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

Chris Juul Hedegaard¹, Anne-Sofie Ballegaard¹, Nanna Ræjel¹, 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, Frederiksberg, Denmark. 2. Upfront Chromatography A/S, Copenhagen, Denmark. 3. KBIF ApS. 4. Multimerics ApS. *contact: PMHH@vet.dtu.dk

**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 Diarizoneae 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).

**Immunoglobulin isolation:**
Primary antibody: biotinylated mouse anti-pig Fc antibody (BD, clone F007).
Complexes were either visualised by silver staining or Western blotting; 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).

**Figure 2:**
- A: SDS PAGE (non-reduced)
- Silver stain:
- pH 6
- pH 7
- pH 9
- Multimeric Ig
- Monomeric Ig
- B: Gel filtration
- pH 6
- pH 7
- pH 9
- 0 mM
- 5 mM
- 10 mM
- Western blot:
- pH 6
- pH 7
- pH 9
- pH 6
- pH 7
- pH 9
- C: Competitive ELISA
- Salmonella Diarizoneae (human, sheep)
- Escherichia coli O149
- pH 6
- pH 7
- pH 9
- p<0.001
- p<0.05

**Figure 3:**
- Pepsin induced changes
- pH 9
- pH 7
- pH 6
- pH 5
- 0 mM
- 10 mM
- 25 mM

Swine IgG reactivity to bacterial antigens measured in a competitive ELISA. The results are indicated as the change in inhibition (%).

**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 induced changes
- pH 9
- pH 7
- pH 6
- pH 5
- 0 mM
- 10 mM
- 25 mM

Swine IgG reactivity to bacterial antigens measured in a competitive ELISA. The results are indicated as the change in inhibition (%).