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Optimizing Stability Towards Gut Conditions

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

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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 at pH 9 using 5-10 mM NaIO₄, which confers to increased reactivity towards Salmonella Diarizonae 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 slaughterhouse. 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 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).

Results
IMMUNOglobulin MULTIMERIzATION:
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 captured during gelfiltration (Fig. 2B, 20 mM).

Figure 1: Sodium Periodate (NaIO₄) multimerisation

Figure 2:
A: SDS PAGE (non-reduced)
Silver stain:

B: Gelfiltration

C: Competitive ELISA

Figure 3: Pepsin induced changes

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 read out was dependent on the ability of the swine Ig to inhibit the signal by interfering with the binding of conjugated antibody to its ligands.

In vitro (piglet) stomach conditions:
According to Petschow & Talbott (1 ped gastoent nutr: 1994) the stomach pepsin concentration is 13 units/ml; this was mixed with swine Igs and incubated in 50mM sodium acetate pH 3 for 3 hrs, at 37°C where after the pepsin was inactivated by increasing the pH to 5.6 by adding Na₂CO₃.

ELISAs:
In the competitive ELISAs, the swine Ig concentration was varied from 0.1 to 100 mg/ml. The optimal conditions for Igs-multimerisation were observed to multimerised at 5 mM NaIO₄ gain an advantage as compared to non-periodate aggregates (black line on Sephacryl S300 gelfiltration). (D) Swine Ig-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 swine Igs.

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 preferable.

Figure 3: Pepsin digestions of the porcine Igs. Pepsin-digested and non-digested samples were mixed 1:1 with HRP-conjugated anti-salmonella antibody and incubated in wells coated with salmonella antigens. Based on the degree of inhibition of the HRP-signal, any changes between the non-digested and digested samples were recorded.