In vitro Assessment of Bacteriostatic Potency of Egg Yolk Immunoglobulin against Salmonella Typhimurium

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ABSTRACT

The present study was carried out in commercial layer chickens to assess the bacteriostatic potency of egg yolk immunoglobulin IgY against food poisoning pathogen. The O antigen of food poisoning pathogen Salmonella was prepared and used to immunize commercial layer chickens. The eggs which contain anti-Salmonella IgY were collected on 30th day of first injection and stored at 4°C. The antibacterial IgY was separated by water dilution method (10 times diluted with distilled water, pH 5.0-5.5, incubated at 4°C for 6 hr) and purified by 60% ammonium sulphate. The recovery of IgY was in range of 57-62 %. The pathogens in tryptic soya broth (approx. 6x10^8/ml) were cultured with specific IgY's @ 20 mg /ml and inhibitory effect was measured in UV spectrophotometer at 550 nm. The resultant growth curve indicated that the application of polyclonal antibodies (IgY) on meat could be used to prevent the Salmonella food poisoning.

Keywords: Anti-Salmonella IgY, food poisoning, Salmonella,

Introduction

Foodborne diseases are important infectious diseases affecting millions of people in developing countries. Food poisoning can occur due to contamination of food and water with bacteria pathogens. Salmonella is one of the major pathogen which causes the food poisoning in notable manner.

Traditionally cleansing of the food sources was carried out by irradiation, chemical preservatives and use of antimicrobial agents to prevent the foodborne infection. Indiscriminate use of these practices, leads to development of resistance and residues in the food sources.

The above situation necessitated the need of alternate strategies. Of the available options for control of food poisoning, use of immunoglobulins against infections was first evaluated by Bartz et al. (1980). Oral administration of avian immunoglobulins (IgY) to prevent rotaviral infection was demonstrated by Ebina et al. (1990).

Scientific studies were made on use of IgY for its non-invasive procedure, abundance source, phylogenetic distance (Jensensius et al., 1981), lower cost and convenience (Polson et al., 1980), long self life (Larsson et al., 1993) over the conventional production of hyper immune sera from laboratory animals. The food poisoning incidence that occurs due to spoilage by Salmonella can be prevented by application of specific monoclonal antibodies against Salmonella at appropriate time resulting in reduction in proliferation of organisms Shimizu et al. (1988).

Keeping the above in the mind, the present study was formulated with the objectives to produce and
purify chicken egg yolk antibodies (IgY) against Salmonella and assess their bacteriostatic potency against Salmonella.

Materials and Methods
Six, 18 weeks old layer chickens purchased from a commercial layer farm in Namakkal were used for raising the anti-Salmonella IgY. The serotype Salmonella Typhimurium maintained in the laboratory was used for antigen preparation and testing the antibacterial effect of IgY. The stock cultures of the serotype Salmonella Typhimurium was revived by peptone water and enriched with selenite and tetrathionate broth. After incubation for 24 hr at 37°C, it was streaked on selective (Brilliant green agar) and differentiated agar (MacConkey agar and bismuth sulphite agar) and incubated at 37°C for 24-48 h, and was confirmed by biochemical tests (Barrow and Feltham, 1993).

Preparation of Salmonella somatic antigen was done as per the method described by Barrow (2000). The prepared Salmonella antigen was stored at -20°C until further use.

The method of Sriram and Yogeswaran (1999) with slight modifications was used for preparation of anti-Salmonella IgY. One ml of Salmonella O antigen was homogenized with one ml of (1:1) of Freund’s complete adjuvant and one ml of this emulsion was given I/M to 18 weeks old chickens. Two booster doses of 0.5 ml, one with Freund’s incomplete adjuvant, one without adjuvant at 14th day and 21st day, respectively were given by the same route. A week following last injection, test bleeding was done to assess the antibody response by slide agglutination method.

When the results were found satisfactory, the chickens were bled, the serum separated and stored at -50°C in small quantities to be used as known positive serum to compare the efficacy of IgY. After obtaining satisfactory results by slide agglutination test, the eggs were collected daily and stored at 4°C until analysis.

Separation and purification of anti-Salmonella IgY
The separation was done as per the method described by Akita and Nakai (1993). IgY containing water soluble fragments was purified by salt precipitation method described by Hansen et al. (1998).

Estimation of globulin
The Biuret method was used to estimate the globulins. To obtain higher concentration the IgY was dialyzed against heavy material like poly ethylene glycol. The corresponding serum and saline were used as positive and negative control respectively.

Assessment of bacteriostatic potency of anti-Salmonella IgY
The bacteriostatic potency was evaluated as per method described by Sunwoo et al. (2000) with slight modifications.

The Salmonella Typhimurium was cultured in tryptic soya broth and concentration was adjusted with Mcferland standard No. 2 (Approximately 6x10^8 bacteria/ml). Then cultured with specific anti Salmonella IgY or control at concentration of 20 mg/ml at 37°C for 1-6 h. The growth curve was plotted at hourly interval by measuring the turbidity at 550 nm spectrophotometer.

Results and Discussion
In the present study, the birds were given pre calculated Salmonella antigen just before lay. The booster doses with Freund’s incomplete adjuvant and without adjuvant were given during the laying period at 14th and 21st day, respectively.

ECVAM (1996) also recommended that it is preferable to immunize chickens before they begin to lay, because stresses induced by handling them could have an adverse effect on egg production. They also opined that the booster doses can be given during the laying period and FCA is the best choice.

The eggs with anti bacterial IgY were collected daily and stored at 4°C. Akita and Nakai (1992) reported that the major advantage in using chickens for IgY production as it was easy and non-invasive procedure.

Sunwoo et al. (2000) reported a weak antibody activity in egg yolk on day 7 or first injection, rapidly
increased on day 14, and gradually increased thereafter to reach the peak on day 56.

In the present study, the collection of immunized eggs started from day 30 after first injection, with high titre by day 35 and remained higher for approximately one to six months.

The water dilution method was used for the separation of IgY from egg yolk after it was diluted 10 times with distilled water. The pH of the diluted egg yolk was found to be 6.2 to 6.5. The egg yolk dilution were adjusted to the desire pH 5-5.5 by adding 0.1N HCL and incubated at 4°C for 6 h.

Akita and Nakai (1992) investigated the effects of dilution by diluting the egg yolk with 4, 6, 8, 10, 12, 14, 16, 18, 20 and 40 times with distilled water. They found that egg yolk diluted 10 times gave relatively clear supernatants with slight lipid contamination. In the present study, clear supernatant was obtained in 10 times dilution of egg yolk.

In the present study the pH of the diluted yolk was adjusted to 5-5.5 and obtained a very clear water soluble fragment. Akita and Nakai (1992) suggested that the highest yield of IgY could obtained at the pH 5-5.2.

In this study, the pH adjusted diluted yolk was incubated at 4°C and clear filtrate was obtained after 6 hr. The effect of time of incubation on 10 times diluted egg yolk, pH 5.0-5.2 was investigated by Akita and Nakai (1992) over a period of 49 h at 4°C. They found out a clear filtrate was obtained after 2 h. The IgY concentrations were found to remain almost constant after 5.5 h. They also suggested that incubation at 4°C for 6 h was sufficient to obtain clear WSF without lipid contamination.

The storage/incubation of diluted yolk samples at freezing temperature (-20°C) or in the cold (4-6°C) was important for removal of lipoprotein from WSF (Kim and Nakai, 1998). The WSF thus obtained was subjected to estimation of protein concentration by Biuret method. The protein content of the WSF was in the range of 35-40 mg/ml. Sriram and Yogeswaran (1999) obtained the same 50% - 55% recovery when they used modified WD method.

The biuret estimation of resultant anti-Salmonella IgY was 20-25 mg/ml and the recovery was 57% - 62%.

In the present study, a concentration of 200 mg of IgY/egg was observed and it was aggregated with the result obtained by Leslie and Clem (1969) who also reported the recovery of IgY from one egg was in the range of 100 to 200 mg.

**Inhibitory effects of anti-Salmonella IgY**

The resultant growth curve (Fig. 1) indicated the
inhibitory effect of anti Salmonella IgY, which was raised against Salmonella Typhimurium O antigen. In the similar study Sunwoo et al. (1996) reported the anti-liposaccharide specific IgY has inhibitory effect against Salmonella Typhimurium and it found useful for prevention of Salmonella food poisoning.

The study indicated that the anti-Salmonella IgY against their O antigens can be effectively produced in chicken eggs. The method of plotting of inhibitory growth curve as described by Sunwoo et al. (2000) is effective for the assessment of inhibitory effect of IgY. Specific assays such as quantitative ELISA must be developed for the assessment of concentration of antigen specific IgY and their antibody activity.

Based on the results of the present study, it is inferred that the face value and safety index of the food product can further be increased by the application of polyclonal antibodies (IgY) against Salmonella Typhimurium.

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