

*Full Length Research Paper*

# The potential application of avian egg antibodies with emphasis on immunotherapeutic and immunodiagnostic purpose

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Avian eggs present an ideal alternative antibody source to mammals as the IgY in the chicken's blood is transported to the egg and accumulates in the egg yolk in large quantities. The existence of an IgG-like molecule in avian eggs, referred to as IgY, has been well documented, and extensive research has been carried out on its characterization, production and purification. Although it is the functional equivalent of mammalian IgG, the major serum antibody found in mammals, IgY is structurally different, and has been found to exhibit several important differences when compared to mammalian antibodies, including its physicochemical properties and immunological capabilities. Recently, considerable research has focus sedon the use of IgY as an alternative to mammalian antibodies for several applications, including for immunotherapeutic applications, especially for the oral passive immunization against various bacteria and viruses. Much research has also been carried out on the use of IgY as a replacement for IgG in various immunodiagnostic and immunoaffinity purification purposes. The use of IgY offers several advantages over polyclonal antibodies produced in mammals, including providing a much more hygienic, cost efficient, convenient, humane and plentiful source of antigen-specific antibodies.

**Key words:** Avian, egg yolk antibody, immunodiagnostic, immunotherapeutic, IgY

## INTRODUCTION

The avian egg contains all the necessary nutrients and growth factors required for the developing embryo, including antibodies that are transported from the blood of the hen into the egg yolk to provide immunity to the chick (Yigani and Korver, 2010). The production of antibodies (Abs) in chickens and the extraction of specific Abs from egg yolk (IgY Abs) are increasingly attracting the interest of the scientific community as demonstrated by the significant growth of the IgY literature. Avian eggs present an ideal alternative antibody source to mammals as the IgY in the chicken's blood is transported to the egg and accumulates in the egg yolk in large quantities. Maternal antibody can be transferred from hens to the chicks either through the placenta, colostrum, milk, or egg (Grindstaff et al., 2003). Birds transmit maternal antibodies to their offspring by depositing the antibodies in the egg (Brambell, 1970). There are three classes of antibodies in chickens, namely IgY (IgG), IgA, and IgM. Chicken IgA and IgM

are similar to mammalian IgA and IgM in terms of molecular weight, structure, and immunoelectrophoretic mobility. In eggs, IgY is present predominantly in the egg yolk (Leslie and Clem, 1989) whereas IgA and IgM are present in the egg white as a result of mucosal secretion in the oviduct (Rose et al., 1994).

Hen eggs consist of approximately 9.5% egg shell (including shell membrane), 63% albumen, and 27.5% yolk. The main components are water (75%), proteins (12%), lipids (12%), as well as carbohydrates and minerals (1%) (Burley and Vadehra, 1989). The proteins are distributed throughout the egg with the majority found in the egg yolk and egg white and a small proportion in the egg shell and shell membrane (Watkins, 1995). The lipids are found almost exclusively in the egg yolk, mainly in the form of lipoproteins (Burley and Vadehra, 1989). Several minerals have also been found in eggs, most of them in the egg shell. Carbohydrates are a minor egg component, present

throughout the egg, both as free and conjugated forms, attached to proteins and lipids (Watkins, 1995).

Immunoglobulin Y (IgY) in avian egg has many applications in the medical and research fields, including in the areas such as diagnostics and proteomics. However, the most valuable and promising areas of IgY research is its use for passive immunization to treat and prevent human and animal diseases. Antibodies from eggs may have also many applications against microorganisms in humans and livestock or poultry (Gibbins, 1977). Serum antibodies of hyper-immunised hens are efficiently transferred and accumulated in the egg yolk (Fichtali et al., 1994). There are also efficient cation exchange chromatographic techniques for separating these antibodies from egg yolk. Coleman (1998) reported that antibodies from eggs can be effectively used to treat mastitis in dairy cows and may also have potential in treating HIV/AIDS.

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies (Tizard, 2002). The chicken immune system has been studied for many years, and these studies have contributed substantially to the understanding of the fundamental concepts of immunology and the development of different immunoglobulin classes (Carlander et al., 1999). Immunoglobulin Y (IgY) is the major antibody in birds, reptiles, and lungfish (Warr et al., 1995). In birds, the IgY is found mainly in blood and in the fluid fraction of the egg providing protection to newly hatched chick (Schade et al., 2005).

When animal welfare became more relevant in scientific studies, researchers began to seek alternatives to reduce the indiscriminate use of animals for research and diagnostic purposes. Although IgY and IgG are sometimes used as synonyms in the scientific literature, the term IgY has become universally accepted based on its unique features (Tizard, 2002). Although functionally similar, there are several important differences between mammalian IgG and avian IgY (Sharma, 1997), and the use of avian antibodies offers many advantages over mammalian antibodies. The production of specific IgY against many different antigens has been studied, and its application as an immunotherapeutic agent including its use for the oral passive immunization against enteric pathogens has been extensively reported. Due to its distinctness from IgG, IgY has also been found to be advantageous in several techniques as well as in immunoaffinity purification, in many cases replacing IgG. Recently, the chicken has attracted considerable attention as an alternative source of antibodies. IgY is deposited in the egg yolk in large quantities (Janson et al., 1995), and it can be easily purified from the yolk by simple precipitation techniques, making chickens an ideal source for specific polyclonal antibodies (Gassmann et al., 1990).

Antibody purification involves selective enrichment or specific isolation of antibodies from serum (polyclonal

antibodies), ascites fluid or cell culture supernatant of a hybridoma cell line (monoclonal antibodies). The need to develop effective, economical and rapid purification methods of monoclonal and polyclonal antibodies from a variety of biological fluids becomes imperative for *in vitro* or *in vivo* application. Antibody purification can be divided into two main groups: precipitation methods and chromatographic methods. Purification of immunoglobulin from mammalian blood is time-consuming and expensive. Today, hens are recognized as a convenient and inexpensive source of antibodies. It has been reported that the amount of immunoglobulin that can be yielded from one egg of an immunized hen is as much as that can be obtained from 300 ml of rabbit blood. Chicken egg yolk antibodies (IgY) have been applied successfully for scientific, diagnostic, prophylactic and therapeutic purposes. Because of the phylogenetic distance between birds and mammals, mammalian proteins are often more immunogenic in birds than in other mammals and antibody synthesis readily stimulated in hens (Bizhanov et al., 2004).

IgY and IgG egg yolk antibodies have been used in many diagnostic and biomarker discovery applications as a result of immunoreactivity difference. However, much research has focused on the use of IgY for passive immunization application. Passive immunization has recently become an even more attractive approach because of the emergence of new and drug resistant microorganisms and individuals with impaired immune system who are unable to respond to conventional vaccines. Passively administered antibodies have the ability to provide rapid and immediate protection; for example, against agents of bioterrorism (Casadevall et al., 2004). The reduction of antibiotics use in the livestock industry and increasing evidence that resistant organism may pass from animals to humans, resulting in infections that are harder to treat (Yegani and Korver, 2010).

Therefore, this paper aims:

- To review several aspects of avian immunoglobulins and the avian immune system, including the structure, production and purification of IgY,
- To outline many current and potential applications of IgY, especially in the areas of immunotherapy and immunodiagnostics.

## AVIAN EGG FORMATION

The hen's reproductive system is a very complex system that can produce an egg in 24 hours. The formation of an egg involves the conversion of the feed into egg constituents through a number of intricate and highly coordinated steps as a storehouse of nutrients. The formation of an egg occurs in the ovary and oviduct. Although two sets of ovaries and oviducts are present during embryonic development only the left set fully develop in chickens. When the chicken becomes mature

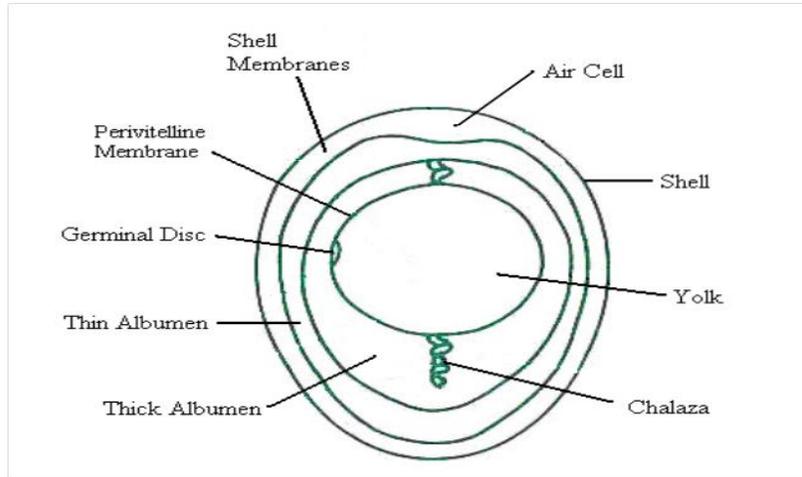


Figure 1. Formation of the hen's egg

Table 1: Comparisons of mammalian IgG and chicken IgY

Animals	mammals (IgG)	Chicken (IgY)
Source of antibodies	blood serum	egg yolk
Kind of antibodies	polyclonal	polyclonal
Antibody sampling	bleeding	collecting egg
Antibodies amount	200 mg/blood	100-150 mg/egg
Quantity of antibody	1400 mg	40,000mg

Source: Schade et al., 1991.

(about 150 days old), the ovary has grown to about 7 g and rapidly increases to about 40 g (around 170 days old) (Burley and Vadehra, 1989). The mature ovary will have several follicles in different development stages at any one time and the largest follicle is the one to be ovulated to produce an egg firstly. Yolk constituents are synthesized in the liver and they are transported to the follicular walls in the blood. The follicle undergoes a rapid development during which most of the yolk is deposited 6-10 days prior to ovulation, when sufficient yolk has accumulated. The follicle in the ovary is ovulated into the oviduct where the yolk is enveloped in albumen and the shell. It takes 24-27 hours for this development. In laying hens, the oviduct is 40-80 cm long with an average weight 40 g and consists of five regions, infundibulum, magnum, isthmus, uterus and vagina (Burley and Vadehra, 1989). The infundibulum is the top portion of the oviduct; with a broad funnel shaped anterior end (8-9 cm) and a narrow posterior end to receive the ovulated follicles (Burley and Vadehra, 1989). An egg consists of the yolk (30-33%), albumen (~60%), and shell (9-12%) (Figure 1).

The total solids content of egg yolk is generally around 50%, but can vary with the age of the hen and the storage of the shell eggs. The major constituents of the solid matter of yolk are proteins and lipids, present mainly in the form of lipoproteins (Li-Chan et al., 1995).

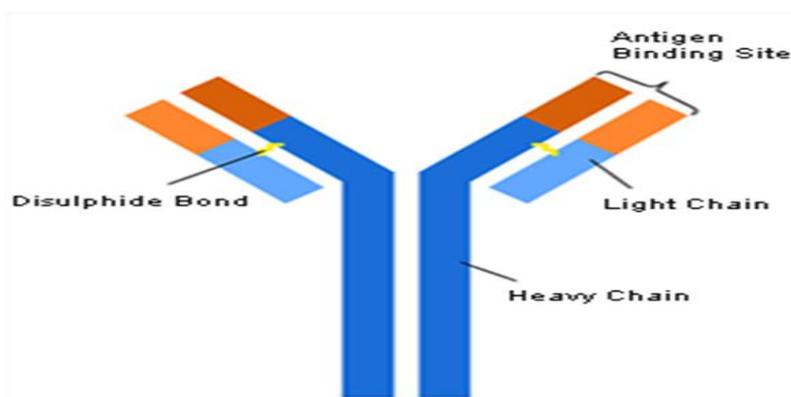
Their relative amounts can be seen in Table 1. The yolk can be separated by high speed centrifugation into sedimented granules and a clear fluid supernatant called plasma. Granules are composed of 70%  $\alpha$ - and  $\beta$ -lipovitellins, 60% phosvitin, and 12% low-density lipoprotein. The plasma is divided into the low-density lipoprotein fraction (33%) and the water soluble fraction (WSF) (5%), which contains the livetins, which are lipid-free globular proteins, including g-livetin, also referred to as IgY (Li-Chan et al., 1995).

## AVIAN EGG ANTIBODIES

### Avian immune system

The chicken immune system consists of the Bursa of Fabricius, bone marrow, spleen, thymus, the Harderian gland, lymph nodes, circulating lymphocytes, and various lymphoid tissues. The thymus serves as the primary lymphoid organ for T-cell differentiation while the antibody-synthesizing B-cells are produced in the Bursa of Fabricius (Carlander et al., 1999). The spleen is the centre for plasma cell proliferation and memory B-cells (Carlander et al., 1999).

Previously antibodies presently available for research, diagnostic and therapies were mostly mammalian



**Figure 2:** Structure of Antibodies

monoclonal or polyclonal antibodies, but now a day chicken egg yolk antibodies (IgY) have been applied successfully for scientific, diagnostic, prophylactic and therapeutic purposes (Bizhanov et al., 2004). Chicken IgY is highly concentrated in egg yolk than it is in serum. The chicken is an excellent producer of antibodies. Even though avian IgY has been applied, it is underuse according to some literature. This may be due to lack of information concerning the different methods and applications where IgY is more advantageous compared to the traditional mammalian IgG antibodies (Larsson et al., 1993). Avian antibodies contain both heavy (H) and light (L) chains that are encoded by two unlinked loci. In the light chain locus there are only single gene segments each for the V and J regions. The heavy chain has only one segment each for V and J regions, and about 15 D segments (Sharma, 1997). Therefore, rearrangement contributes little diversity in chicken B-cells, in contrast to mammals, because there are only single gene segments for the V and J regions. Only the D segments serve to introduce a combinatorial factor of diversity (Reynaud et al., 1989). Birds instead attain antibody diversity using sequences of pseudo genes (25 for the light chain and around 100 for the heavy chain) in a process of gene conversion in which segments of pseudogenes are inserted into the V-region (Sharma, 1997). In this way, despite the fact that chickens have an extremely limited number of immunoglobulin genes, compared to mammals, they are capable of producing a wide range of immune responses and diverse antibody molecules (Sharma, 1997).

### Biosynthesis

Three immunoglobulin classes have been shown to exist in chicken are: IgA, IgM, and IgY. The IgA and IgM are similar to mammalian IgA and IgM. Chicken IgY is the functional equivalent of IgG, the major serum antibody found in mammals, and makes up about 75% of the total antibody population (Carlander et al., 2000). In mammals, the transfer of maternal antibodies can take place after birth, however in the chicken; the maternal antibodies must be transferred to the developing embryo

to give acquired immunity to the chick (Sim et al., 2000). Antibody, specifically IgA and IgM, is secreted into the ripening egg follicle and is incorporated into the egg white in the oviduct along with the egg albumen secretion. Serum IgY is selectively transferred to the yolk via a receptor on the surface of the yolk membrane which is specific for IgY translocation (Morrison et al., 2002). Egg white contains IgA and IgM at concentrations of around 0.15 and 0.7 mg/ml, respectively, whereas the yolk may contain from 5 to 25mg/ml of IgY (Li et al., 1997). Mammalian equivalents of IgE and IgD have not been identified in chickens (Sharma, 1997).

### Structure of immunoglobulin Y

The structure of IgY is significantly different from that of mammalian IgG even though there is similarity in their function (Carlander et al., 1999). IgY contains two heavy (H) and two light (L) chains and has a molecular mass of 180 kDa, larger than that of mammalian IgG (159 kDa). IgY possesses a larger molecular weight H chain (68 kDa) as compared to that from mammals (50 kDa). The H chain of IgG consists of four domains: the variable domain (VH) and three constant domains (C<sub>V</sub>1, C<sub>V</sub>2 and C<sub>V</sub>3). The C<sub>V</sub>1 domain is separated from C<sub>V</sub>2 by a hinge region, which gives considerable flexibility to the Fab fragments. In contrast, the H chain of IgY does not have a hinge region, and possesses four constant domains (C<sub>V</sub>1- C<sub>V</sub>4) in addition to the variable domain. Sequence comparisons between IgG and IgY have shown that the C<sub>V</sub>2 and C<sub>V</sub>3 domains of IgG are closely related to the C<sub>V</sub>3 and C<sub>V</sub>4 domains, respectively, of IgY, while the equivalent of the C<sub>V</sub>2 domain is absent in the IgG chain, having been replaced by the hinge region (Warr et al., 1995). The content of  $\beta$ -sheet structure in the constant domains of IgY has been reported to be lower than that of IgG, and the flexibility between the C<sub>V</sub>1 and C<sub>V</sub>2 domains, corresponding to the hinge region of IgG, is less than that of IgG (Shimizu et al., 1992). Unlike IgG, IgY has two additional Cys residues, Cys 331 and Cys 338, in the C<sub>V</sub>2 C<sub>V</sub>3 junction, which likely participate in the inter-chain disulfide linkages (Warr et al., 1995) Figure 2 above.

### Origin of Immunoglobulin Y

Although IgM is the only universally distributed antibody and is believed to be the precursor for all immunoglobulin classes, current evidence suggests that IgY may have instead been the immediate progenitor of both IgG and IgE (Warr et al., 1995). The comparisons of IgY and IgG are listed as follows (Table: 1). It does not also have the ability to precipitate or agglutinate multivalent antigens unless at high salt concentrations (around 1.5 M), perhaps due to steric hindrance caused by the closely aligned Fab arms of the IgY molecule. High salt concentrations may serve to release the Fab arms, permitting agglutination.

### Production and purification of immunoglobulin Y

Chickens can be used for antibody production throughout their entire egg laying period. Animals that are used for antibody production for more than three months should be given booster immunizations every other month to assure that the antibody titer remain high. Chickens can produce high avidity antibodies already after one immunization, compared to sheep whose avidity becomes similar after four immunizations (Landon and Woolley, 2005). Chicken eggs present an ideal alternative antibody source to mammals, as the IgY in the chickens' blood is transported to the egg and accumulates in the egg yolk in large quantities. The amount of antigen specific antibodies of the total pool of antibodies in an egg has been reported to be up to 10 %. However, the actual amount of specific antibodies probably varies depending on the individual animal, immunization procedures and the immunogenicity of the antigen itself (Carroll and Thalley, 1990).

The major problem in isolating IgY from egg yolk is separating the lipoproteins from egg yolk prior to purification of the IgY. There are several methods of purification of IgY described. These IgY separation methods include: lipoprotein precipitation by polyethylene glycol (Svendsen et al., 1995), sodium dextran sulphate and natural gums such as xanthan gum (Akita and Nakai, 1993), and dextran blue (Bizhanov and Vyshniausskis, 2000), and sodium alginate (Hatta et al., 1990). Chang *et al.* (2000) recently reported the precipitation of over 90% of lipoproteins from yolk using l- carrageenan, sodium alginate, carboxymethyl cellulose, and pectin. Ion exchange chromatography has also been reported as a final step in IgY purification (Fichtali et al., 1993), as well as hydrophobic interaction chromatography (Hassl and Aspöck, 1988). Immobilized metal ion affinity chromatography (Greene and Holt, 1997), thiophilic interaction chromatography (Hansen et al., 1998), affinity chromatography using alkaline conditions (Kuronen et al., 1997), and synthetic peptide ligands, designed specifically for immobilizing antibodies (Verdoliva et al., 2000). As well, Erhard *et al.* (1996) described a method for the purification of mouse IgG

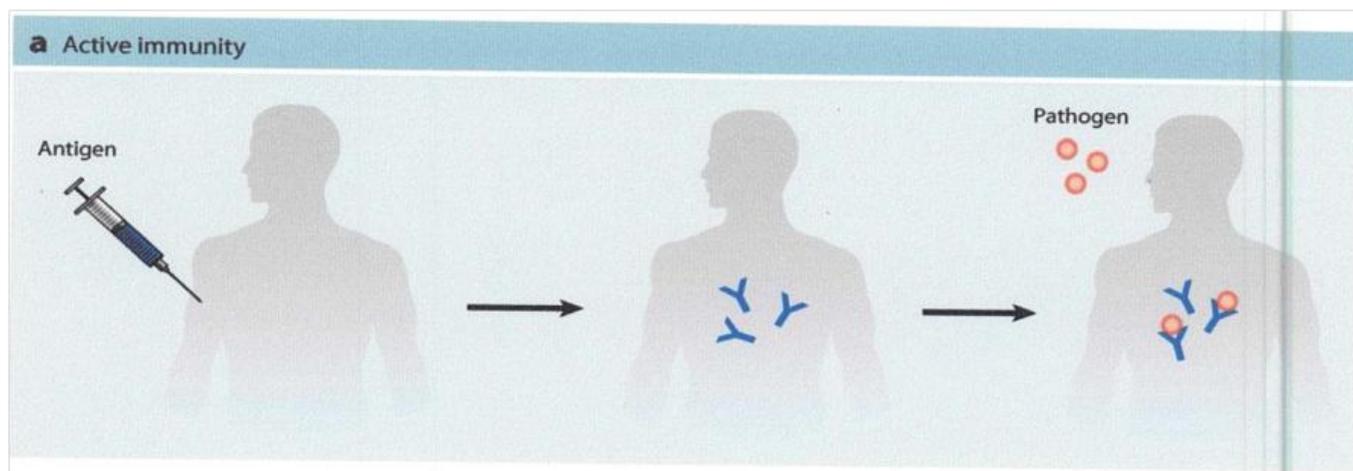
subclass specific IgY using indirect affinity chromatography with protein G Sepharose.

The choice of the methods is a matter of yield and purity desired, final use of the IgY as well as material cost and labor skills. The best way to obtain antibodies is to purify them from the yolk. Several methods can be used, even for large-scale purification, of functionally active chicken antibodies from egg yolk. Over 100 mg of purified IgY can be obtained from a single egg and it is also possible to purify specific antibodies by affinity-chromatography (Akita and Nakai, 1998).

### Physico-chemical properties

IgY and IgG differ not only in structure, but also in their stability to pH, heat, and proteolytic enzymes. Although the stability of both immunoglobulins was similar when subjected to alkaline conditions, IgY showed much less stability than that of rabbit IgG to acid denaturation. Shimizu et al. (1993) found that the activity of IgY was decreased by incubating at pH 3.5 or lower and completely lost at pH 3. The rabbit IgG antibodies, on the other hand, did not demonstrate a loss of activity as the unit of the pH decreased to by 2, and even then some activity still remained. Similar results were also observed by Hatta *et al.* (1993), using IgY produced against human rotavirus. Similarly, the IgY was significantly more sensitive to heating than the rabbit IgG. Shimizu *et al.* (1992) found that the activity of IgY was decreased by heating for 15 minutes at 70 °C or higher, whereas that of the IgG did not decrease until 75-80 °C or higher. Hatta *et al.* (1993) found, using differential scanning calorimetry (DSC), that the temperature corresponding to the maximum of denaturation endotherm ( $T_{max}$ ) was 73.9 °C for IgY and 77.0 °C for IgG. Shimizu *et al.* (1994), however, described the addition of sugar to an IgY solution, and found high concentrations of sugar allowed the IgY to maintain activity when subjected to high heat (75-80 °C), low pH (3), or high pressure (5000 kg/cm<sup>2</sup>). IgY, like IgG, has been found to be relatively resistant to trypsin and chymotrypsin digestion, but sensitive to pepsin digestion (Shimizu et al., 1988). Hatta *et al.* (1993) found that almost all of the IgY activity was lost following digestion with pepsin, however activity remained even after 8 hours incubation with trypsin or chymotrypsin. Otani *et al.* (1991) found that IgY was, however, more susceptible to digestion with trypsin, chymotrypsin and pepsin than IgG. The proteolytic digestion of antibodies is a common technique, used to remove the cross-reacting Fc portion of the antibody molecule. Akita and Nakai (1993b) noted further differences between IgY and IgG, with the peptic digestion of IgY resulting in mainly monovalent Fab' fragments, while the peptic digestion of IgG yields the bivalent (F(ab')<sub>2</sub>) fragments.

The structural factors resulting in the stability differences of the two immunoglobulin's are unknown, as



**Figure 3:** Active immunity involves immunizing, or vaccinating, an individual with an antigen to generate an adaptive response targeting the pathogen of interest.

immunoglobulins are large, complicated molecules, composed of heterogeneous polypeptides. Shimizu *et al.* (1992) predicted that the lower content of b structure in IgY may indicate that the conformation of IgY is more disordered and therefore less stable than mammalian IgG.

#### Advantages of immunoglobulin Y

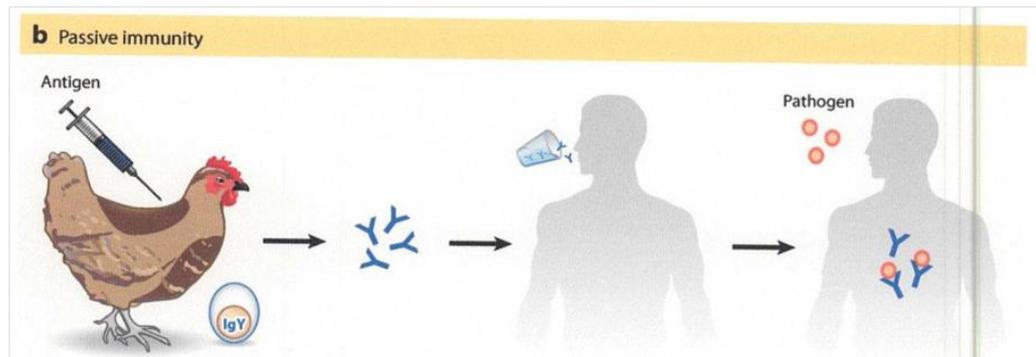
The use of chickens for the production of polyclonal antibodies provides several advantages over the traditional method of producing antibodies in mammals. In contrast to mammalian serum, egg yolk contains only the single class of antibody, IgY, which can be easily purified from the yolk by simple precipitation techniques (Gassmann *et al.*, 1990). The phylogenetic distance between chickens and mammals renders possible outcomes on the production of antibodies, in chickens, against highly conserved mammalian proteins, that would otherwise not be possible in mammals, and much less antigen is required to produce an efficient immune response (Larsson *et al.*, 1988). Chicken antibodies will also recognize different epitopes than mammalian antibodies, giving access to a different antibody repertoire than with mammalian antibodies (Carlander *et al.*, 1999). As well, the method of producing antibodies in hens is much less invasive, requiring only the collection of eggs, rather than the collection of blood, and is therefore less stressful on the animal (Schade *et al.*, 1991), and sustained high titres in chickens reduce the need for frequent injections (Gassmann *et al.*, 1990).

The animal care costs are also lower for the chicken compared to that for mammals, such as rabbits (Carlander *et al.*, 2000). Hens therefore provide a more hygienic, cost efficient, convenient, and plentiful source

of antibodies, as compared to the traditional method of obtaining antibodies from mammalian serum (Carlander *et al.*, 2000). Nakai *et al.* (1994) estimated that the productivity of antibodies in hens is nearly 18 times greater than that by rabbits based on the weight of antibody produced per head. Because of the high yolk IgY concentrations, over 100 mg of IgY can be obtained from one egg (Akita and Nakai, 1992). A laying hen produces approximately 20 eggs per month; therefore over 2 g of IgY per month may be obtained from a single chicken (Carlander *et al.*, 1999). In the egg, IgY is stable for months, and once purified it may be stored for years in the cold (Larsson *et al.*, 1993). As the industrial scale automated collection and separation of eggs is currently carried out, the large-scale production of specific IgY for immunotherapeutic purposes is feasible (Cotterill and McBee, 1995). Similarly, vaccination of chicken flocks has long been used to control avian infections (Sharma, 1999), making the injection of chickens required for large-scale antibody production also feasible.

#### Passive and Active Immunization of Immunoglobulin Y

Active immunity refers to the process of exposing the individual to an antigen to generate an adaptive immune response. This response takes days or weeks to develop but may be long lasting. While, passive immunity refers to the process of providing preformed antibodies to protect against infections, and it provides immediate but short-lived protection lasting several weeks to three or four months at most (baxter, 2007) Figure 3 above and 4 below.



**Figure 4:** In passive immunization, antibodies are isolated from another source (e.g. the egg yolk of immunized hens) and administered to susceptible individuals to provide pathogen-specific immunity. (Source: Baxter, 2007).

## THE POTENTIAL APPLICATIONS OF IMMUNOGLOBULIN Y

### Immunotherapeutic applications of immunoglobulin Y

Passive immunization using specific antibodies is a recent concept which presents an attractive approach to establish passive immunity against pathogens in both humans and animals (Carlander et al., 2000). Previously immunotherapy was carried out via the systemic or intravenous administration of specific antibodies for such applications as a targeting agent for cancer diagnosis and therapy, the inactivation of toxic substances including drugs and as passive immunotherapy for neoplastic or infectious diseases (Reilly et al., 1997). However, there has been increasing interest in the oral administration of specific antibodies for localized treatment of infections (Reilly et al., 1997). The increase in antibiotic-resistant bacteria and the desire to treat pathogens that do not respond to antibiotics such as viral pathogens, along with the escalating number of immune-compromised individuals has prompted much research into the administration of specific antibodies as an alternative to antibiotics and antimicrobial chemotherapy to treat infections. It is for this reason that much of the IgY research carried out has been with regard to immunotherapy (Carlander et al., 2000).

Now a days there is progress to use chicken egg as source of antibodies for prevention and treatment of gut associated infections wherein, after immunization, the specific antibodies, otherwise, known as IgY are transported to the egg yolk and they can then be separated without scarifying the bird. Oral administration of IgY has been tried and found useful in treatment of human and animals against microbes. The potential applications of IgY for prevention and treatment of infections caused by pathogenic bacteria and viruses have been studied at length (Michael et al., 2010) and are discussed below.

### Veterinary applications of immunoglobulin Y

Feed grade antibodies derived from the egg yolks of immunized hens have the advantage of being easily accessible, inexpensive and a rich source of polyclonal antibodies (Cook and Trott, 2010). Because of the ability of laying hens to produce large quantities of egg yolk antibodies on a relatively ongoing basis they have been promoted and tested as potential feed grade prophylactic agents (Cook and Trott, 2010). They have been administered as potential inhibitors of the enzyme uricase to reduce nitrogen emissions in poultry due to the excess production of uric acid in the manure by microorganisms (Kim et al. 2013). The ability to generate specific antibodies in fairly large quantities has also proven advantageous for therapeutic prevention of microbial pathogen colonization. Incorporating feed grade egg yolk antibodies into animal diets has been examined extensively to attempt to limit pathogenic diarrhea causing *Escherichia coli* (*E.coli*) in swine, and limit *Salmonella* establishment in calves and mice, as well as *Campylobacter*, *Clostridium*, and *Salmonella* in poultry (Al-Aldawani et al., 2013).

Egg yolk antibodies have also been developed for attempts to prevent establishment of food borne pathogens that commonly colonize food animals. *Campylobacter jejuni* is one of the major food borne disease causing microorganisms that also happens to be very well adapted to the ecological conditions prevalent in the poultry gastrointestinal tract (Pendleton et al., 2013). In an attempt to isolate antibodies that could limit *C. jejuni* colonization Al-Aldawani et al. (2013) generated chicken egg-yolk-derived antibodies (IgY) in laying hens against the five different *C. jejuni* colonization-associated cell surface proteins. These proteins were produced in sufficient quantities by first expressing the respective protein in *E. coli* and subsequently purifying the proteins for intramuscular injection as a water-oil mixture in combination with Freund's complete adjuvant into *C. jejuni*-free laying hens. Eggs were collected up to 10

weeks post-immunization and egg yolks were lyophilized for eventual purification and quantization of specific egg yolk antibodies reactive to each of the *C. jejuni* proteins. After characterizing specificity and reactivity of the individual egg yolk antibodies generated against the specific cell surface proteins they demonstrated that several of these egg antibodies limited attachment of *C. jejuni* to chicken hepatocellular carcinoma cells and concluded that these were candidate egg yolk antibodies with potential to reduce *C. jejuni* colonization in chickens (Al-Aldawani et al., 2013).

Bovine rota virus (BRV) is an important cause of diarrhea in new born calves and local passive immunity is the most efficient protective strategies to control the disease (Vega et al., 2011). More recently, it was shown that anti-BRV IgY-containing yolk provided up to 80% protection against BRV-induced diarrhea in neonatal calves when compared with calves given non-immunized egg yolk suggesting that supplementing new born calves' diets for the first 14 days of life with BRV-specific IgY may be promising strategy to prevent BRV-related mortality (Vega et al., 2011).

Diarrhea due to enterotoxigenic *Escherichia coli* (ETEC) is a major health problem in humans and animals. IgY could be an alternative source of immunoglobulins for the prevention of ETEC infection as it has been found to inhibit the binding of *E. coli* to the intestinal mucosa (Jin et al., 1998). IgY raised against ETEC antigen has been administered orally to piglets and has offered a potential prophylactic and therapeutic approach for controlling ETEC-induced diarrhea (Marquardt et al., 1999). Marquardt et al. It was found that the IgY titre was much higher when *E. coli* fimbrial antigen was used rather than the whole cell (Marquardt et al., 1999). Imberechts et al. (1997) raised IgY against *E. coli* F18ac fimbriae and in vitro adhesion tests demonstrated that the IgY inhibited attachment of F18ac positive *E. coli* to the intestinal mucosa. The anti-F18ab antibodies were also found to diminish diarrheal cases and death in animals infected with F18ac positive *E. coli*. Yokoyama et al. (1992) studied the passive protective effect of IgY against ETEC infection in neonatal piglets. IgY was administered to the piglets in milk three times a day for 2 days. Control piglets developed severe diarrhea within 12 hours and 30% of the pigs died. In contrast, the pigs given IgY exhibited no sign of diarrhea 24 or 48 hours after treatment (Marquardt et al., 1999). The passive protective effect of anti- ETEC IgY, in neonatal calves, against fatal enteric colibacillosis, has also been studied (Ikemori et al., 1992). Prevention of ETEC in rabbits through the oral administration of anti-ETEC IgY. Because the oral administration of anti-ETEC IgY has proven to be successful for the treatment of gastrointestinal infections of animals and also the clinical application of passive immunization of IgY against diarrhea is now being examined to prevent and treat ETEC infection in infants (O'Farrelly et al., 1992).

*Salmonella Enteritidis* (SE) and *Salmonella Typhimurium* (ST) are the main cause of outbreaks in human and infectious in chickens (Lee et al., 2002a). Chalghoumi et al. (2009) found that IgY against the outer membrane proteins of SE and ST reduce salmonella spp. adhesion to intestinal epithelial cells in vitro, which suggests that passive immunization with salmonella-specific IgY could be useful to prevent salmonella colonization in broiler chickens. Moreover, feeding chickens egg powder containing SE-specific antibodies was found to reduce fecal shedding, cecal colonization and the rate of *Salmonella*-contaminated eggs in experimentally infected chickens (Rahimi et al., 2007).

*Streptococcus mutans serotype c* is thought to be the principal causative bacterium of dental caries in humans. The molecular pathogenesis of *S. mutans* associated dental caries involves a series of binding events that eventually lead to the accumulation of sufficient numbers of these carcinogenic bacteria to cause disease (Hamada and Slade, 1980). Chicken antibodies against *S. mutans* MT8148 serotype c or cell-associated glucosyltransferase were prepared and tested against dental caries (Chang et al., 1999). Consumption of a carcinogenic diet containing more than 2% IgY-containing yolk powder resulted in significantly lower caries scores (Otake et al., 1991) and effective passive protection for the prevention of colonization of *S. mutans* in the oral cavity. It has also been reported that mouth rinse containing IgY specific to *S. mutans* was effective in preventing the dental plaque of humans in vitro and in vivo (Hatta et al., 1997a). Recently, Smith et al. (2001) produced IgY against the glucan binding protein B (GBP-B) of *S. mutans*. GBPs are believed to be involved in *S. mutans* biofilm development, and antibodies against GBP-B appear to have the potential to modulate infection and disease caused by *S. mutans*. Using a rat model of dental caries, they found that those rats treated with anti-GBP-B IgY displayed a decrease in *S. mutans* accumulation, as well as a decrease in the overall amount of dental caries, as compared to control rats. These studies indicate that IgY against *S. mutans*, or its components, may act to interfere with *S. mutans* accumulation, and control plaque and the subsequent oral health problems associated with plaque accumulation (Smith et al., 2001).

In addition, specific IgY has been shown to be effective at preventing and treating several other pathogens. It was found that specific IgY was capable of preventing the pathogenesis of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Its use has also been suggested for passive protection of chicks against infectious bursal disease virus (IBDV) (Etteradossi et al., 1997), and for the protection against porcine epidemic virus (PEDV) in piglets (Kweon et al., 2000).

## Applications of Immunoglobulin Y in human medicine

IgY has been found to be effective against a number of human diseases causing pathogens both in-vitro and in laboratory animal studies and clinical settings. One of the most successful clinical applications of IgY has been in the prevention of *pseudomonas aeruginosa* (PA) colonization in the airways of cystic fibrosis (CF) in patients. In 2008, orphan drug designation was granted for IgY antibody against PA for the treatment of CF in humans by the European Medicines Agency. PA is the major cause of morbidity and mortality in CF patients and once a chronic infection has been established it is very difficult to eliminate, even with the use of antibiotics (Kollberg et al., 2003). Furthermore, there is increasing risk of developing antibiotic-resistant strains (Nilsson et al., 2008). In ongoing trials in CF patients, a mouth rinse containing purified anti-PA IgY given on a continuous basis could significantly reduce or prevent PA colonization, thereby reducing the need for antibiotics (Nilsson et al., 2008). These studies have shown that specific IgY is effective for immunotherapy for long treatment periods without negative side effects (Nilsson et al., 2007). The stability of the anti-PA IgY in the saliva of healthy individuals was also examined and antibody activity was shown to remain even after 8 hrs supporting the potential application of IgY for other localized infections such as the common cold and tonsillitis (Carlander et al., 2002).

Another promising clinical application of IgY in human is for prevention of *Helicobacter pylori* (HP) infection. *H. pylori* is common cause of gastritis and gastric ulcers and the emergency of antibiotic-resistant strains has prompted the investigation in to alternative treatment methods (DeLoney and Schiller, 2000). In vitro, IgY against *H. pylori* reduced bacterial adhesion, growth and urease activity, and decreased *H. pylori* induced gastric mucosal injury and inflammation in an animal model. Because antibodies produced against whole-cell *H. pylori* might also cross-react with normal flora (Shin et al., 2003), the production and efficacy of IgY against immunodominant *H. pylori* proteins and peptides, including urease and urease-driven peptides (Nomura et al., 2005) and a 58 kDa highly reactive *H. pylori* antigen (Hp58) (Attallah et al., 2009), have been also examined. A functional drinking yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium* species and supplemented with 1% antiurease IgY was produced commercially and given to volunteers testing positive for *H. pylori* (Horie et al., 2004).

## Immunodiagnostic Applications of immunoglobulin Y

The production came to be called "IgY Technology" (Warr et al., 1995), which is the internationally accepted

term for describing the production and use of this antibody. Furthermore, the "European Centre for the Validation of Alternative Methods" (ECVAM) strongly recommends that yolk antibodies should be used as an alternative to mammalian antibodies for the animal welfare (Schade et al., 1996). The IgY can be harvested from the egg yolk instead of serum, thus making blood sampling outdated. The antibody productivity of an egg laying hen is greater than a similar sized mammal (Hau and Hendriksen, 2005) and the IgY concentration in the serum of adult hens can reach approximately 5-7mg/ml. As a laying hen produces approximately 20 eggs per month, over 2 grams of IgY can be isolated during this period corresponding approximately the IgY content of 300ml of serum or 600ml of blood. Only larger mammals can produce equal amounts of serum antibodies. Chicken antibodies, therefore, constitute a much less expensive vehicle for use in diagnostic purposes (Carlander, 2002).

The use of IgY can also be advantageous in immunological tests where the interference caused by IgG antibodies can be problematic, particularly, the sensitivity of the assay increases. One example is the rheumatoid factor (RF) that reacts with IgG from different mammalian species and also with mouse monoclonal antibodies (Carlander, 2002). RF is usually found in serum samples from patients with rheumatoid arthritis, but can also be found in patients with other diseases and even in 3-5% of healthy individuals. Interference by anti-IgG antibodies and antibody-binding substances have been demonstrated in approximately 40% of serum samples from healthy individuals in an immunoradiometric assay (Carlander, 2002).

Another important advantage arises from the phylogenetic distance and genetic background that distinguishes birds from mammals improving the likelihood that an immune response will be elicited against antigens or epitopes that may be non-immunogenic in mammals (Spillner et al., 2012). Due to the evolutionary distance between chicken and mammalian immunoglobulins, IgY recognizes more epitopes when the immunogen used is a mammalian protein highly conserved. This feature can result in amplification of the signal, emphasizing the advantages of using IgY over IgG as the first antibody in some types of immunological reactions (Carlander, 2002). It is a well-known concept that a stronger immune response is elicited when the distance between the antigen source and the immune system increases. It has also been shown that chicken antibodies have 3-5 times more affinity to antibodies of pigs than the rabbit IgG for signal amplification in immunological test (Olovsson and Larsson, 1993). The limited flexibility of the avian IgY may account for the inability to precipitate antigens at physiological salt concentrations (Warr et al., 1995). IgY and IgY(Fc) both possess two antigen-binding sites and should precipitate or agglutinate multivalent antigens but this does not always occur. Most chicken antibodies bind

antigen strongly but display precipitating properties only at raised salt concentrations. Duck antibodies generally fail to exhibit efficient precipitation or agglutination reactions (Higgins, 1988). The non-precipitating duck antibodies do not acquire the ability to precipitate antigen at raised salt concentrations (Warr et al., 1995).

More recently chicken antibodies libraries have attracted scientific interest with increased reports on the isolation of chicken derived antibody fragments. In other words, avian species utilize a unique mode of DNA recombination, named gene conversion, resulting in a large and diverse antibody repertoire upon antigen priming (Spillner et al., 2012). This could be exemplified by the development of a humanized chicken monoclonal anti-IL12 antibody (Nishibori et al., 2006). It is also important to keep in mind that recombinant technologies currently available can generate monoclonal IgY or IgY like antibodies from combinatorial libraries, sometimes without animal immunization (Spillner et al., 2012). Taking together, all these characteristics clearly show substantial advantages of IgY technology in many medical areas, especially for diagnosis. Specific chicken antibodies have been successfully raised against a wide variety of antigens including proteins, peptides, lipid hormones and carbohydrate components from viruses, bacteria, fungi, plants and animals (Schade et al., 1994). Several studies have also shown promising results in the development of techniques for immunodiagnostic using IgY, such as immunoassays tests to detect circulating antigen of *Schistosoma japonicum* (Cai et al., 2012), development of IgY antibodies against proteins of *Pythium insidiosum* (Rangel, 2010), use in antigen capture-ELISA (Veerasingam et al., 2008).

### Immunoglobulin Y in immunoaffinity chromatography

Immunoaffinity chromatography involves the isolation and purification of target molecules using immobilized antibodies directed against the target molecule. Due to the highly specific nature of the antibody-antigen interaction, immunoaffinity chromatography allows for the purification of specific molecules from complex starting materials. The widespread use of this process in large scale, however, has been limited by the high cost of the technique and parameters relating to the production of antibody and the efficiency of immobilization (Li-Chan, 2000). Immobilized yolk antibodies have been used for the isolation of value-added proteins from dairy products, including the purification of lactoferrin (Li-Chan et al., 1998) and the isolation and separation of IgG subclasses from colostrum, milk and cheese whey (Akita and Li-Chan, 1998). Although IgY is more sensitive to low pH than IgG, Akita and Li-Chan (1998) reported that using standard affinity chromatography conditions (i.e. elution at low pH), an IgY immunoaffinity column was stable and

could be reused over 50 times without significant decreases in binding capacity. Alternative eluents have been examined, including highly alkaline conditions (Kuronen et al., 1997) and high concentrations of guanidine hydrochloride (Otani et al., 1991). To extend the use of IgY immunoaffinity columns, Kim *et al.* (1999) also examined the reusability of avidin-biotinylated IgY columns, in which biotinylated IgY is held by strong non-covalent interaction on columns containing immobilized avidin. A number of other applications using IgY immunoaffinity columns have been described for the purification of biological molecules from human serum, including the purification of tetrachlorodibenzo-p-dioxin (Shelver et al., 1998), prekallikrein (Burger et al., 1986), and human alpha-2 antiplasmin (Lee et al., 1997).

### Other applications of immunoglobulin Y

It has been estimated that 1.7 million people are bitten or stung by venomous snakes, scorpions, jelly fish, or spiders each year, resulting in 40,000-50,000 fatalities. The most widely used treatment of envenomation is the use of specific anti-venoms to neutralize the toxic and potentially lethal effects of the venom. Chicken anti-venom IgY has been produced, and was found to have a higher bioactivity than anti-venoms raised in horses (Almeida et al., 1998). IgY also has a lower likelihood of producing significant clinical side effects, such as serum sickness and anaphylactic shock, which can occur upon administration of mammalian serum proteins (Larsson et al., 1993). Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases, which are an increasing burden to hospitals and society in terms of the cost of medication and treatment, and time lost due to illness (Hay and Hay, 1992). Standard medical care for these diseases includes anti-inflammatory drugs, immunosuppressants, and antibiotics, but their use is limited by side effects, immunosuppression, and incomplete efficacy. Immunotherapy using monoclonal mouse antibodies directed against tumor necrosis factor (TNF) has been approved for use, however it can be costly and adverse side effects have been reported in patients receiving systemic anti-TNF therapy (Sandborn and Hanauer, 1999). Recently Worledge *et al.* (2000) reported that anti-TNF antibodies produced in chickens were capable of effectively treating acute and chronic phases of colitis in rats, and were also found to neutralize the treatment of inflammatory bowel disease in humans in the future. Human TNF invitro, indicating its possible use for the treatment of inflammatory bowel disease in humans in the future.

### CONCLUSION AND RECOMMENDATIONS

Chickens like mammals are capable of producing antigen specific IgY which functions similar to IgG in

response to an antigenic stimulus. It was not until recently, however, the particular immunological properties of IgY were recognized and IgY began replacing mammalian antibodies in such applications as immunodiagnostic assays, immunotherapeutics and affinity purification techniques. Yolk antibodies do not activate the mammalian complement system or interact with mammalian Fc receptors that could mediate inflammatory response in the gastrointestinal tract. As these immunotherapeutic applications often require the continuous or frequent administration of antibodies, large quantities are required. IgY is, therefore, the ideal choice for the production of large quantities of conveniently purified antibodies. The use of IgY is also cost-effective with IgY costing less than \$10 per gram compared to IgG which can cost upwards of \$20 000 per gram. This technology will allow for new potential applications of IgY in medicine, public health, veterinary medicine and food safety. Chickens useful for the production of specific IgY and it also needs to demonstrate the deposition of recombinant human antibodies into the egg yolk of transgenic chickens suggesting an extension of the production of specific IgY in eggs.

In line of the above conclusive remarks the following recommendations are forwarded:

- Researches should be continued to be carried out on the potential methods of production and application of egg yolk antibodies.
- To increase the use of IgY, techniques for both direct and indirect labelling must be optimized.
- When antibodies are to be used for therapeutic purposes, the use of free from specific pathogens chicken is compulsory.
- It is preferable to immunize chickens before they begin to produce egg, because the stresses induced by handling them have an adverse effect on egg production.
- Further study on the application of IgY in immunotherapeutic and diagnostic purposes should be undertaken.

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