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New Dimension in Fish Immunotherapeutics: Avian Egg Yolk Antibody (IgY)

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A B S T R A C T

Intensification in aquaculture practices has resulted the development of various diseases caused due to pathogens and water quality issues. In recent times, the usage of antibiotics for controlling bacterial diseases in aquaculture has been proven unsustainable and barren due to development of antibiotic resistance in pathogens one of the greatest human health challenges of the 21st century. Many measures have been used as potential alternatives to antibiotics including organic and inorganic acids, antibodies, probiotics and herbal products. Oral administration of Immunoglobulin (IgY) has attracted significant attention worldwide as a means of controlling infectious diseases of bacterial and viral origin in fishes due to its high specificity. In the present review, a holistic approach was studied to summarize the mode of action of IgY, advantage and application of IgY in aquatic organisms.

Introduction

Antibodies are called immunoglobulins, a set of glycoprotein secreted by plasma cells in response to antigen exposure. Since last few decades antibodies are being used widely in the field of disease diagnosis, quantification and identification of specific antigens (Amro *et al.*, 2018). Till recent year animal species were primary choice for researchers to produce specific antibody which involves steps like immunizing the animal with specific antigen, collection of blood, purification of antigen. Such process most of the times leads to death of the animal due to excessive loss of blood. With the discovery of IgY in the bird egg it has started to gain importance and broader application due to several advantages over mammalian antibodies produced. Chicken serum IgY transported to egg following similar pattern of placental transfer of IgG in mammals to protect the newly hatched chicken by providing passive immunity. Following similar function like

IgG, molecular structure of IgY is different from IgG, but shows similarity with mammalian IgE and IgA (Warr *et al.,* 1995, Ambrosius, 1996). Thus, it is postulated that IgY is phylogenetic progenitor of mammalian IgE and IgA (Carlender, 2002). In this review we will discuss about the multidimensional application of IgY in the field of aquaculture and fish health management.

Structure and Properties

IgY contain two heavy chain and two light chain resembles a Y like structure. Unlike mammalian IgG, this antibody has higher molecular weight due to presence of extra constant (CV2) domain in heavy chain (Warr *et al.,* 1995). CV3 and CV4 constant region of heavy chain of IgY shows some similarity with CV2 and CV3 of IgG (Shizimu *et al.,* 1992). However, the hinge region of IgY is not well developed. The undeveloped hinge region alters the flexibility of Fab region which may cause some difference in the epitope recognition for IgY and IgG (Cser *et al.,* 1982 and Noll *et al.,* 1982). To consider a candidate for oral administration stability to lower pH is one of the important properties of an antibody. IgY unlike IgG has lower stability in low pH (Calzado *et al.,* 2005). IgY rapidly lost its activity near pH- 4.0 to pH- 3.0 (Lee *et al.,* 2002; Hatta *et al.,* 1993). To suppress the inactivation of IgY at lower pH scientists have developed various techniques so that it can be prevented. Addition of 50% sorbitol with IgY found to have prevented low pH inactivation (Lee *et al.,* 2002). Increases use of alkaline (Sodium Bicarbonate) and egg white found effective to provide resistance against acid inactivation and proteolytic cleavage. IgY shows more resistance against trypsin and chymotrypsin (Calzado *et al.,* 2005). IgY is stable at higher temperature like 60-70°C. Some scientist has also found 100% stable IgY even at 100 degree centigrade (Lösch *et al.,* 1986).

Transport to egg

Concentration of IgY is much higher (5 to 15 mg / ml) than IgM (1 to 3 mg/ml) in blood serum (Rose *et al.,* 1974; Kowalczyk *et al.,* 1985). It took 5-6 days for the IgY to appear in the egg yolk after detection in serum (Davison *et al.,* 2008). Amount of IgY in egg is positively correlated with amount in serum. However, concentration of IgY differ with species and within chicken lines (Carlander, 2002). The Fc and hinge region play important role in transfer of IgY from serum to egg (Mohammed *et al.,* 1998; Morrison *et al.,* 2001). Though there are some contradictions in studies shows presence of IgM and IgA in egg yolk but recent studies show that IgY is exclusively transported to egg via a receptor mediated endocytosis (Kitaguchi *et al.,* 2008). Studies found that all immunoglobulins transported to yolk have a characteristic HEAL (His-Glu-Ala-Leu) sequence (Calzado *et al.,* 2005). Some birds and reptiles contain truncated form of IgY which is devoid of terminal domain in heavy chain. Such truncated IgY transported to egg via fluid based endocytosis (Kitaguchi *et al.,* 2008). Schade *et al.,* (1994) found that around 100-200 mg of IgY found in egg with 2-10% antigen specific IgY.

Advantages of using IgY

One of the major advantages of using IgY over other antibodies is their non-invasive way of production and purification. The production of IgY involves a non-invasive method that includes immunizing host with specific antigen. The antibody will be produced in the chicken mother will eventually transferred into egg. A chicken produces 260-300 eggs per year. From each egg 100-200 mg of antibody can be produced with 2-10 % of with Ag specific antibody (Schade *et al.,* 1994; Carlander, 2002). Thus 20-40 mg of IgY can be produced from single bird without causing

any damage. A large proportion of antibody lost in the guts when administered orally. This is largely due to enzymatic inactivation of antibody and lower pH of guts. Thus, a high quantity of Ab required for oral administration. Higher yield of IgY antibody in cheaper cost make it possible for the treatment of animals orally.

Specially in case of fishes, where individual injection becomes tedious job, oral treatment provides greater advantage because target species can be administered specific IgY mixing with feed.

Stability at higher temperature is necessary for making antibodies in powder form. IgY is stable at higher temperature of 60-70˚C. Some scientist has also found 100% stability of IgY even at 100 ◌̊C. Birds are phylogenetically distant to fishes, which reduces any threat of cross reactivity of IgY with fish epitopes, Fc segment etc. Besides that, birds need small amount of antigen to produce large quantity of IgY which shows remarkably high affinity, high avidity and highly neutralizing ability against infectious pathogen (Zorriehzahra *et al.,* 2016).

Mode of Action

Mode of action of IgY is yet to determine. However, four principal mode of action of IgY have been proposed by the scientists includes agglutination, adherence blokade, opsonization followed by phagocytosis and neutralization of pathogen.

Agglutination

Agglutination of virus, parasites and bacteria causes immobilization, thereby prevent their growth. Tsubokura *et al.,* (1997) observed prevention of growth of bacteria and reduced the CFU count due to agglutination following the treatment of IgY.

Adherence Blokade

Inhibition of adherence in the cell surface seems to be the principal mechanism of IgY in preventing bacterial infection (Lee *et al.,* 2002; Jin *et al.,* 1998). IgY binds with the cell elements of bacteria such as pili or fimbriae, lipopolysaccharides and other membrane bound proteins that are responsible for adherence for the bacteria into host tissue (Xu *et al.,* 2011). Such binding not only reduce the adherence but also stop the cell signaling process responsible for release of toxins and other growth enhancing protein (Xu *et al.,* 2011).

Phagocytosis followed by opsonization

IgY reported to have increase the phagocytosis against pathogen. Improved phagocytosis of Staphylococcus aureus by neutrophils were observed after treatment with IgY (Nie *et al.,* 2004). Presence of IgY increase phagocytosis activity of E. coli by milk macrophages and polymorphonuclear neutrophil leukocytes (Zhen *et al.,* 2008). Binding of IgY in the surface of Salmonella typhimurium and E. Coli O111 make them susceptible to phagocytosis (Lee *et al.,* 2002).

Neutralization

IgY prevent internalization of S. aureus by mammalian epithelial cell leads to neutralization of toxin. Wang *et al.,* (2011) suggested possible treatment of mastitis may only be possible by blocking of internalization of S. aureus or by neutralizing the toxin rather than focusing on growth inhibition by IgY.

Application of IgY in Fisheries and Aquaculture

Application in disease control

One of the major threats for the growth of aquaculture industry is outbreak of diseases.

Intensification of the culture systems makes the sector more vulnerable to diseases. Resistance to most of the antibiotics drives the scientific community to find an alternative solution to control and manage diseases. Passive immunity is a method to transfer specific antibody against a bacteria into the healthy fishes even before the occurrence of disease is an ideal technique for treatment of fish and other aquatic animals.

Treatment with antibody is getting importance in the aquaculture especially due to its non-harmful nature and also there is no threat of bacteria developing resistance. IgY due to is high production potential and easy extraction methods make itself the ideal antibody to carry out such treatment. Several scientists have tried to use IgY for both, providing passive immunity to fishes and also therapeutics treatment and found surprising results. Applications of IgY in fisheries are summarized in Table 1.

WSSV in shrimp causes severe economic loss over the years. Passive immunization of shrimp against WSSV by producing antiviral antibody against VP28 and VP19 protein neutralize viral particle (Lu *et al.,* 2009). Two different methods have been tried to produce antiviral IgY. Chicken Injected with inactivated WSSV and also by introducing DNA vaccine encoding specific gene. However, the result shows higher affinity by IgY for WSSV when obtained through inactivated WSSV than IgY obtain through DNA vaccine. Effectiveness of treatment studied in Crayfish (*Procambarus clarkii*) and it was found that intramuscular injection, oral and immersion all were effective (Lu *et al.,* 2009; Xu *et al.,* 2011). *Yersinia ruckeri* causes enteric red mouth disease in Rainbow trout. These microbes get released by carrier fish with faeces and persist for longer period. Oral administration of anti *Y. ruckeri* antibody followed by immersion in water containing bacteria results in lower mortality (Lee *et al.,* 2000). Oral treatment also leads to lower count of this bacteria into guts. *Aeromonas salmonicida* causes serious ulcer in Koi carp, erosion of skin and subsequent exposure of muscle. *A. salmonicida* specific IgY antibody produced and purified. Purified antibody injected into Koi carp, immunised fishes with anti *A. salmonicida* antibody is then immersed in water containing *A. salmonicida*. Immunised fishes show significant protection (Gan *et al.,* 2015). Li *et al.,* (2006) inhibited the growth of *Aeromonas hydrophila* in *Carassius auratus* gibelio using anti *A. hydrophila* antibody. Formalin killed bacteria used to immunise hen. ELISA test confirms peak antibody production after 56 days. 60% fishes show survivability after challenged with *Aeromonas hydrophila*. It was also observed complete inactivation of bacteria at 1 mg/ml concentration of specific Ab in bacterial culture. Another study of *Vibrio anguillarum* in Japanese Ayu (*Plecoglossus altivelis*) shows a survivability of 64% and 55% incase of passive immunization and therapeutics treatment by specific IgY Ab respectively (Li *et al.,* 2014). Besides that Oral administration of specific antibody could leads to increase in the phagocytosis activity of macrophages, decreases expression of groups of cytokines includes TGF-**β**, TNF-***α*** and IL-**β** (Li *et al.,* 2014; Zorriehzahra *et al.,* 2016). Another treatment of *Vibrio anguillarum* in half smooth tongue sole (*C. semilaevis*) with encapsulated specific anti-IgY shows 70% survivability when challenged with bacteria (Gao *et al.,* 2016). Encapsulation were done to prevent enzymatic inactivation of antibody in intestinal gut. *Edwardsiella tarda* causes infection through intestinal mucosa in Japanese eel. Oral treatment with specific IgY shows good survivability with no disease syndromes. Similarly, abalone when treated with encapsulated specific IgY shows 65-70% survivability (Hatta *et al.,* 1993; Hatta *et al.,* 1994).

Table.1 Application of IgY in fisheries and aquaculture

Application in Disease Control				
Disease	**Pathogen**	**Target species**	**Effects of IgY**	**Reference**
White spot syndrome	White spot syndrome virus	Shrimp (*P. monodon)* Crayfish (*Procambarus clarkii*)	Passive immunization of shrimp against WSSV infection Provide protection to Crayfish from WSSV	Lu *et al.*, 2009 Xu *et al.*, 2011
Vibriosis	*V. alginolyticus*	Abalone (*Haliotis* Sp.)	Increase resistance against vibriosis, Reduced mortality rate	Lee *et al.*, 2000
Furunculosis	*A. salmonicida*	Koi carp	Immunized fishes show significant protection against *A. salmonicida*infection	Gan *et al.*, 2015
Enteric red mouth disease **Aerominasis**	*Yersinia ruckeri* *A. hydrophila*	Rainbow trout *Carassius auratus* gibelio	Oral administration of IgY results in reduced mortality rate Increased solvability by 60%	Lee *et al.*, 2000 Li *et al.*, 2006
Edwardsiellosis	*E. tarda*	Japanese eel Abalone	Oral treatment shows good survivability, Prevented paracolo disease Encapsulated specific IgY shows 65-70% survivability	Hatta *et al.*, 1993 Hatta *et al.*, 1994
Vibriosis	*Vibrio anguillarum*	Half smooth tongue sole (*C. semilaevis*)	Encapsulated specific anti-IgY shows 70% survivability when challenged with bacteria.	Gao *et al.*, 2016
Application in Detection and Control of food borne disease				
Food borne illness/Toxin	***Causative Agent***	**Method to detect/Control**	**Effect of IgY**	**Reference**
Ciguatoxin	*Gambierdiscus toxicus*	Sandwich ELISA	Reliable results in detection	Campora *et al.*, 2008
Specific Spoilage Bacteria	*Shewanella putrefaciens, Pseudomonas fluorescens*	Addition of IgY as preservative	Increase shelf life of fish product in cold storage	Xu *et al.*, 2012
Meningitis	*Listeria monocytogenes*	Addition of IgY as preservative	Inhibits growth in smoked and fresh salmon flesh	Sui *et al.*, 2011

Application in detection and prevention of seafood contamination

A major sea food borne illness caused by Ciguatoxin in human after consumption of reef-based fishes. This toxin is released by *Gambierdiscus toxicus*, a dinoflagellate accumulates in the skin, head, muscle and viscera of fishes which cannot be destroyed by cooking at higher temperature. IgY based sandwich ELISA developed to detect Ciguatoxin gives very reliable result (Campora *et al.,* 2008). Thus, IgY based immunodiagnostic techniques provide a cheap and more reliable platform in the field of detection of seafood contamination and immunodiagnostics kits can also be developed.

Specific spoilage organism (SSO) like *Shewanella putrefaciens*, *Pseudomonas fluorescens* responsible for spoilage of fishery product in aerobic cold storage. Use of SSO binding IgY antibody significantly prevent the microbial activity and thus significantly increase the shelf life of fishery product (Xu *et al.,* 2012). *Listeria monocytogenes*, a bacterium responsible for food borne illness in human causes severe infection in the central nervous system. Contamination of seafood with *L. monocytogenes* has led to product recall. Therefore, bacteria possess serious threat to the seafood industry. Sui *et al., (*2011) inhibited the growth of bacteria in the smoked and fresh salmon flesh with specific antibody.

Limitation

One of the major limitations of IgY is acidic inactivation of IgY at lower pH. When administered orally for passive immunotherapy, IgY reaches to the small intestine, which is the main target site. Upon reaching the small intestine IgY become very susceptible to proteolytic degradation and inactivation due to acidic environment. IgY is fairly resistant to proteolytic degradation (Hatta *et al.,* 1993; Shimizu *et al.,* 1988). But activity tends to decrease at pH- 3.5 and it completely lost its activity at pH-3.0. Microencapsulation of antibody can be used as an effective method to prevent acidic inactivation and results showed a good portion of antibody is protected which resulted in better survival of fishes (Lee *et al.,* 2000; Chang *et al.,* 2002; Cho *et al.,* 2005; Li *et al.,* 2007; Wu *et al.,* 2011). However, such measures will add additional amount of financial burden. Application of IgY as food preservative tend to decrease the microbial load for initial days but after few days the microbial concentration tend to increase which proves IgY is not fully effective food preservative. Further research is required in this area to use IgY a food preservative for longer time.

In the decade of increasing pressure of productivity, a sustainable remedial measure is the need of the hour. Many researchers have explored many alternatives but at present we can pompously say that IgY poses the great potential to stand high to solve all the issues in this scenario of crisis. The increasing resistance to antibiotics already leads to loss of crops and their residual effects also seem to impact the humans and other animals. Use of several chemicals also did not give encouraging results in addition to that it poses severe environmental coercions. In this scenario of high need for a long term, economical and effective control measure egg derived IgY will definitely boast the aquaculture production in coming future with its multidimensional advantages. The avian egg can accumulate a large quantity of antibodies which is highly effective as immunotherapeutic. It has been found that at a very short period of time high quality and quantity of antibodies can be obtained which is not possible by any other means. There are

many encouraging results showing the effectiveness of IgY in fish against some devastating pathogens. There are many advantages of IgY which will attract the pharmaceutical companies to formulate monospecific or multifunctional antibodies. By using IgY we can reduce the cost of production many folds as its production cost is less and effectivity against pathogen is very high. There is no doubt about its effectiveness, at this point of time we need some more scientific evidence before it comes to the commercial market by overcoming some of its limitations. The future of fish and fisheries health management will be highly dependent on the success of avian egg derived IgY.

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