials & Methods
Immunoglobulin isolation:
Porcine Igs were purified from blood plasma at UpFront Chromatography A/S (Copenhagen) by high-volume Expanded Bed Adsorption with a proprietary adsorbent. Plasma was obtained from a Danish slaughter house. The immunoglobulins were multimerised by controlled periodate oxidation of immunoglobulin-bound carbohydrate (Fig. 1). The multimerisation process was stopped by increasing pH to 12. Cross-coupled Ig-species were analysed by non-reduced 12% Bis-Tris SDS PAGE or gel filtration (S300 Sephacryl).

ELISA:
For testing the reactivity of the swine Igs on pathogenic bacterial antigens a competitive ELISAs were applied. Along with the swine Igs either Genway Biotech’s anti-E. coli (18-511-245057) or anti-salmonella (18-511-245055) HRP-conjugated antibodies were used. Initially, antigens were coated in the wells before a mix of swine Igs and HRP-conjugated antibody was added. The signal was recorded.

GUT CONDITIONS:
The pepsin concentration applied was not strong enough to digest the non-multimerised nor the multimerised swine Igs (Fig. 2A). By comparing the different levels of inhibition between the digested and non-digested samples, in the competitive ELISA, it appears that Igs multimerised at 5 mM NaIO4 gain an increased ability to inhibit binding of the conjugated antibody (Fig. 3), thus multimerisation at pH 9 and 5 mM could be preferable.

Results
IMMUNOGLOBULIN MULTIMERISATION:
The degree of Igs-multimerisation was tested at 5, 10, and 20 mM NaIO4, and at different pH values (6, 7 and 9), and all conditions subjected to NaIO4 oxidation resulted in multimerisation (Fig. 2A-B). As the increasing multimerisation was associated with lower signal on Western blot (developed with anti-porcine Fc-antibody) suggests that the Fc moieties are situated in the centre of the complex shielded from the anti-Fc-antibody (Fig. 2A, Western blot). The lower level of protein in the samples multimerised with 20 mM NaIO4 might be due to aggregated Igs caught during filtration of the samples preceding gelfiltration (Fig. 2B, 20 mM). NaIO4-multimerisation seems to sacrifice some Ig-reactivity (Fig. 2C, 5 mM, pH 9) but on the other hand gain some reactivity by size and complexity (Fig. 2C, 10-20 mM).

Figure 1: Sodium Periodate (NaIO4) multimerisation

Figure 2: (A): The samples (grouped by pH) were crosslinked by reacting them with periodate (NaIO4) at different concentrations (coloured lines) as compared to non-periodate aggregates (black line) on Sephacryl S300 gelfiltration. (B): Different periodate concentrations were used (coloured lines) as compared to non-periodate aggregates (black line) on Sephacryl S300 gelfiltration. (C): Swine IgG reactivity to bacterial antigens measured in a competitive ELISA. The results are indicated as the degree of inhibition of the conjugated anti-Salmonella or anti-E. coli antibodies by the same Igs.

Figure 3: Pepsin induced changes

Figure 4: Silver stain:

Figure 5: Western blot:

Figure 6: Competitive ELISA: