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Industrial Application of IgY

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INTRODUCTION

Egg is the largest cell known which originates from one cell division(Burley et al 1989) and is composed of various important chemical substances that form the basis of life. Avian egg is a store house of nutrients for proteins, lipids, carbohydrates and biologically active substances including growth promoting factors which are needed to become chicken. Besides above components, the immunoglobulin G of yolk which is called IgY is accumulated in higher concentrations. The ability of chickens to produce high levels of precipitating antibodies following injections of heterologous serum proteins. Fraser et al.(1934) and Brandly et al.(1946) have demonstrated the passage of antiviral antibodies from the serum of chickens to egg yolk. This transport of antibodies from laying hen to egg yolk is mechanism for transfer of maternal antibodies to the offspring.

The effectiveness of the passive immunization by oral administration of antibody against bacteria or virus has demonstrated to prevent corresponding infections disease.

The application of this passive immunization therapy requires the development of an effective process of preparing antibody, because the preparation of large amount of antibody may be desired for specific appl cations. A convenient and large scale supply of antibody will also promote the use of antibody not only in pharmaceutical but also in food and cosmetic industries. Commercial available antibodies have usually been produced in mammals such as mouse, rat, rabbit, goat, and horse after immuization of these animals. These antibodies can not be prepared on industrial scale and are not cost effective because of the difficulty to obtain large amount of blood and collecting blood requires too much of care.

We report here the production of IgY against several antigens such as *Streptococcus mutans* (a causative bacteria of dental caries), *Edwardsiella tarda* (a causative bacteria of fish disease), *E.coli* (a causative bacteria of diarrhea), *Salmonella enteritidis* (bacteria of food poison), porcine epidemic diarrhea virus (PEDV), *Rotavirus* (virus of childrens' diarrhea).

IMMUNOGLOBULIN YOLK AGAINST *EDWARDSIELLA TARDA*

Edwardsiella tarda infection of Japanese eel and flounder is called "paracolo disease". The main pathological change is abscess formation either in the liver or kidney. The causative bacterium can infect the fish via intestine. Paracolo disease of the eel and flounder has been a serious problem. This disease was well controlled by treatment with antibiotics and chemotherapeutants (tetracyclines and oxolinic acid). However, this disease has been found to cause mass mortalities due to the appearance of drug-resistant strains, known since 1977(Aoki et al 1977). Vaccination has been investigated as an alternative control method for this disease. Various vaccination methods have been tried, including oral administration, spray, direct immersion, hyperosmatic infiltration and intra peritoneal injection with bacteriua of *E. tarda*(Salati, 1988). Some succcess has been achieved in immunization of eels and flounders with the bactrial lipopolysaccharide (Salati& Kusuda, 1986). However, these methods are not of practical value for fish farmers and effective control methods against paracolo disease are still required.

Gutierrez MA(1993)reported protective properties of egg yolk IgY containing anti-*Edwardsiella tarda* antibody against paracolo disease in the Japaneses eel, *Anguilla japonica*.

Immunized hens are known to contain a high level of immunoglobulin Y(IgY) in their egg yolk. . The IgY was stable against eel digestive factors, and therefore, was orally administered with viable *E. tarda* to the japanese eels and the efficacy of protection against *E. tarda* infection was evaluated. The fish orally administered with IgY showed reduced morbidity and mortality. These results suggest that egg yolk containing anti-*E. tarda* IgY is effective in preventing edwardsiellosis.

PRE-CLINICAL TEST

Mortalities of flounder fishes artificially infected with *Edwardsiellosis* (10^8/ml) and *Streptococcus iniae* (10^8/ml) in control group were 96% and 92%, respectively. However, in IgY (water soluble fraction powder) feeding group, mortalities of flounders were significantly lower than in control group.

It is possible to use water soluble fraction separated from IgY eggs to replace of antibiotics in order to prevent flounder' s disease.

IMMUNOGLOBULIN YOLK AGAINST *STREPTOCOCCUS MUTANS*

Streptococcus mutans, one of the prime pathogens of dental caries, produces at least two kinds of glucosyltransferase(G Tase) that synthesize water-soluble and water -insoluble glucans. The ability to produce water-insoluble glucan adherent to solid surfaces has been demonstrated to be a virulence factor of *Streptococcus mutans* in dental caries development (Hamada et al., 1980). *S. mutans* strains are classified serologically into seven serotypes, a to g.

Hatta(1996) reported passive immunization against Dental plaque formation in Humans: effect of a mouth rinse containing egg yolk antibodies specific to *Streptococcus mutans*.

Table 1. The protection effect of *Edwardsiella tarda* and *Streptococuus iniae* by IgY in flounder fishes

Fish group	Number dead / total (% mortality)	
	Edwardsiellosis	Streptococcal infection
	Non-treated group	
0*	48/50(96)	46/50(92)
	Treated group	
10	40/50(80)	42/50(84)
20	16/50(32)	12/50(24)
40	14/50(28)	10/50(20)

* ; Water soluble fraction mg/kg of fish body weight(Egg Biotech, 2001).

The antibodies were derived from egg yolks obtained from hens immunized with whole cells of *S. mutans*. Immune IgY inhibited *S. mutans* adherence to saliva-coated hydroxyapatite discs by 59.2%. In the short-term test using a mouth rinse containing 10% sucrose, immune IgY decreased the ratio of the percentage of *S. mutans* per total streptococci in saliva. These results support the effectiveness of IgY with specificity to *S. mutans* grown in the presence of sucrose as an efficient method to control the colonization of mutans streptococci in the total cavity of humans.

Chang (1999) reported productivity and some properties of immunoglobulin specific against *Streptococcus mutans* serotype c in chicken egg yolk (IgY).

Hens were immunized on thighs by using whole cells of *Streptococcus mutans* MT8148 serotype c strain as antigen through intramuscular (im) and subcutaneous (sc) routes to investigate the difference of immunization reactions and the changes in yolk antibody activities against antigen after initial immunization. Several properties of crude IgY were examined to evaluate the stability during food processing. Results showed that the specificity of IgY of intramuscular treated hens was nearly 10 times as high as those of subcutaneous treated antibody. IgY from the hens immunized with the serotype c strain showed significant cross-reactions against serotypes e and f, in thermal stability tests, IgY

activity in both yolk and crude IgY decreased with the increasing temperature, from 70 to 80 degrees C, but the thermal denaturation rates between those two samples were not significantly different.

INHIBITORY ACTIVITY OF IGY AGAINST *Streptococcus mutans* : COMPARISON WITH CATECHIN AND ANTI-STREPTOCOCCUS IGY

▶ Inhibitory activity against *Streptococcus mutans*

Catechin and anti-S.mutans IgY were mixed with 4 × 10^8 cell of *Streptococcus mutans* suspended with sterilized saline. Supernatant was measured O.D value on 660nm at 4, 24 hour-post-reaction.

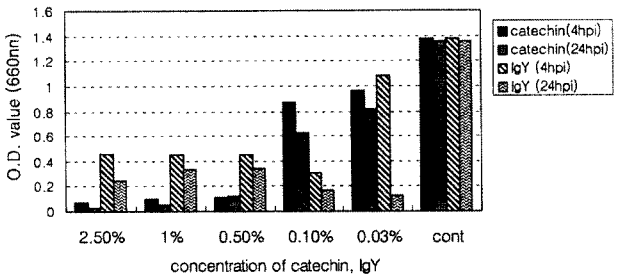


Fig. 1. Inhibitory activity against *S. mutans*

▶ Inhibitory of growth rate against *Streptococcus mutans*

Catechin and anti-S.mutans IgY were mixed with 1 × 10^6 cell of *Streptococcus mutans* suspended with Tryptic soy broth and incubated in 37℃. Mixture was measured O.D value on 660nm at 24 hour-post-reaction.

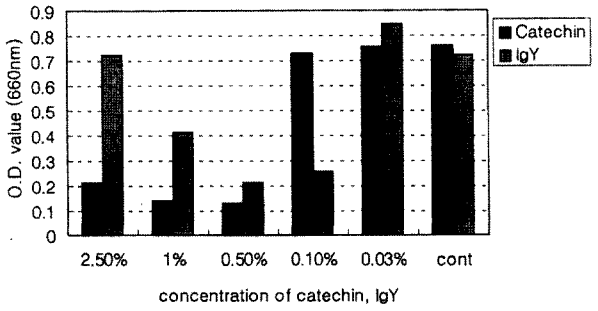


Fig. 2. Inhibitory of growth rate against *S. mutans*

IMMUNOGLOBULIN YOLK AGAINST PORCINE DIARRHEA AGENTS

Diarrheal disease caused by enterotoxigenic *E. coli* (ETEC) is the most common enteric colibacillosis encountered in neonatal piglets. Colonization of the small intestine off the piglet by ETEC adhering to the epithelium accounts for most gastrointestinal disorders (Arbuckle, 1970; Bertschinger et al., 1972). The fimbrial K88,k99, and 987 P antigens of porcine ETEC that are associated with intestinal colonization have been extensively investigated with respect to their genetic background, protein chemistry, and immunological properties (Klemm, 1985; Mooi, 1985). They have been widely employed with promising results as vaccine antigens in controling porcine colibacillosis. In passive immunization expeiments, antibodies raised against these fimbrial antigens have been administered orally to piglets and have offered potential therapeutic value in controlling the disease.

Oral administration of antibodies derived from serum and colostrum and even with monoclonal antibodies has been very successful. However, it is expensive to obtain the large amounts of antibodies required (Kuhlman et al., 1988).

Veterinary interest is the use of chicken egg yolk antibodies for the treatment of porcine colibacillosis. Vaccination of laying hens provides a cheaper and good alternative antibody source; the eggs collected after a high level of antibodies is reached in the egg yolk. This principle is not new. Some authors have used chicken egg yolk antibodies in the prevention or control of rotaviral infection in mice and cats, and their promising results have led to the suggestion that egg preparations might serve as a source of antiviral antibodies for humans(Yolken et al., 1988).

Imberechts(1997)reported chicken egg yolk antibodies against F18ab fimbriae of *Escherichia coli* inhibit shedding of F18 positive *E. coli* by experimentally infected pigs.

F18ab and F18ac are antigenic variants of a colonizing fimbria commonly found on *E. coli* associated with postweaning diarrhea and edema disease in pigs. Chicken F18ab antibodies were obtained by immunizing hens with purified F18ab fimbriae. *In vitro* adhesion tests demonstrated that the chicken F18ab antibodies inhibited attachment of F18ab positive *E. coli* bacteria to the intestinal mucosa. The animals were infected on the second day of a period during which chicken F18ab antibodies were added to their feed. The F18ab antibodies diminished the cases of diarrhea and death in animals infected with F18ac positive *E. coli*.

Erhard (1996) reported prophylactic effect of specific egg yolk antibodies in diarrhea caused by *Escherichia coli* K88 (F4) in weaned piglets.

The protective effect of specific egg yolk antibodies on diarrhea caused by *Escherichia coli* K88 (F4) was investigated with 179 weaning piglets. The egg powder was offered in a 5% feed ration. Compared with the control groups, the piglets of the antibody group showed significant differences ($P < 0.05$, chi 2-test) in the parameters rate of diarrhea (17.2%) (antibody group) to 60.7% (control egg group) or 56.7% (control group without egg powder), severity of symptoms (5.2~39.3% or 26.7%) and frequency of additional treatments (8.6~55.7% or 51.7%).

Wiedemann (1991) reported chicken egg antibodies for prophylaxis and therapy of infectious intestinal diseases. V. *In vivo* studies on protective effects against *Escherichia coli* diarrhea in pigs.

A field study and a controlled infection trial showed the protective effect of egg yolk lyophilisate and whole egg lyophilisate against enterotoxic *E. coli* germs. In a first field study using egg yolk antibodies, 92% of 299 diarrhea affected piglets were cured. The infection trial showed, that whole egg lyophilisate of immunized hens was as successful as a common antibiotic therapy in curing piglets, orally infected with 5×10^{10} *E. coli*/feeding and animal. The present data show that chicken egg antibodies can be used for treatment of infectious diarrheal diseases in young animals. So far they represent a good alternative to the common used antibiotic therapy.

Kweon (2000) reported immunoprophylactic effect of chicken egg yolk immunoglobulin (Ig Y) against porcine epidemic diarrhea virus (PEDV) in piglets.

Porcine epidemic diarrhea virus (PEDV) is the causative agent of neonatal diarrhea in piglets, which causes high mortality rates. In this study, the immunoprophylactic effects of chicken egg yolk immunoglobulin (Ig Y) against PEDV were investigated in neonatal pigs. Ig Y was found to reduce the mortality in piglets after challenge exposures. The results in this study indicated that Ig Y against PEDV could be an alternative way of supplementing prophylactic measures like colostral antibodies from sows.

IMMUNOGLOBULIN YOLK AGAINST ROTAVIRUS

Rotaviruses are major causes of infectious gastroenteritis in infants and young children living in developed and developing countries(Kapikian et al., 1979).

The importance of rotaviruses in human disease has led to the development of stragies for prevention of rotavirus gastroenteritidis by means of active immunization with attenuated strains of human or antigenically related animal rotaviruses. However, there are persons at high risk for serious consequences of rotavirus infections who might not be expected to respond to active immunization with live attenuated viruses. These persons include newborns, children with congenital or acquired immunodeficiency syndromes, and virtue of chemotherapy, malnutrition, or aging(Yolken et al., 1982, Saulsbury et al., 1980).

One alternative method for the prevention of infectious gastrroenteritidis in individuals unable to mount an active immune response involves the administration of preformed antibodies capable of neutralizing microbial pathogens. Egg yolks provide an additional source of immunoglobulins that is suitable for consumption by humans. It is likely that chicken eggs contain antibodies capable of neutralizing human rotaviruses. It is also possible that chickens might be immunologically

primed by previous infection and thus be directed to produce antibodies specific for human rotaviruses by immunization with antigens derived from human rotavirus strains(Yolken et al., 1988).

Sarker et al.,(2001) reported randomized, placebo-controlled, clinical trial of hyperimmunized chicken egg yolk immunoglobulin in children with rotavirus diarrhea.

Antibodies derived from eggs of immunized hens may be a less expensive and more practical alternative. In this study, children with proven rotavirus diarrhea were treated with immunoglobulin extracted from eggs of chicken immunized with human rotavirus strains. 79 children with known rotavirus diarrhea were assigned to receive either 10g hyperimmune egg yolk (HEY) daily in four equally divided doses for 4 days (HEY group) or a similar preparation obtained from nonimmunized chicken (placebo group). The daily stool frequency and amount, oral rehydration solution (ORS) intake, and presence of rotavirus in the stool were monitored for 4 days. : In the HEY-treated group, there was significant reduction in stool output , and significant reduction of ORS intake on day 1 and clearance of virus on day 4 (HEY vs. placebo; 73% vs. 46%, P = 0.02). These results indicate an encouraging role of HEY in the treatment of rotavirus-induced diarrhea in children.

Hatta (1993) reported productivity and some properties of egg yolk antibody (IgY) against human rotavirus compared with rabbit IgG.

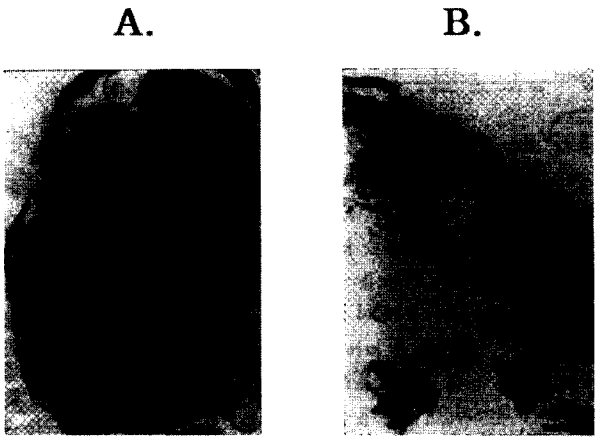
Productivity and some properties of anti-Human Rotavirus (HRV) hen egg yolk antibody (IgY) were compared with those of anti-HRV rabbit serum antibody (IgG). The hens immunized with HRV (Wa strain, serotype 1 and Mo strain, serotype 3) were found to continuously to lay eggs and the yolk of the eggs laid over a year showed a high level of neutralization titer against HRV. The production of anti-HRV IgY by a hen (one year) was at least 15 times (anti-Wa) and 120 times (anti-Mo) more effective than those by an immunized rabbit in the neutralization titer of the antibodies.

Yolken et al(1988) reported antibodies to rotaviruses in chickens' eggs: a potential source of antiviral immunoglobulins suitable for human consumption.

The prevalence of antibodies to human rotaviruses in commercially available eggs and egg products that are suitable for human consumption was investigated. The yolks of virtually all of the individual eggs and pasteurized pooled egg preparations contain antirotavirus antibodies detectable. Also, the eggs and egg preparations are capable of inhibiting the growth of two strains of rotaviruses in tissue culture. The antibody levels in eggs can be increased by the immunization of hens with purified rotavirus preparations, and the immunoglobulins isolated from the eggs of immunized hens can prevent the development of rotavirus gastroenteritis in experimentally infected animals. Egg preparations might serve as a practical source of antiviral antibodies suitable for consumption by infants and young children.

PREVENTION OF ROTAVIRUS INFECTION IN MICE

1. Hematoxylin-eosin-stained section of the small intestine



A. Anti-rotavirus IgY inoculated intestine.
B. Rotavirus-infected intestine.
- a loss of the microvilli on enterocytes
- interstitial edema of villi
- degenerated enterocytes with intracytoplasmic vacuoles.

2. Protection with anti-rotavirus IgY against rotavirus-induced diarrhea in mice

Protection with anti-rotavirus IgY against rotavirus-induced diarrhea in mice

Sample (anti-rotavirus IgY)	No. of mice	Clinical signs (affected/total)			
		depress	bad hair	diarrhea	
WSF: (IgY concentration: 0.2mg/day)	10	1/10	1/10	0/10	0%
Egg yolk : (IgY concentration: 0.2mg/day)	10	0/10	0/10	0/10	0%
Control (PBS)	10	9/10	10/10	4/10	40%

* All mice of group were challenged with rotavirus and then treated with IgY containing sample and control solution(Egg Biotech, 2001).

CONCLUSION

Hens transfer blood serum immunoglobulin G which accumulate in the egg yolk during oogenesis. The antigen-specific antibody can be obtained in large quantities from eggs laid by hyper immunized hens.

We have industrialized the manufacturing technology for the functional foods of egg yolk containing IgY .

Mass production at industrial scale strongly suggest the possibility for IgY as a practical reality to be applied by oral administration to prevent various infectious disease.

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