

Survival of Yolk's Immunoglobulins Directed against *Salmonella* Enteritidis and *Salmonella* Typhimurium in the Gastro-intestinal Tract of the Broiler Chicken

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Introduction

Salmonella represents an important source of human food-borne illnesses which are commonly associated with the consumption of contaminated broiler chicken meat [1]. Oral immunotherapy, using pre-formed pathogen-specific antibodies, has been examined as a method of establishing passive immunity against enteric pathogens (e.g. *Salmonella*) in poultry. For that purpose, avian egg immunoglobulin (IgY) has shown promising results [2]. However, its application is limited by its sensitivity to gastro-intestinal conditions including pH or proteolytic enzymes [3]. To be therapeutically active against *Salmonella* in the chicken intestine, antibodies orally administered must survive their passage through the gastro-intestinal tract. This led us to investigate by *in vitro* and *in vivo* approaches the gastrointestinal stability of IgY directed against *Salmonella* Enteritidis (SE) and Typhimurium (ST) and presented under different forms.

Material and Methods

Egg yolk powders containing high IgY titers directed against SE and ST were obtained from laying hens hyperimmunized as previously described [4]. Three feed additives derived from these hyperimmune eggs were used: freeze-dried yolk powder (FYP), spray-dried yolk powder (SYP), and freeze-dried water-soluble fraction of yolk powder (WSFP). The amount of IgY contained in those powders resisting to acidic conditions (pH 2.0, 3.0, 4.0 and 7.0) has been determined *in vitro* by measuring total IgY using enzyme-linked immunosorbent assays (ELISA) after 30, 60 and 120 minutes. An *in vivo* force-feeding assay has been conducted with 48 five-week-old males Ross broiler chicken (*Salmonella* spp.-free status). Birds were fasted 24 hours then they were orally gavaged with wet feed containing one of the yolk powders (50 g/kg of diet) or without any yolk powder for control group. At 1:30 and 3 hours post-administration, 6 birds from each group were euthanized. Intestinal contents (duodenum, jejunum, ileum, cecum) were collected, and immediately diluted in an enzyme inhibitor solution. After centrifugation, supernatants containing antibodies were subjected to ELISA to measure total and specific (anti-SE/ST) IgY levels.

Results and Discussion

In vitro incubations indicated IgY were stable at neutral pH but were rapidly degraded at low pH. The activity of total IgY decreased by 74.2, 67.3 and 83.2 % for FYP, SYP and WSFP respectively after 30 minutes at pH 2.0 and by 13.7, 33.7 and 37.8 % after 30 minutes at pH 3.0. This suggests digestion in the proventriculus and gizzard (pH level between 1.8 and 2.5) could have a great impact on antibodies. Interestingly, whole yolk revealed a potentially protective effect on IgY when subjected to acidic conditions while degradation was more important at pH levels of 2.0 when IgY were under WSFP form ($p < 0.05$).

IgY activity observed *in vivo* in the samples from intestinal tract cannot be expressed as a percentage relative to the initial dose administered in contrast with the *in vitro* approach. When IgY were distributed in the WSFP or FYP form, the levels of total and specific immunoglobulins found throughout the intestine were dramatically reduced: for WSFP, IgY activity reached same level than for SYP whereas its initial level was almost ten times higher and for FYP, no difference was observed with intestinal contents of control animals ($p > 0.05$). Concerning SYP, total and specific antibody activity remained detectable in all intestinal segments including the cecum which represents the major site of infection in poultry. Comparison between SYP and WSFP confirmed the protective effect of whole yolk observed *in vitro*. The lower resistance of FYP *in vivo* could possibly be explained by proteolytic enzymes not present in the *in vitro* trial.

Conclusion and Perspectives

Adding antibodies in the form of spray-dried whole egg yolk powder to poultry feed may be the most effective way of inclusion to maintain immunological activity. Nevertheless, additional protections should be searched to limit observed digestive deactivation of IgY and maximize the anti-*Salmonella* effects of this feed additive. Furthermore, these results concern undigested antibodies and must be completed by ELISA performed with a secondary antibody recognizing Fab or F(ab')₂ fragments. Fab fragments, which might be released by pepsin digestion, are more resistant to the digestive processes than the rest of the molecule and conserves immunological functionality since it contains the antigen binding site.

Acknowledgement

This study was supported by the Directorate General of Agriculture of the Walloon Region (DGA, Namur, Belgium).

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