Bioengineering Research

Investigation the Production Rate of Anti-Streptococcus Mutans Immunoglobulin Y in Serum and Egg Yolk of Hens

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Abstract

Introduction: *Streptococcus mutans* (*S. mutans*) is a Gram-positive bacterium that lives in the mouth and contributes in to the formation of dental caries. Production and evaluation of hens egg yolk and serum antibodies in poultry industry are important.

Objectives: The serum and egg yolk of immunized hens were evaluated for producing of anti-*S. mutans* immunoglobulin Y (IgY).

Materials and Methods: A booster injection was performed 10 days after the initial infusion for producing of anti-*S. mutans* IgY. The effects of anti-*S. mutans* IgY antibody was detected in serum and egg yolk samples of immunized hens were evaluated by broth dilution. Finally, the tube agglutination test was used for the detection of *S. mutans* antigens in serums and eggs yolk. Sixteen days after initial immunization, IgY concentration was reached to maximum range.

Results: The tube agglutination test was showed IgY against whole cell antigen of *S. mutans* at 1:8 antibody titration. A 25 µg/mL of IgY was completely inhibited the growth of *S. mutans* and immunization against *S. mutans* by IgY that obtained from serums and eggs yolk of immunized hens. The results were showed this concentration was significant and more effective and can decrease the growth of this bacterium in its culture compare to normal antibodies obtained from unimmunized hens ($p<0.05$).

Conclusion: Anti-*S. mutans* IgY antibody which obtained from immunized hens decrease the bacterial growth rate and it could help to detect tooth decay and treating dental caries caused by this bacterium.

Keyword: Immunoglobulin Y; anti-Streptococcus mutans; Hens

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1. Introduction

Streptococcus *mutans* is a Gram-positive facultative anaerobic bacterium commonly found in the human oral cavity and it is one of the significant agents in human dental decay [1]. This pathogen was first described by Clarke in 1924 and responsible for human dental plaque [2, 3]. *S. mutans* serotype C is a principal causative agent of dental caries and infective endocarditis. Decreasing the consumption of sugar can help to prevent the growth of some bacteria but it

is not possible to eliminate all bacteria from the mouth. *S. mutans* adhesions interact with the tooth surface and help to an accumulation of the bacteria to generate biofilm. These bacteria produce glucose and glucans by glucosyl transferase and convert sugars into lactic acid through fermentation [4, 5]. Many methods include early surgical intervention; fluoride treatment, mouth rinses, and dental sealants are used for caries management but the best procedure for inhibition and decreasing of tooth decay is limiting sugar consumption [6, 7]. In kinds of toothpastes presence of fluoride and peroxide inhibited the growth of *S. mutans* [1]. To prevent the attachment of bacteria special *S. mutans* on to the surface of teeth, antibodies via their antigen-binding site can block the antigenic adhesion site of the pathogen [4, 8]. For developing of avian embryo their eggs contain all of the necessary nutrients and growth factors [9]. Chicken egg yolk antibodies (IgY) is a major antibody in bird, reptile, and lungfish blood. IgY is the functional equivalent to immunoglobulin G (IgG) in chickens and in immunoglobulins arising during the immune response, only IgY is found in chicken eggs [10, 11]. Also, IgA and IgM antibodies are present in chicken egg yolks but at low levels [9].

Chicken egg yolk is an important antibody source against many different bacteria like *S. mutans* and IgY activity against an antigen of *S. mutans* has been demonstrated. So, the purpose of the present study was to investigate the production and evaluation of anti-*S. mutans* hens egg yolk antibodies (IgY) in serum and eggs laid.

2. Materials and Methods

2.1. Organism used and preparation of antigen

A Standard strain of *S. mutans* C (MT8148) was obtained from the Department of Preventive Dentistry, Faculty of Dentistry, Islamic Azad University, Khorasgan Branch (Isfahan province, Iran). *S. mutans* C was cultivated on Brain Heart Infusion (BHI) broth supplemented with 5% sucrose and incubated at 37°C for 48 hours aerobically. After cell concentration reaches 2 $\times 10^9$ CFU/mL, 0.5% formalin was added to the culture for 24 hours and used for killing whole cells. Then, the bacterial cells pellet was collected by the centrifugation (10000 g, 15 min). The plates were then washed three times by sterile saline containing 0.5% formalin and the pellet resuspended in sterilized saline, using a vortex mixer.

2.2. Hen immunization

For *in vitro* studies, 20 white leghorn hens (18 weeks old) were used and were divided intofour groups. Five hens in group 1 were immunized by intramuscular injection of an emulsified mixture (1 mL) of formalin-treated whole cell suspension (10^9 cells per 0.5 mL) and 0.5 mL of Freund's completes adjuvant (FCA-Difco Laboratories, Detroit, Mich). A booster injection was done 10 days after the initial immunization. Group 2 including 5 hens were similarly immunized and boosted with an emulsified mixture (1 mL) of formalin-treated whole cell suspension (10^9 cells per 0.5 mL) and FCA (0.5 mL), while hens in group 3 were immunized with an emulsified mixture of formalin treated whole-cell suspension (10^9 cells per 0.5 mL) and FCA (0.5 mL). Group 4 of hens consist of 5 hens were sham-immunized (SI) with an emulsion of saline (0.5 mL) and FCA (0.5 mL). Eggs were collected, labeled and stored at 4°C until processed for determination of IgY.

2.3. Antibodies preparation from egg yolks

Antibodies were prepared by the modified method of Fulton et al., 2002 [12]. In brief, egg yolks were separated from the eggs of immunized or SI hens and resuspended in equal volume of PBS (Na_2HPO_4 (0.02 mM), NaCL (0.15 M) pH=7.2) using a Vortex mixer for 3 minutes. This mixture was centrifuged at 1800 g for 25 min at 4°C and the lower layer and supernatant consist of lipid layer were removed and the middle layer was separated and frozen.

2.4. Inhibitory test by broth dilution

The effects of anti-*S. mutans* IgY obtained from serum and egg yolk of immunized hens were evaluated by broth dilution. After culture of *S. mutans* in Brain Heart Infusion Broth (BHI) supplemented with 5% sucrose the serial dilution of anti-*S. mutans* IgY with an initial concentration of 30 µg/mL of IgY were done in each wells and the plate and was incubated overnight at 37°C, anaerobically. In this test, PBS was negative control and Amoxycillin (50-

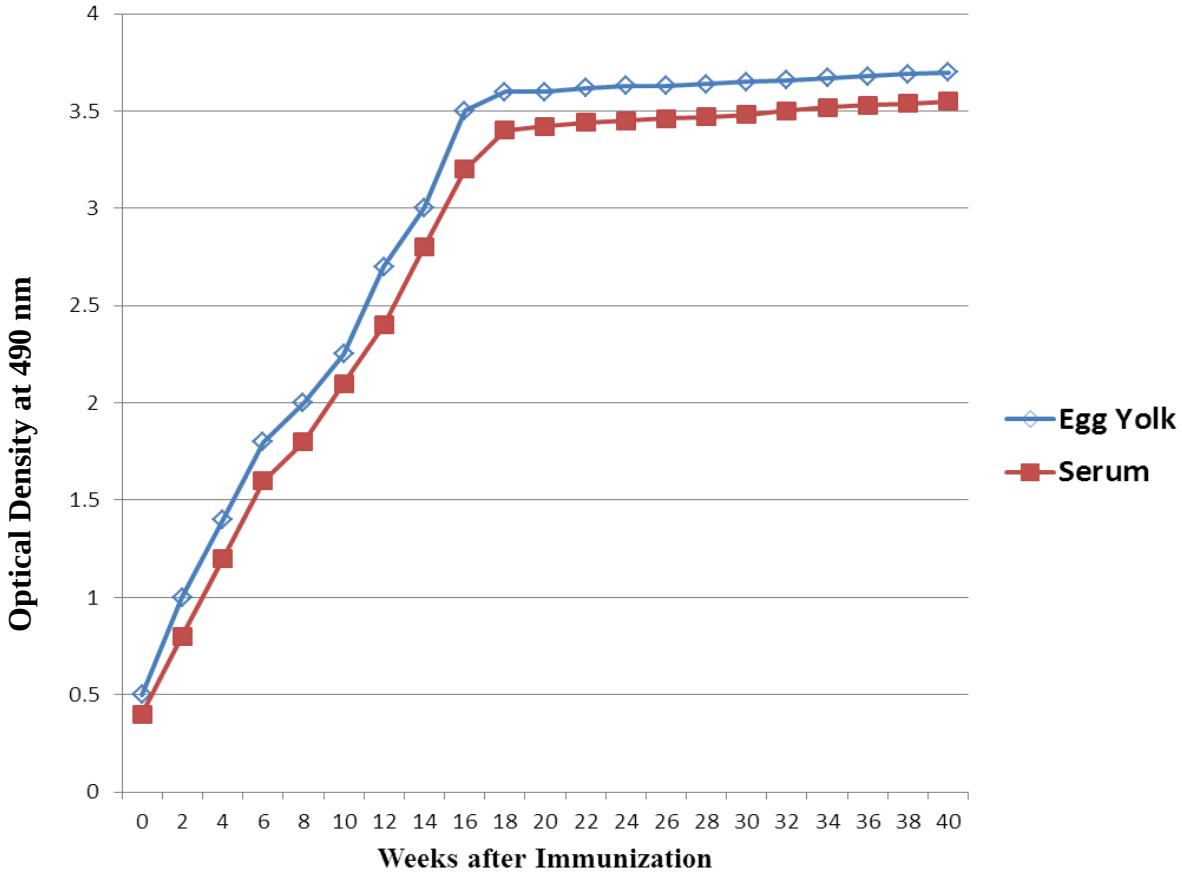

Figure 1. The IgY antibody level against *S. mutans* monitored in both hen serum and egg yolk. Ten days after the initial immunization, IgY in serum and egg yolk were increased and in day 16 was reached to maximum range.

Table 1. Minimum agglutination assay for detection of IgY concentration against whole cell antigen of *S. mutans*.

Antibody dilution or titration	Agglutination of IgY against *S. mutans* whole cells antigen
1:8	+++
1:64	++
1:512	++
1:4096	+
1:32768	-
Negative control (IgY from egg yolk of a non-immunized hen)	-

-No agglutination
+Minimum agglutination titer
++Presence of agglutination
+++ Strong agglutination reaction

70 µg/mL) antibiotic was served as a positive control. After incubation, the media were subcultured again onto BHI agar and incubated overnight at 37°C, anaerobically. The optical density (OD) and turbidity of grown *S. mutans* cells in each concentration of IgY in the tubes were measured at 600 nm using Shimadzu spectrophotometer (Shimadzu UV-160). The concentration and number of cells were compared to negative and positive controls.

2.5. Tube agglutination test of S. mutans cells by anti- S. mutans IgY

Tube agglutination test was done on serum and egg yolk for detection of *S. mutans* antigens using anti-*S. mutans* IgY. Whole-cell of *S. mutans* that titrated by formalin (0.5 %) were suspended in saline (2 mg (dry weight) per mL) and the cell suspension and serum was mixed with an equal volume of ten fold-diluted IgY. The plate was then placed in the incubator at 37°C for 2 hours and finally for overnight at 4°C, and agglutination was visually determined. The minimum concentration of IgY that gave positive agglutination it was found as antibody titration in the reaction mixture.

3. Results

In immunized hens, the IgY level was evaluated after every booster dose in both serum and egg yolk. IgY antibody against *S. mutans* in the serum and egg yolk were increased 10 days after the initial immunization and monitored for 40 days by tube agglutination test. The antibody level was remained stable 60 days after the initial immunization (Figure 1).

The concentration level of IgY in egg yolk was sharply increased during the immunization period and 16 days after initial immunization reached to maximum range. The result of the tube agglutination test was showed the minimum agglutination concentration of IgY in serum and egg yolk against whole cell antigen of *S. mutans* was 1:8 (Table 1). These results indicated that titration of IgY immunization against *S. mutans* and its activity in the serum and egg yolk was significant and more effective and decrease the growth of this bacterium in its culture compare to normal antibodies obtained from unimmunized hens ($p<0.05$). The results were showed the growth of *S. mutans* was completely inhibited at a concentration of 25 µg/mL of IgY.

4. Discussion and Conclusion

In the present study, the production of anti-*S. mutans* IgY in serum and eggs yolk of immunized hens were evaluated. The findings of this study were showed 10 days after the initial immunization IgY antibody against *S. mutans* in the serum and egg yolk increased. The IgY concentration increased in the serum and egg yolk with subsequent booster doses and reached to maximum range in day 16. The results of the tube agglutination test were showed IgY against whole cell antigen of *S. mutans* at 1:8 antibody titration. Moreover, this study indicated that IgY antibody titration in immunization hens against *S. mutans* and its activity in the serum and egg yolk in this poultry was so high and decrease the growth of this bacterium in its culture compare to normal antibodies obtained from unimmunized hens ($p<0.05$). In a study of Dinesh and colleagues on IgY against *S. mutans* in micro-agglutination test, the reaction was observed up to 1:1280 dilution and antibodies were generated in chicken against this bacteria's antigens with the high peak titre of 1:10000 antibody on 63rd day of observation [13], while in the present research, this ratio by tube agglutination test was 1:8 antibody titration and maximum level of IgY concentration in day sixteen were observed in the serum and egg yolk. Also, IgY obtained from yolks of eggs by immunized hens is an excellent source of polyclonal antibodies and useful tool in research and diagnostics [14]. In another study high titer of more than 1:10000 antibodies were detected by indirect antigen capture ELISA and observed at 150th day [15], while, in our study IgY concentration 16 days after initial immunization reached to maximum range. In a study of Moreno et al. 2011, the highest IgY level was obtained on the 42nd-day post-immunization by ELISA test [16] whereas in the present work after the initial immunization IgY in serum and egg yolk were reached to maximum range in day 16 by tube agglutination test. In a study of Rajan and co-workers on a generation of egg yolk antibodies in chicken (IgY) against *S. mutans* the high titer of more than 1:10000 antibodies were detected by indirect antigen

capture ELISA at 150th day of observation. They showed *S. mutans* growth with increasing concentration of antibodies (IgY) was decreased [4]. In our study, the IgY antibody level against *S. mutans* in the serum and egg yolk were increased 10 days after first immunization and 16 days after initial immunization reached to maximum range.

The findings of the present work indicated that anti-*S. mutans* IgY obtained from serum and egg yolk of immunized hens decrease the *S. mutans* growth in culture and it could be used for diagnosing dental caries caused by this bacterium and as a therapeutic approache.

Conflicts of interest

The authors declare no conflict of interest for this article.

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