Invitro Assessment of Bacteriostatic Potency of Egg Yolk Immunoglobulin against *Escherichia coli*

Vikrama Chakravarthi. P[1], Shanmugasundaram. K[2] and Malmarugan. S.*

Department of Microbiology,
Veterinary College and Research Institute, Namakkal. India- 637001.
1. Asst. Product Manager, Natural Remedies Pvt. Ltd., Banglore, India.
2. Scientist, Agricultural Research Services, Hisar, Haryana
* Corresponding author email : micromals@rediffmail.com, Cell: +91 9443172607

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 Escherichia coli was prepared and used to immunize commercial layer chickens. The eggs which contain anti-E.Coli IgY was collected on 30 th day of first injection and stored at 4 0 C. The antibacterial IgY was separated by water dilution method (10 times diluted with distilled water, pH 5.0 - 5.5, incubated at 4 0 C for 6 hrs) and purified by 60 % ammonium sulphate. The recovery of IgY was in range of 57-62 %. The pathogens in Tryptic soya broth (approx. 6X108/ ml) were cultured with anti-E.coli IgY @ 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 E.coli food poisoning.

Keywords: - Food poisoning, *E.coli*, Anti- *E.coli,* IgY, Layer chicken, Immunoglobulin.

Introduction

Food poisoning is any disease of an infectious or toxic nature caused by the consumption of food or water. Food borne diseases is still one of the most important communicable and highly infectious diseases affecting millions of people in developing countries. Many outbreaks of food borne diseases that were once contained within a small community may now take place on global dimensions. The rapid globalization of food production and trade has increased the potential likelihood of food contamination.

Food poisoning can occur due to contamination of food and water sources with food intoxicating bacteria namely Clostridium, Salmonella and Escherichia. *Escherichia coli* is one of the major ubiquitous 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 food borne 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 Rota viral infection was demonstrated by Ebina et al. (1990).

Scientific studies were made on use of IgY for its non-invasive procedure, abundance source, phylogenic distance (Jensenius 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 *E.coli* and Salmonella can be prevented by application of specific monoclonal antibodies against *E.coli* and Salmonella at appropriate time resulting in reduction in proliferation of organisms (Shimizu et al. 1988).

Based on the above reports, the present study was formulated with the following objectives,

1. To produce and purify chicken egg yolk antibodies (IgY) against *E.coli.*
2. To assess the bacteriostatic potency of specific IgY against *E.coli.*

Material and Methods

1.Procurement of Chickens:

Twelve, 18 weeks old layer chickens were purchased from a commercial layer farm in Namakkal. One group of six birds were used to raise the anti *E.coli* IgY. Remaining six birds were used as a control group.

***E.coli* isolates**: The pathogenic *E.coli* was revived by

enriched BHI broth and subcultured on Macconkey agar and was confirmed by biochemical tests (Barrow and Feltham, 1993)

2. Preparation of E.coli O antigen and Anti-*E.coli* IgY:

Preparation of E.coli O antigen was done as per the method described by Barrow (2000). After satisfactory concentrations obtained, the *E.coli* antigens was stored at -20 0C until further use.

3. Preparation of anti-E.coli IgY:

The method was described by Sriram and Yogeswaran (1999) with slight modifications. One ml of *Escherichia coli* 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 14 th day and 21st day respectively 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 bleed, the serum separated and stored at -50 0 C in small quantities to be used as known positive serum to compare the efficacy of IgY. After obtain satisfactory results by slide agglutination method the eggs were collected daily and stored 40C until analysis.

4. Separation and Purification of anti-*E.coli* 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).

5. 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.

6. Assessment of bacteriostatic potency of anti-*E.coli* IgY:

The bacteriostatic potency was evaluated as per method described by Sunwoo et al. (2000) with slight modifications. The *E.coli* was cultured in Tryptic Soya Broth and concentration was adjusted with Mcferland standard No.2. (Approximately 6x108 no. of bacteria / ml). Then they cultured with specific anti-*E.coli* IgY or control at concentration of 20 mg / ml at 37°C for 1-6 hrs. The growth curve was plotted at hourly interval by measuring the turbidity at 550 nm of bacteria in culture by using UV Spectrophotometer.

Results and Discussion

1. Production of antigen specific IgY:

In the present study, the test birds were given pre calculated *E.coli* 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.

2. Collection of eggs:

After satisfactory results obtained the eggs with anti bacteria IgY were collected daily and stored at 40C. 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 a., (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.

3. Separation and purification of IgY:

3.1. Separation of IgY: The water dilution method was used for the separation of IgY from egg yolk after it was diluted 10 times with distilled water. In the present study the pH of the diluted yolk was adjusted to 5.0 -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 5.0-5.2.

3.2 Effect of dilution: 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.

4. Estimation of globulin:

The globulin (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 water dilution method.

5. Purification of IgY:

The biuret estimation of resultant anti-*E.coli* IgY was 20- 22 mg/ ml respectively and the recovery was 57-62%.

In the present study, a concentration of 200mg of IgY/egg was observed 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.

6. Inhibitory effects of anti-*E.coli* IgY

The resultant growth curve (Figure. 1) indicated the inhibitory effect of anti-E.coli IgY, which was raised

against *Escherichia coli* O antigen.

Earliest the inhibitory activity effect of anti-*E.Coli* IgY was shown by Akita and Nakai (1992).In their study, they measured anti-*E.Coli* IgY ,which was raised against enterotoxigenic Escherichia coli (ETEC) strain and reported that the water soluble IgY inhibited the growth of ETEC.

Sunwoo et al. (2000) also reported the inhibitory effects of anti *E.Coli* IgY at the concentration of 5 mg/ml. While in the present study, higher concentration (20 mg/ml) of IgY was used with appreciable results.

The following conclusions were drawn from this study:

a. The anti-*E.Coli* IgY against their O antigens can be effectively produced in chicken eggs.

b. 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 *Escherichia coli.*

Acknowledgements

The authors are thankful to Tamil Nadu State Council for Science and Technology for providing funding for this student project. The authors are also thankful to the Dean, Veterinary College and Research Institute, Namakkal and Dr. Durairajan, Professor and Head, Department of Microbiology, VC&RI, Namakkal for providing necessary facilities to carry out the project work.

References

1. Akita, E.M. and Nakai, S. (1992). Isolation and purification of immunoglobulin from egg yolk. *J. Food. Sci.* 57: 629-634.
2. Akita,E.M. and Nakai,S. (1993). Comparison of four purification methods for the production of immunoglobulins from eggs laid by hens immunized with an enteotoxigenic *E.coli* strain. *J. Immunol. Methods.* 160: 207-214.
3. Barrow, P.A. (2000). Serological diagnosis of *E.coli* by ELISA and other tests: In E.coli in domestic animals, Wray. C and Wray.A (Eds). CAB Publishing International, Oxon, UK pp: 420-421.
4. Barrow,G. I. and Feltham,R.K.A. (1993). Characters of Gram positive bacteria: In Cowan and Steel's Manual for the identification of Medical Bacteria, 3 rd Edn, Barrow, G.I., and Feltham, R.K.A (Eds). Cambridge University Press, UK. Pp: 94-150.
5. Bartz,C. R., Conklin, R.H., Tunstall, C.B. and Steele,J.H. (1980). Prevention of murine rotavirus infection with chicken egg immunoglobulin. *J . Infec. dis.* 142 : 439.
6. Ebina,T.,Tsukada,K.,Umezu,K., Nose, M., Tsuda, K., Hatta, H., Kim, M and Yamamoto,T. (1990).Gastroenteritis in suckling mice caused by human rotavirus can be prevented with egg yolk Ig (IgY) and treated with a protein-bound polysaccharide preparation. *Microbiol. Immunol.* 35:617-629.
7. ECVAM. (1996).The production of Avian (Egg yolk) antibodies: IgY. The report and recommendation of European centre for the validation of alternative methods (ECVAM) workshop 21st. 22-24, March, 1996, Berlin, Germany. 24: 925-934.
8. Hansen,P., Scoble,J. A. Hanson, B. and Hoogenraad, N.J. (1998). Isolation and Purification of immuno-globulin from chicken eggs using thiphilic interaction chromatography. *J. Immunol . Methods*. 215: 1-7
9. Jensensius, J. C., Anderson,I.,Han,J.,Crone,M and Koch, C. (1981). Eggs. Conveniently packaged antibodies. Methods for purification of yolk IgG. *J. Immunol. Mthods.* 46: 63-68.
10. Larsson,A.,Balow,R.,Lindahl,T.L. and Forsbery,P. (1993). Chicken antibodies: Taking advantage of evolution. *Poultry Science*.72: 1807-1812.
11. Leslie, G.A. and Clew, L.M. (1969). Phylogeny of immunoglobulin structure and function. *J. Experimental medicine.* 130: 1337-1352.
12. Polson, A.,Von Wechmar,M.B. and Van Regenmortal,M.H.V. (1980). Isolation of viral IgY antibodies from egg yolks of immunized chicken. Immunol.Commun. 9: 475.
13. Shimizu, M., Fitzsimmons, R. C. and Nakai,S. (1988). Anti E. Coli immunoglobulin Y isolated from egg yolk of immunized chicken as a potential food gradient. *J. Food. Sci.* 53: 1360.
14. Sriram, V. and Yogeswaran, G. (1999). Improved recovery of immunoglobulin fraction from egg yolk of chicken immunized with AsialoGM 1. *Russ J Immunol.* 4: 131- 140.
15. Sunwoo, H. H., X. Li, E. N. Lee, Y. K. Kim, and J. S. Sim. (2000). Preparation of antigen-specific IgY for food application: In Egg Nutrition and Biotechnology. J. S. Sim, S. Nakai., and W. Guenter, (Eds). CAB International, New York. pp 311–322.

* * * * * * * *