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

Hedegaard, Chris Juul; Ballegaard, Anne-Sofie; Røjel, Nanna; Bendix Hansen, Marie; Kjær Lindved, Bodil; Bisgaard Frantzen, Kirsten; Larsen, Lars Erik; Lihme, Allan; Heegaard, Peter M. H.

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
**SWINE PLASMA IMMUNOGLOBULINS FOR PREVENTION AND TREATMENT OF POST-WEANING DIARRHOEA: OPTIMIZING STABILITY TOWARDS GUT CONDITIONS**

Chris Juul Hedegaard¹, Anne-Sofie Ballegaard¹, Nanna Rejel¹, Marie Bendix Hansen², Bodil Kjær Lindved³, Kirsten Bisgaard⁴, Marie Bendix Hansen², Bodil Kjær Lindved³, Kirsten Bisgaard⁴

1. National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark. 2. Upfront Chromatography A/S, Copenhagen, Denmark. 3. KiBiF ApS. 4. Multimerics ApS. *contact: PMH@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 be at pH 9 using 5-10 mM NaIO₄, which confers to increased reactivity towards Salmonella Diiarizonae 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 SDS PAGE or Western blotting; primary antibody: biotinylated mouse anti-pig Fc antibody (BD, clone F007-1241); developed by alkaline phosphatase-streptavidin and NBT/BCIP.

**Figure 1: Sodium Periodate (NaIO₄) multimerisation**

![Image of Sodium Periodate (NaIO₄) multimerisation](image)

**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.

**GUT CONDITIONS:**

The pepsin concentration applied was 13 units/ml; this was mixed with swine Igs and incubated in 50 mM sodium acetate pH 3 for 3 hrs. at 37°C where after the pepsin was inactivated by increasing the pH to 9.6 by adding Na₂CO₃.

**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)
- **B:** Gelfiltration
- **C:** Competitive ELISA

![Image of ELISA results](image)

**Figure 3:**

![Image of Pepsin induced changes](image)

**Change in Reactivity (%)**

- **pH 6**
- **pH 7**
- **pH 9**

**Figure 4:**

![Image of Pepsin induced changes](image)

**Change in Reactivity (%)**

- **pH 6**
- **pH 7**
- **pH 9**

**Table 1:**

<table>
<thead>
<tr>
<th>pH</th>
<th>Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6</td>
<td>50</td>
</tr>
<tr>
<td>pH 7</td>
<td>60</td>
</tr>
<tr>
<td>pH 9</td>
<td>70</td>
</tr>
</tbody>
</table>

**Figure 5:**

![Image of Pepsin induced changes](image)

**Change in Reactivity (%)**

- **pH 6**
- **pH 7**
- **pH 9**

**Table 2:**

<table>
<thead>
<tr>
<th>pH</th>
<th>Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6</td>
<td>50</td>
</tr>
<tr>
<td>pH 7</td>
<td>60</td>
</tr>
<tr>
<td>pH 9</td>
<td>70</td>
</tr>
</tbody>
</table>