Swine Plasma Immunoglobulins for Prevention and Treatment of Post-Weaning Diarrhoea
Optimizing Stability Towards Gut Conditions

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**Background**

Post-weaning diarrhoea (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 be 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 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).

**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).

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