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Egg Yolk Antibodies: Production and applications in the diagnosis and treatment of animal diseases - A review

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Abstract

Antibodies are extensively used in molecular biology, protein detection, cell isolation, immunopathology as well as in the diagnosis and treatment of various diseases in humans and animals. Rabbit and mice are the common mammalian species used for the production of both polyclonal and monoclonal antibodies. However, there are many advantages of raising antibodies in poultry. Chicken are phylogenetically distant from mammals and reacts strongly against highly conserved mammalian antigens. Also, chicken has a natural tendency to concentrate immunoglobulins in higher concentrations in egg yolk which makes subsequent collection and separation of antibodies easier by non-invasive methods than the separation from serum as in rabbits or mice. Moreover, by laying eggs each day a hen can act as a small antibody production unit. Eggs or egg yolk can be included as dietary components and egg yolk antibodies (EYA) have minimal toxic or side effects. At the same time, chicken immunoglobulin (IgY) can resist almost all gastric barriers in food animals, help in reducing gastrointestinal pathogen attachment and colonization, and improve digestion, thus can act as an excellent feed additive. The EYA can also be produced against various bacterial, fungal, or other immunogenic toxins and snake venoms. This review presents an overview of the production and purification techniques of EYA, its challenges and the applications in the diagnosis and treatment of animal diseases along with the conjectures on the future of IgY technology.

1. Introduction

The discovery of chemotherapeutic agents during the first half of the twentieth century was a milestone in the prophylaxis and treatment of many deadly infectious diseases. However, a variety of bacterial species and many other pathogens like plasmodium, mycoplasmas *etc.* are increasingly becoming resistant to a variety of such agents. The emergence of multiple drug resistance (MDR) has been observed in many bacterial species due to selective pressure created by their indiscriminate use both at prophylactic and therapeutic levels (Begum et al. 2021). Also, applications of chemotherapeutic agents in food animals have been found to cause the accumulation of drug residues in animal byproducts. Concurrent with the increasing antibiotic-resistant bacterial types, the emergence of new and novel pathogens as well as changing climatic conditions and ecological niches of pathogens there are ever-growing challenges to managing such unpredictable health issues. Many novel therapeutics like bacteriophage therapy, gene therapy, stem cell therapy, intravenous and oral immunoglobulin therapy, use of prebiotics and probiotics, plant-based edible vaccines, oncolytic viruses *etc.* are being explored for such challenges (Dhama et al. 2011; Tiwari et al. 2011; Lee et al. 2009a). Among these approaches, oral immunoglobulin therapy is a very easy, acceptable and highly desirable method. Oral immunoglobulin prevents the entry of the infectious agent into the host as most of the infections have the oro-faecal route. This therapy may neutralize the toxins or viruses before their attachment or entry into the susceptible cell types/ tissues in the body. Therefore, the use of immunoglobulin therapies has the added advantage that they are effective in the prophylaxis of many viral diseases by causing their agglutination than antiviral therapies which are less developed due to the intracellular nature of viruses.

For a long time and after the invention of the monoclonal antibody technique animals are exploited for the production of polyclonal and monoclonal antibodies for therapeutic and diagnostic purposes. Both ways of antibody production, however, raise serious animal welfare concerns and thus most recent endeavours in biological sciences are focused on minimum use of animals in experimentation. Interestingly ingenious and novel methods are nowadays being explored to produce antibodies in transgenic plants and animal hosts like silkworm larvae and lactating animals. Very recently, the use of transgenic chickens producing antibodies in the egg ovalbumin is a particularly attractive technique where laying hens act as bioreactors in a similar way as transgenic lactating cows (Lillico et al. 2005). Production of antibodies using transgenic hens or after hyperimmunization with specific pathogenic antigen has the added advantage that the egg yolk and white are the ideal and sterile bio-receptacle system. The downstream processing technology for fractionating the various proteins from egg yolk is available. Modern intensive mechanization and highly automated systems can make the collection and processing of thousands of eggs per day possible in a highly efficient and cost-effective manner. Available literature suggests that specific EYA have the promising potential not only in prevention and treatment but also in the detection and diagnosis of various bacterial, viral, and other pathogenic diseases of humans, animals, poultry, aquatic animals, and other species (Xu et al. 2011).

2. Characteristics and advantages of EYA

There are three different classes of immunoglobulin; IgM, IgY, and IgA are present in chicken (Karlsson et al. 2004). The IgM is the primary immunoglobulin produced against invading foreign antigens while IgA is present in mucosal secretions and both these immunoglobulin classes are present in less concentrations. On the other hand, IgY represents the main immunoglobulin class in chicken serum and is homologous to mammalian IgG. It differs structurally from classical mammalian IgG, however, resembles in function to both mammalian IgG and IgE antibodies (Alexander et al. 2009). The chicken egg also contains all these antibody types but the type and concentration of antibodies vary with the egg component. The IgY secreted from B cells are transferred from serum into the egg at the time of ova formation in the ovary of the hen and the main class of antibodies in egg yolk (Hamal et al. 2006). The other types of IgM and IgA are secreted with other secretory components of egg white during the egg formation in the oviduct (Hincke et al. 2019). The purpose of immunoglobulin transfer into eggs and thus future offspring is to provide protection (passive humoral immunity) against various infectious diseases during immunologically immature status (Hatta et al. 1997; da Silva and Tambourgi 2010).

There are many advantages of using chicken for the production of polyclonal antibodies over the mammalian production systems. Chicken are phylogenetically distant from mammals which makes the production of antibodies against mammalian epitopes more successful in chicken than in other mammals (Gassmann et al. 1990). Additionally, chicken antibodies tend to react and detect similar protein antigens in several mammalian species which makes them more widely useful and applicable in diagnosis and therapy. Also, the most important property is that IgY does not activate mammalian complement (Carlander et al. 2000). These properties bring great advantages to the application of IgY in their applications to xenotransplantation, diagnostics and antibiotic-alternative therapy (Fryer et al. 1999; Carlander et al. 2000). Apart from these facts, laying hens act as efficient producers of antibodies through their eggs. A single hen can lay 280-300 eggs in a year with each egg of yolk volume of about 15-17 ml and the total amount of IgY present in egg yolk is about 20-25 mg/ml (Rose and Orlans 1981), of which 2 to 10 % are specific antibodies against a particular antigen (Tini et al. 2002; Schade et al. 2005). Such a huge egg number can supply over 100 g hen/ year of antibodies of a particular type. The collection of antibodies from egg yolk has the added advantage of being a non-invasive method compared to the collection of IgG from mammalian blood/ serum. It is also easy and economically viable to rear a several laying chicken than mammals such as guinea pigs, rats or rabbits (Schade et al. 2005). Thus in comparison to mammalian serum for the collection of specific antibodies, egg yolk can act as a rich, convenient, easy, economically cheap and hygienically sterile source of antibodies (Table 1).

There are many more characteristics of IgY which make it more efficient and advantageous than mammalian IgG. It is not surprising that due to the high body temperature of chickens, 41°C, the avian immunoglobulins are more resistant to temperature fluctuations and thus retain their biological activity for several months at room temperature. It has been found that thermal denaturation and loss of antigen-binding capacity of IgY occurs at temperatures higher than 75°C (Chang et al. 1999). Due to such characteristics, IgY is more resilient and can withstand repeated cycles of freezing/thawing compared to mammalian immunoglobulins without affecting its biological activity for up to six months at room temperature. Moreover, chicken IgY can withstand the denaturing stomach environment and can tolerate a wide pH range of about 4-11. IgY are also more tolerant to several gastrointestinal enzymes like trypsin and pepsin and can resist its degradation and retain its activity in acidic environments. All these features make IgY fit for oral administration and its use to prevent many gut microbial infections. At the same time, the use of IgY has the least effects on the environment with no toxic and residual effects as compared to chemotherapeutics agents (Coleman 1999).

3. Production and purification of EYA

Antigen-specific EYA are produced after repeated administrations or hyper-immunizations of a specific purified

Table 1 Comparison between rabbit (mammalian) IgG and chicken IgY (EYA)

Remarks	Rabbit (mammalian) IgG	Chicken IgY
Minimum Number of animals required	one	one
Source of antibody collection	Serum	Egg yolk
Kind of antibody produced	Polyclonal	Polyclonal
Sampling Method	Invasive (Blood collection 15 to 20 mL/ week)	Non-invasive (Daily egg Collection)]
Steps inducing physical suffering to the antibody-producing animal	Antigen inoculation, blood collections, animal restrain	Antigen inoculation
Sample volume collected in one week	20 ml of blood	15-20ml of yolk /egg X 7 eggs = 100to 140 ml
Antibody amount per week	200 mg/bleed	up to 2500 mg
Antibody produced against mammalian antigens	Generally low	Usually high
Quantity of antibody (per year)	1300 to 1500 mg	up to 127 g
Presence of other Ig	IgM, IgA, IgE	None
Day of Sustained antibody production	After 60th day	After 30th day
Antibody source collection	Complexes and expensive several steps	Simple and inexpensive few steps
Protein–A/G binding capacity	Yes	No
Chances of contamination with IgA or IgM during preparation/ separation	Yes	No
Cross reactivity with proteins from mammals	More	Less

antigen or a microbe of interest usually through intramuscular or subcutaneous inoculation route into the chicken (Sun et al. 2001). However, many factors affect the quality as well as quantity of antibodies produced in the egg yolk. In general, the type of microbial antigen, dose and frequency of administration, route of inoculation, adjuvant types used and genetic differences among chicken lines considerably alter antibody production in the egg yolk (Levesque et al. 2007). The intramuscular route is the most common and economical route for hyper immunization and it results in the generation of higher levels of specific IgY (Woolley and London 1995, Chang et al. 1999). These immunized chickens can continuously produce particular antibodies for more than six months. Many modifications can be done in the antigen itself or in the process of inoculations through genetic manipulations like recombinant DNA technology, chimaera proteins or reverse vaccinology. DNA vaccines encoding microbial antigens of interest are also used for IgY production and have the advantage of eliminating the tedious and costly purifying processes and stability (Cova 2005).

Although the generation of a specific antibody type and its collection *via* egg yolk is easy, the downstream purification and separation of IgY from the yolk lipoproteins involves several tedious steps (Hatta et al. 2008). Several methods have been used by various workers with varying results on the final EYA outcomes in terms of yields and purities. In general, the first step involves the separation of specific antibodies (IgY) along with soluble proteins from the lipid portion of egg yolk either by the water dilution (Verdoliva et al. 2000) or freeze-thaw cycle and centrifugation method (Devi et al. 2002) or polyethylene glycol lipid precipitation (Sarah et al. 2003). In the next step separation of specific antibodies from soluble proteins is done by affinity chromatography using a synthetic ligand (TG19318) (Verdoliva et al. 2000). Further isolation and purification of immunoglobulins from chicken eggs can also be done by a single thiophilic interaction (Hansen et al. 1998) or ion exchange chromatographic step after ammonium sulfate fractionation (Ko and Ahn 2007) or a two-step procedure using hydrophobic interaction chromatography and gel filtration. Ultrafiltration- diafiltration method is the new method wherein two different membranes are used for the purification of egg yolk IgY (Liu et al. 2010). Ultrafiltration-diafiltration method is based on ionic concentration and pH of the solution using polyethersulfone (PES) and modified PES (MPES) (Hernndez-Campos et al. 2010). Final characterization and quantification of egg yolk specific antibodies (IgY) are done by various immunochemical methods like agglutination, precipitation, immune-diffusion, immunofluorescence, ELISA for particulate and soluble antigens and neutralization or blocking methods/ assays for toxins, enzymes, hormones or viruses.

4. Antimicrobial mechanisms of Action

The exact mechanism by which IgY counteracts virulent pathogens cannot be attributed to a single specific mechanism of action. Several mechanisms may be involved by which IgY

inhibits or neutralizes the virulence effects of a pathogen. One of the major pathways by which specific antibodies act on a particular pathogen is by their physical interaction with surface antigens of a pathogen. Such reactions lead to pathogen clumping or their agglutination thereby inhibiting their attachment with their receptors. These mechanisms are mainly found in the inhibition of Gram-negative bacterial growth or their colonization of the gut. Intestinal bacteria use many surface components for intestinal colonization like outer membrane proteins, LPS, flagella or fimbriae and their blocking by specific IgY antibodies leading to a reduction in CFU and pathogenic effects (Tsubokura et al. 1997). Chicken IgY has been shown to prevent the adherence of E. coli K88 to the intestinal mucus in piglets (Jin et al. 1998). Moreover, many studies suggested that not only particular pathogenic bacteria are neutralized by a specific IgY but a large number of related pathogens can also be blocked from causing the disease or pathogenic effects in the host (Xu et al. 2011). It is also possible that specific antibodies can block the adherence the viruses to their respective receptors at the site of their entry into their host like on mucosal surfaces. There are many other possible mechanisms for the specific IgY that may be preventi-

Table 2: Use of IgY against various pathogens

Pathogen	Outcome of pathogen	IgY antibody effect	References
Pseudomonas aeruginosa	Cystic fibrosis	Minimized the use of antibiotics against *Pseudomonas* causing cystic fibrosis	(Nilsson et al. 2008)
Helicobacter pylori	Gastritis, ulcers	interference in bacterial adhesion, growth and pathogenic activity of enzymes like urease. Hence bacterial load reduced and prevents from gastritis	(Attallah et al. 2009)
Porphyromonas gingivalis	Periodentitis	Reduced bacterial adherence and hydrolase activity	(Yokoyama et al. 2007)
Streptococcus mutans	Dental caries	Biofilm formation reduced	(Smith et al. 2001)
E. coli	Intoxication	Reduced bacterial adherence	(Wang et al. 2010)
Candida albicans	Opportunistic infections	Reduced growth in mice model	(Fujibayashi et al. 2009)
Clostridium botulinum neurotoxins type A and B	Food intoxication	Block the toxins functional activity.	(Pauly et al. 2009)
S. aureus	Intoxication	Inhibit toxin production	(LeClaire et al. 2002)
Salmonella Enteritidis and *Salmonella* Typhimurium	Food poisoning	Colonization prevented	(Rahimi et al. 2007)
Snake Venome	Venomation	Toxin neutralization	(Liu et al. 2010)
Rabies virus	Rabies	Bind virions and cells infected causing neutralization	(Motoi et al. 2005)
Enterotoxigenic *Escherichia coli*	Diarrhoea and death in calves	Survival of calves, reduction in diarrhoea	(DiLorenzo et al. 2008)
Enterotoxigenic *Escherichia coli* K88	Diarrhoea in piglets	inhibit the growth of *E. coli* K88, prevent bacterial colonization, reduction in diarrhoea	(Wang et al. 2019)
Bovine rotavirus	Neonatal diarrhea	Reduced diarrhea and death	(Vega et al. 2011, Vega et al. 2015)
E. coli (O111) and *S. aureus*	Mastitis in cattle	Inhibition of bacterial entry and multiplication	(Wang et al. 2011)
Streptococcus bovis and Fusobacterium necrophorum	Gut microbiota and faecal shedding of pathogens	Growth promoter effect	(DiLorenzo et al. 2006, 2008)
Enterotoxigenic Escherichia coli	Diarrhoea	Antidiarrheal effects	(Li et al. 2009)
Porcine epidemic diarrhea virus (PEDV)	Diarrhoea	Prevents piglets against diarrhoea	(Kweon et al. 2000)
Salmonella Enteritidis (SE) and *Salmonella* Typhimurium	Diarrhoea	Prevent colonization and prevent fecal shedding	(Rahimi et al. 2007)
E. coli O78:K80	Gut health and immunocompetence	Gut health improvement. Good feed utilization	(Mahdavi et al. 2010)
Influenza virus	Avian influenza	Protects mice against H1N1, H5N1 and H5N2 challenges	(Nguyen et al. 2010)
Infectious bursal disease virus	IBD	Protects chicken against infection	(Yousif et al. 2006)
Eimeria spp	Coccidiosis	Protects chicken against infection	(Lee et al. 2009b Juárez-Estrada et al. 2021)
Canine parvovirus-2	Parvoviral diarrhoea	Protection against diarrhea	(Van Nguyen et al. 2006)
Gallibacterium anatis	Peritonitis and salpingitis in chickens	lower lesion scores for the peritoneum, liver, and duodenum; improve the protective response	(Zhang et al. 2019)

-ing bacterial infections by altering cellular signalling pathways thereby leading to a decrease in pathogenesis or toxin production (Table 2).

Egg yolk antibodies also play a major role in the virus and toxin neutralization thereby preventing the pathology of the disease. There are several examples by which EYAs have been found to act by mechanism. Neutralization by specific EYA may be the major mechanism responsible for preventing viral diseases like bovine coronavirus and bovine rotavirus diarrhoea in food animals (Kuroki et al. 1994; Ikemori et al. 1997). Diseases in poultry like infectious bronchitis or inclusion body hepatitis or Newcastle disease and the toxin neutralization of cobra venom or other venoms can be controlled by this particular mechanism (Pereira et al. 2019; Nguyen et al. 2020). Not all pathogens can be blocked by antibody binding. The other process by which particulate antigens are rendered more susceptible to phagocytosis is called opsonization. Antibody many times functions as an opsonin thereby enhancing phagocytosis of pathogenic microbes. It has been found that IgY improves the phagocytosis of *Staphylococcus aureus* by neutrophils as well as milk macrophages and neutrophils show enhanced phagocytic activity against the *E coli* possibly by enhanced opsonin mechanism (Nie et al. 2004; Zhen et al. 2008).

5. EYA in the control of animal diseases

The control of infectious diseases in livestock is of utmost importance as it is directly related to the economy and human health. Most of the diseases in livestock are transmitted through the oro-feacal route or involve the respiratory system. Oral admiration of EYA through eggs without purification is an easy and convenient method to prevent the entry of the pathogen into the systemic circulation. Previously many studies were carried out in this direction and had shown promising results in controlling infections both *in vitro* and *in vivo* experiments. It was found that specific IgY antibodies against *O111 and* K99-piliated entero-toxigenic *Escherichia coli* (ETEC) strains increased the phagocytic activity of milk macrophages and polymorphonuclear cells *in vitro* (Zhen et al. 2008), and protected the neonatal calves against ETEC-induced diarrhoea (Ikemori et al. 1992). Likewise, specific egg yolk powder from immunized hens was found to control bovine coronavirus and rotavirus induced calf diarrhoea (Ikemori et al. 1997; Ozpinar et al. 1996). Moreover, in the era of increasing drug resistance EYA have shown better results than penicillin in clinical and experimental mastitis when injected via the intra-mammary route (Zhen et al. 2008, 2009). Specific hen EYA therapy has also proven to protect the newborn piglets from porcine transmissible gastroenteritis virus (TGEV) (Fan et al. 2009; Zuo et al. 2009), fatal enteric colibacillosis (Yokoyama et al. 1992; Ronald et al. 1999), post-weaning diarrhoea and oedema disease (Imberechts et al. 1997). Similarly, in feedlot animal husbandry practices EYA are used to improve health and body weight gain by preventing infections at prophylactic levels. The use of a specific immunoglobulin-rich egg yolk powder in weaning piglets has been shown to improve their body weight (Vila et al. 2010). Also, specific EYA against pig adipose tissue plasma membranes resulted in improved growth and carcass composition of pigs, and changes in serum insulin and leptin levels (Jianga et al. 2007).

6. EYA in the control of poultry diseases

Among animal food production systems poultry industry has gained utmost importance as it can provide sufficient quality animal protein to ever-growing human populations. However, intensive poultry production systems with compelling cost-benefit ratios and the selection of prolific genetic lines are posting intense pressure on the immune system of birds here by making them highly susceptible to many infections. To counter such infections indiscriminate prophylactic antibiotics are being used during the early stages of commercial chick-rearing which has led to the emergence of new antibiotic-resistant bacteria. Researchers in the poultry industry are looking for viable alternatives including the use of specific egg yolk antibody therapy. Studies have shown that specific egg yolk antibodies protect against poultry pathogens like *Salmonella enteritidis* and *Salmonella typhimurium* both *in vivo* (mice) and *in vitro* (Yokoyama et al. 1998). Likewise, the cross-reactivity of anti-salmonella EYA protects the food animals against the colonization of common pathogenic Salmonella strains (Biswas et al. 2010). Similarly, *in vitro* studies have shown inhibitory effects against *E. coli* O157:H7 (Sunwoo et al. 2002) and *E. coli* O78:K80 (Mahdavi et al. 2010) by specific EYA. Not only against bacterial pathogens specific EYAs are effective against parasitic and viral diseases as well. It has been found that IgY conferred protection against coccidiosis caused by *Eimerai tenella* or *E. maxima* infections (Lee et al. 2009b), Infectious Bursal Disease (IBD) and New Castle Disease Virus (NDV). Specific egg yolk antibodies have protected the birds against Avian Influenza subtype H9N2 and thus eliminating the source of human infections (Shaban et al. 2007). Moreover, IgY has been found to reduce the food born zoonosis like anti-*Campylobacter jejuni*. Oral EYA prophylactic administration has been found to significantly reduce the *C. jejuni* faecal count in poultry (Tsubokura et al. 1997).

7. Diagnostic applications

The use of IgY in immunodiagnostic procedures is gaining importance day by day and research in this field is still unabated. Due to phylogenetic distance chicken immune system reacts more strongly against highly conserved mammalian antigens than the mammalian immune system. This helps in the improvement of assay sensitivity, accuracy and performance with less interference and signal to noise ratio (Greunke et al. 2008). Specific EYA are used in various formats depending upon the convenience and resources available like in immunochromatographic assay (Wilmar and

Tambourgi 2010; Xiulan et al. 2005; Kaur et al. 2007), rocket immunoelectrophoresis, quantitative microcomplement fixation tests (van Regenmortel and Burckard 1985) *etc.* for the qualitative and quantitative measurements. Moreover, in assays like quantification of cancer biomarkers (Xiao et al. 2010), detection of Indian cobra (*Naja najanaja*) venom in the biological samples of forensic laboratories (Brunda et al. 2006), detection of cathepsin D in immunogold labelling of human tissues (Fortgens et al. 1997), surveillance of avian influenza (Jeong et al. 2010), cartilage glycoprotein (gp-39) in guinea pig serum (de Ceuninck et al. 2001), detection of LPS of bacteria and protein antigens of *Campylobacter jejuni* and *E. coli* by SDS-PAGE (Chandan et al. 1994) IgY are used. Therefore, IgY could supersede mammalian IgG in immune-biotechnological applications in future.

8. Limitations of IgY technology

The stability of IgY at acidic pH limits its use for oral passive immunotherapy as IgY completely loses its activity at pH less than 3.0 (Shimizu et al. 1998; Hatta et al. 1993). IgY also undergoes proteolysis in the small intestine, the primary site for protein digestion. However, microencapsulation is one of the effective methods to overcome this problem but the process will add additional costs (Chang et al. 2002; Cho et al. 2005; Li et al. 2009). The affinity of IgY to mucosal receptor exploited by E. coli K88+MB is lower than the organism itself (Jin et al. 1998) and the absorption of IgY into circulation decreases with the increase in the age of the animal (Yokoyama et al. 1993). These antibodies are unable to activate the classical pathway of the complement system unlike mammalian IgG and therefore, IgY are less effective in killing bacteria directly. There are limited reports regarding the application of IgY against Gram-negative bacteria (Zhen et al. 2009; Wang et al. 2011). In some cases, the main aim of IgY technology is the reduction of infectious agents rather than their complete elimination or treatment of the disease (Kuroki et al. 1994). This could result in the survival of the potential pathogens in the environment. As the production cost of high-quality IgY antibodies is higher than the cost of routine antibiotics, the development of methods for large scale production with high purity of IgY is needed (Casadevall and Scharff 1994). Storage stability, viscosity and passage rate in the intestinal tract, and dietary composition are the major hurdles in commercial applications (Malik et al. 2006). However, protease-resistant IgY may increase the fraction of immune-reactive antibodies delivered orally (Reilly et al. 1997). Also, the EYA technology may take a long time to get approval from regulatory authorities.

9. Conclusion and future perspectives

The immunization of laying hens with specific antigen for the production of EYA is one of the cost-efficient methods for the production of large quantities of specific antibodies. The specific EYA have a promising potential in controlling entero-pathogenic diseases of bacterial and viral origin as eggs can be fed directly as the feed component. The EYA technology has several other advantages which include: (a) treatment is safe, environment-friendly and live organisms are not used; (b) reduction in animal welfare concerns since an egg is used as an antibody source; (c) the technique is non-invasive and showing less cross-reactivity with mammalian proteins; (d) No toxic residues are produced and there is no development of resistance and (e) isolation process is efficient and economical. Despite its general applicability, IgY technology has scarcely been used on a large scale. It seems therefore of great importance to publicize IgY technology and its inherent advantages. Further research is needed especially related to the problems like stability, applicability, and efficient delivery so that significant and sustainable beneficial impact on production performance, and safety for poultry, animal, and human health can be achieved. Techniques like lyophilisation to overcome storage problems and the encapsulation in chitosan-alginate microcapsules to improve stability under gastric conditions are to be addressed. It is expected that in future, EYA technology will play an increasing role in research, diagnostics and immunotherapy.

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