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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

Chris Juul Hedegaard1, Anne-Sofie Ballegaard1, Nanna Rejel1, Marie Bendix Hansen2, Bodil Kjaer 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. KiBif 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 the Igs were observed to be a moderate multimerisation at pH 9, which confers better pepsin-resistance and increased reactivity towards E. coli O149.
- 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 gel filtration using a S300 Sephacryl column and non-reduced 12% Bis-Tris SDS PAGE.

Figure 1: Sodium Periodate (NaIO4) multimerisation

ELISA:
A ‘Sandwich ELISA’ was applied to observe the reactivity of the purified Igs used as the ‘bottom’ antibody. Extract of Serotype O149 E.coli was used as antigen and a biotinylated rabbit anti-E.coli (Abd Serotec) was used as ‘top’ antibody.

In vitro gut conditions:
Porcine Igs and 1000 units/ml pepsin were incubated in 50mM sodium acetate pH 3 for 3 hrs. at 37°C whereafter the pepsin was inactivated by adding Na2CO3 buffer that increased pH to 9.6. Next one pepsin treated sample was chosen (pH 9, 0 mM NaIO4), which then was treated either with trypsin, chymotrypsin or with both. Both proteases were titrated ranging from 887-0.05 u/ml trypsin and 127-0.007 u/ml chymotrypsin. Incubation for 2 hrs. at 37°C. The proteolytical reaction was stopped by addition of the Pefabloc® inhibitor (Sigma-Aldrich).

Results

IMMUNOGLOBULIN MULTIMERISATION:
The degree of Igs-multimerisation was tested at 10, 20 and 40 mM NaIO4 and at different pH values. At low to neutral pH, a tendency towards spontaneous aggregation was observed as these Ig species were eluting early from the gel filtration column (Fig. 2A, pH 6-7) and appear as a high molecular smear on SDS PAGE (Fig. 2B, pH 6-7); in contrast, Igs cross-linked at pH 9 eluted in response to the degree of NaIO4 concentration resulting in a transition from right to left on the chromatographs (Fig. 2A, pH 9) due to the increase in size of the multimers (Fig. 2B, pH 9).

Figure 2.

GUT CONDITIONS:
In general, pepsin degraded the porcine Igs extensively (Fig. 2B, pepsin) and especially Igs that had been subjected to periodate oxidation at low/neutral pH conditions were less stable in the presence of pepsin than those samples that had been periodate treated at pH 9. Furthermore, greater multimerisation also resulted in lower stability (Fig. 2B, pepsin).

Figure 3.

Conclusions
1. Developing feed additives for oral provision for protection against PWD based on natural antibodies (immunoglobulins) led to moderate multimerisation at pH 9, which confers better pepsin-resistance and increased reactivity towards E. coli O149.
2. To increase stability (reducing gut proteolysis) by cross-linking the immunoglobulins (Igs).

These results suggest that cross-linked Igs could be used for prevention/treatment of PWD and reduce antibiotic consumption.