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 remains a major cause of human foodborne infections which are commonly associated with the consumption of contaminated broiler chicken meat [1]. This microorganism may be carried asymptomatically in the chicken digestive tract and spread via the slaughter process to raw, finished meat products. In this context, it is important to develop strategies to prevent intestinal colonization of chicken with *Salmonella*.

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 for oral immunotherapy could be limited by its sensitivity to gastro-intestinal conditions including pH or proteolytic enzymes [3]. Indeed, to be therapeutically active against *Salmonella* in the chicken intestine, antibodies orally administered must survive their passage through the gastro-intestinal tract.

Objective

The aim of this study is to investigate by *in vitro* and *in vivo* approaches the gastrointestinal stability of IgY simultaneously directed against *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST) and presented under different forms as poultry feed additives.

Materials and methods

Three egg yolk powders

- Freeze-dried yolk powder (FYP)
- Spray-dried yolk powder (SYP)

} *Whole yolk powders*

- Freeze-dried water-soluble fraction of yolk powder (WSFP) → *Semi-purified immunoglobulins*

Hyperimmune eggs containing high levels of IgY simultaneously directed against SE and ST were obtained through immunization of laying hens as previously described in [4].

Acidic incubations

- FYP, SYP and WSFP (1g) diluted in buffer at various pH levels: pH 2.0, 3.0, 4.0 or 7.0 (control)
- Temperature : 41°C (body temperature of chicken)
- Incubating times : 30, 60 and 120 minutes
- Falcon tubes under constant agitation (n = 6)

In vivo force-feeding trial

- 48 males Ross broiler chicken (*Salmonella spp.*-free status, Broeierij Vervaeke, Tielt, Belgium), five-week-old
- Wet gavage feed = commercial poultry feed (Vital Coq, Scar, Herve, Belgium) + yolk powder (FYP, SYP or WSFP; 50 g/kg of diet) or no powder (control group) + tap water (1.5 kg/kg of diet).

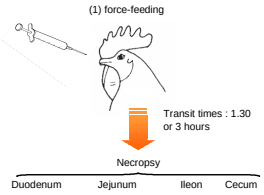

Figure 1. Force-feeding protocol. Chicken were fasted 24 hours then they were gavaged with wet feed following [5]. At 1:30 or 3 hours post-administration, 6 birds from each group (FYP, SYP, WSFP, Control) were euthanized. Intestinal contents were collected, diluted in an enzyme inhibitor solution and centrifuged. Supernatants containing antibodies were subjected to analysis.

This experimental protocol was approved by the Official Animal Care and Use Committee of the Gembloux Agricultural University (protocol N° 08/02 NPC).

ELISA

Enzyme-linked immunosorbent assays were used to check titers of total (Fig.3a) and specific anti-*Salmonella* IgY (Fig.3b) in samples.

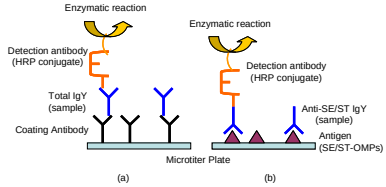

Figure 2. Designs of performed ELISA for total **(a)** and specific **(b)** IgY activity measurements. Total IgY dosage was realized using the "Chicken IgG ELISA Quantitation Kit (Bethyl, E30-104). For specific IgY detection, coating antibody from this kit was replaced by SE/ST-OMP antigen.

Results

In vitro acidic incubations

As shown in Fig.3, IgY levels fell quickly when egg yolk powders were exposed to acidic solutions (results of incubations at pH 2.0 are presented here). On the opposite, IgY were stable at neutral pH (data not shown). This suggests digestion in the proventriculus and gizzard (pH level between 1.8 and 2.5) could have a great impact on antibodies contained in the poultry feed additive. 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 ($p < 0.05$).

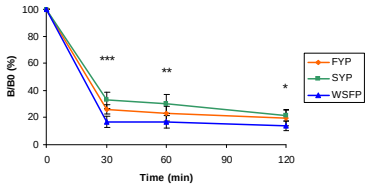

Figure 3. Acid stability of total IgY. IgY was incubated in solutions at pH 2.0 for 2h. Remaining total IgY concentrations during the incubations were measured using ELISA after 30, 60 and 120 minutes and are expressed as a percentage of the initial concentration (n = 6).

In vivo force-feeding trial

Immunological characteristics (Total IgY concentrations and specific anti-SE/ST activity) of wet gavage feeds are given in Table 1. However, for this *in vivo* trial, IgY activity observed 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.

(a)

Feed	Total IgY (ng/g)
Control	0
FYP	306.706
SYP	309.280
WSFP	2.761.500

(b)

Feed	Dilution factor of ELISA	Specific IgY (O.D. 450 nm)	
		ST	SE
Control	1	0,041	0,027
FYP	50	0,533	0,426
SYP	50	0,454	0,393
WSFP	500	0,462	0,394

Table 1. (a) Concentrations of total IgY in wet gavage feeds (ng IgY / g of feed). **(b)** Specific activity of anti-SE/ST IgY in wet gavage feeds (OD at 450 nm). Values are the mean of quadruple samples.

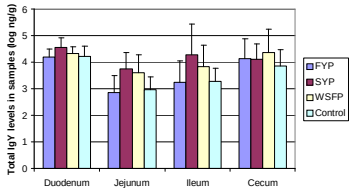

Figure 4. Concentrations of total IgY (log ng IgY / g of sample) in digestive contents of chicken duodenum, jejunum, ileum and cecum after gavage with wet feed containing one of the egg yolk powders. Values are the means for 12 chicken (6 euthanized 1:30h post-administration and 6 euthanized 3h post-administration).

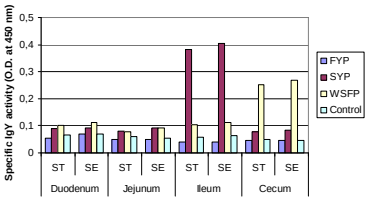

Figure 5. Specific activity of anti-SE / ST IgY in digestive contents of chicken duodenum, jejunum, ileum and cecum after gavage with wet feed containing one of the egg yolk powders. Values are obtained by ELISA performed on digestive samples diluted 10 times.

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. This confirms the protective effect of whole yolk observed *in vitro*.
- 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 especially in the ileum and the cecum which represents the major sites of infection in poultry.

However, these results concern undigested antibodies and must be completed by ELISA performed with a secondary antibody recognizing Fab or $F(ab')_2$ fragments. Indeed, Fab fragment, which might be released by pepsin digestion, is more resistant to the digestive processes than the rest of the molecule and conserves immunological functionality since it contains the antigen binding site. These analyses are in progress.

Conclusions

IgY distributed in dried yolk can partially resist digestive conditions in poultry. 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 because of the protective function of yolk. Nevertheless, additional protections should be searched to limit observed digestive deactivation of IgY and maximize the anti-*Salmonella* effect of the feed additive.

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For further information

Please contact *marcq.c@fsagx.ac.be*. More information on this and related projects can be obtained at *www.fsagx.ac.be/zt*.

Acknowledgments

This study was supported by the Directorate General of Agriculture of the Walloon Region (DGA, Namur, Belgium). We also thank J.-C. Bergen, R. Chalghoumi, S. D'Hoostelaere and P. Peeters for laboratory assistance and Biopole S.A. (Les Isnes, Belgium) and ATISA asbl (Gembloux, Belgium) for their technical help in egg yolk powders production.