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Avian IgY antibodies and its immunotherapeutic applications

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ABSTRACT

Antibodies, also called immunoglobulins, are specialized proteins produced by the immune system in response to the presence of pathogens or foreign substances in the body. These unique proteins are commonly used for diagnostic and therapeutic purposes because they easily bind to antigenic molecules. Polyclonal antibody production currently involves the use of laboratory animals such as rats, rabbits, sheep, goats, and horses. However, the manufacture of these antibodies generally involves practices that cause pain to animals, such as prolonged bloodletting. In recent years, isolating antibodies from egg yolk following hyperimmunization of chickens has emerged as a popular approach for producing significant amounts of antibodies. This approach combines the principles of natural passive immunity and artificial passive immunity. To ensure a continuous accumulation of antibodies in egg yolks, chickens are regularly immunized with specific antigens. Egg yolk antibodies, known as IgY, are extracted and used for immunotherapy and immunodiagnostic purposes in human and animal applications due to their promising antibacterial properties. The antibacterial properties of egg yolk antibodies have been a significant focus in IgY studies. Several reports have shown that IgY helps prevent bacterial transmission or infection in vivo. The production of IgY against mammalian antigens has a higher success rate than IgG production. This is because of the phylogenetic difference between mammals and chickens. Furthermore, these antibodies have a more comprehensive range of antigenic epitope recognition and can respond to more than one species, making them more versatile. This study compiles information on the properties, mechanisms of action, and uses of egg yolk antibodies based on existing literature on IgY technology.

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Introduction

Klemperer (1893) conducted an experiment demonstrating that specific antibodies (Abs) produced after immunization chickens could be transferred to the egg yolk. This finding gained popularity as animal welfare became an increasingly prominent ethical issue in the scientific community. Klemperer's findings were first made known worldwide with the publication of the study titled 'Principles of Humane Experimental Technique' conducted by Russell and Burch in 1959 (Russell and Burch, 1959). Subsequent research by numerous scientists over the next two decades further emphasized the significance of these findings.

In 1992, a research team funded by the German Government and with experience in egg yolk antibodies was established. This team investigated the comparative effectiveness of chicken antibodies with traditionally used polyclonal antibodies produced from mammals, specifically rabbits. As a result of this

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research, it is noteworthy that chicken antibodies were found to be more effective than mammalian antibodies because they contain highly phylogenetically protected antigens. Over six years, IgY technology gained increasing acceptance. Within a few years, it became an accepted alternative to traditional procedures and has been reported to cause less stress to animals (Schade et al., 2000).

The term 'IgY technology' first appeared in 1996 to describe the production and uses of egg yolk antibodies. That same year, the European Center for the Validation of Alternative Methods (ECVAM) recommended using egg yolk-produced IgY antibodies instead of mammalian IgG antibodies to reduce the pain associated with invasive antibody sampling. In addition, it was reported in the ECVAM workshop that using chickens for antibody production improves animal welfare by eliminating the need for blood collection and reduces animal use since chickens produce more antibodies than mammals (Schade and Hlinak, 1996). In 1999, The Swiss Government Veterinary Office (Office Vétérinaire Fédéral) approved IgY technology as an alternative method to support animal health (Schade et al., 2000).

The use of IgY antibodies in basic research has become increasingly common due to their advantages over mammalian antibodies. IgY does not bind to mammalian rheumatoid factors or Fc receptors, making it a preferred choice. However, it should be noted that using chicken antibodies can sometimes result in false positive results in immunochemical tests (Schade et al., 2000).

Studies have shown that the yield of IgY antibodies is considerably higher than that of IgG antibodies. According to Gottstein and Hemmeler (1985), an immunized chicken can produce 18 times more antibodies than an immunized rabbit in one month. Another study reported that a chicken can produce the same amount of antibodies per week as approximately 100 ml of serum or 200 ml of whole blood (Larsson et al., 1993). Similarly, it has been reported that up to 1500 mg of IgY can be collected monthly, of which an average of 2% to 10% is specific IgY (Schade and Hlinak, 1996). During the same period, IgG antibodies collected approximately 200 mg and constituted only 5% of the specific antibody (Tini et al., 2002). Laying hens can produce more antibodies than 4 rabbits in the same period. They are also called 'small factories' due to their ability to produce over 20 g of IgY in approximately one year (Xu et al., 2011). Egg yolk antibodies have the advantage of inducing a lower antigenic response, making them more suitable for long-term production than rabbits (Gassmann et al., 1990). Additionally, IgY technology allows for accessible collection of antibodies from egg laying hens, eliminating the need for painful blood collection. Thus, it meets the principle of reducing practices that cause pain, which is the primary goal of animal welfare (Larsson et al., 1993; Tini et al., 2002). Similarly, keeping chickens is a more cost-effective and manageable alternative to using laboratory mammals for research purposes (Gassmann et al., 1990). The production of IgY is considered a more sterile and efficient system compared to the production of mammalian IgG. IgYs are a sustainable alternative to antibiotics because they do not have any adverse effects, such as resistance or toxic residues (Coleman, 1999).

This study describes IgY technology, including its production, structure, components, and mechanisms of action. Additionally, it discusses the use of egg yolk antibodies in preventing and treating pathogenic infections.

Properties of IgY antibody

Structure

Chickens possess three distinct classes of immunoglobulin: immunoglobulin Alpha (IgA), immunoglobulin Mu (IgM), and immunoglobulin Upsilon (IgY). The IgA and IgM found in chickens are structurally and functionally equivalent to their mammalian counterparts. IgY, the dominant antibody group in blood serum and egg yolk, was initially classified as IgG due to its similar function to mammalian IgG antibodies (Gadde et al., 2015). However 1969, researchers recommended that the immunoglobulins found in chicken egg yolk and serum should be named IgY instead of IgG due to structural differences (Leslie and Clem, 1969). IgY antibodies are also accepted as the evolutionary ancestor of IgG and IgE and are the predominant serum antibodies in amphibians, reptiles, and lung-breathing fish, except birds (Leslie and Clem, 1969; Warr et al., 1995).

It has been reported that within the egg matrix, IgA and IgM antibodies are concentrated in the egg white, while IgY antibodies are concentrated in the egg yolk (Gadde et al., 2015). The structure of the IgY molecule is with two heavy chains (H) weighing 65 kilodaltons (kDa) each and two light chains (L) weighing 25 kDa each (Pereira et al., 2019). IgY has a larger molecular mass (~180 kDa) than IgG (~150 kDa). The IgY antibody's heavy chain is represented by the letter Y, or the twentieth letter of the Greek alphabet, upsilon (υ) (Warr et al., 1995; Gadde et al., 2015). Figure 1 illustrates the molecular structures of IgG and IgY.

The light chain of IgY is similar to that of IgG, as it consists of a constant region (CL) and a variable region (VL). However, the heavy chains of IgY and IgG differ.

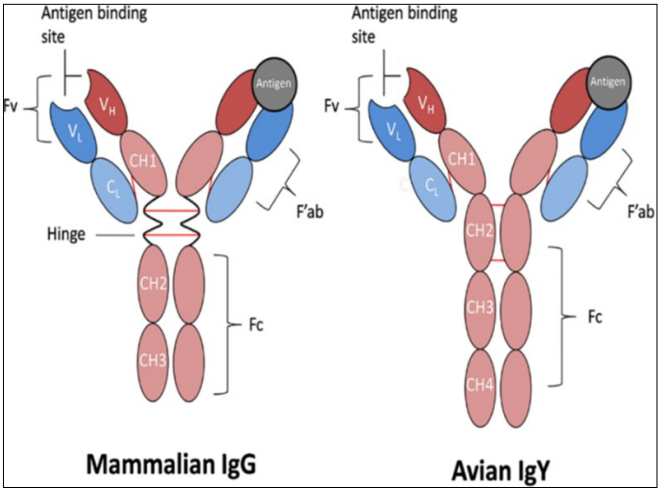

Figure 1. Molecular structures of IgG and IgY

The diagram shows the disulfide bonds connecting the heavy and light chains, indicated by red lines. IgY has an additional CH4 domain, similar to IgE, but lacks the folding region found between CH1 and CH2 in IgG (Lee et al., 2017).

IgG has three constant regions (CH1-CH3) in its heavy chain, while IgY has four constant regions (CH1-CH4) (Pereira et al., 2019). Several studies reported that the CH3 and CH4 regions of IgY correspond to the CH2 and CH3 regions of IgG (Parvari et al., 1988; Fellah et al., 1993; Magor et al., 1994; Warr et al., 1995). However, the CH2 region of IgY does not have a corresponding domain in IgG. This is because it has been replaced by the folding region (Parvari et al., 1988; Fellah et al., 1993). It is important to note that IgY is less flexible than IgG because IgY lacks the folding region located between the CH1 and CH2 domains found in IgG (Shimizu et al., 1992). Additionally, the short proline and glycine-rich regions located at the borders of the CH1-CH2 and CH2-CH3 domains provide a limited degree of inflexibility to the IgY molecule, which does not have a folding region (Warr et al., 1995). Structural and phylogenetic differences between IgY and IgG have distinct effects on their biochemical and molecular interactions. Egg yolk antibodies do not activate rheumatoid factors or mammalian Fc receptors (Larsson et al., 1992). Table 1 summarizes the differences between IgY and IgG.

Stability

Numerous studies have demonstrated that egg yolk antibodies can be stored for extended periods without any activity loss. According to Larsson et al. (1993), egg

Table 1. Characteristics of IgY compared to IgG (Zhang et al., 2021)

Features	IgY	IgG
Species	Birds, reptiles, amphibians and lungfish	Mammals
Antibody subclasses	None	IgG_1, IgG_2, IgG_3 ve IgG_4
Antibody source	Egg	Blood serum
Antibody sampling	Meets 3R animal welfare principles	It can be painful
Average antibody per animal	50-100 mg/ egg yolk	5 mg/ml/blood, up to 40 ml blood collection per month
Monthly antibodies per animal	1000-2800 mg/chicken/month	200 mg/rabbit/month
Amount of antigen-specific antibodies	Total antibody %0,5-10	In serum 50-200 µg/ml
Molecular weight (kDa)	180	150
Isoelectric point	5,5-7,6	up to 8.5
pH stability	pH 3,5-11/ decisive	pH 2-11/ decisive
Proteolytic degradation	Pepsin, papain	Pepsin, papain, tripsin ve kimotripsin
Heat stability	65 °C decisive > 2 month 100 °C > 6 minute 4 °C > 6 month	Generally higher than IgY
Chain type/number of areas/folding area	υ chain/3 fixed fields/none	ɣ chain 2 fixed fields / there is
Binding to Fc receptors	No	Yes
Cross-reactivity with rheumatoid factor	No	Yes
Cross-reactivity with human anti-mouse antibody (HAMA)	No	Yes

yolk antibodies can be stored at +4°C for approximately 5 to 10 years without severe loss of activity. The study also reports that IgY antibodies remained effective after being stored for 6 months at 24°C or 1 month at 37°C. The most effective method for extending the storage time of IgY antibodies is to freeze them at -20°C. It has been reported that IgY antibodies may lose over 50% of their activity when stored for extended periods at -70°C (Schade et al., 2000). Furthermore, it has been reported that the stability of IgY is not significantly affected by lyophilization and freezing processes unless they are repeated multiple times (Shimizu et al., 1988). Many research mentions a minimum loss of antibody activity, but some researchers have reported a significant decrease in IgY solubility after lyophilization (Chansarkar, 1998; Sunwoo et al., 2002). In addition, IgY antibodies can be stored for an extended period without activity loss when spray dried. Moreover, spray drying has been reported as a more cost-effective alternative to lyophilization (Yokoyama et al., 1992).

Egg yolk antibodies are heat-stable. However, their activity and stability decrease when exposed to high temperatures for extended periods. Lösch et al. (1986) reported that IgY activity did not decrease when eggs were boiled for six minutes, but antibody activity decreased after ten minutes. Similarly, another study reported that egg yolk antibodies maintained around 50% of their antibody activity after being heated at 70° C for 30 minutes but only retained 10% of their activity when heated at 80°C for 10 minutes (Hatta et al., 1993). However, according to Shimizu et al. (1992), IgY antibodies can remain active even after being heated to 70°C for 15 minutes, suggesting they can withstand temperatures between 60°C and 70°C.

Egg yolk antibodies exhibit stable activity within the pH range of 4 to 11, and the activity ceases completely at pH 3 Shimizu et al. (1988 and 1992). Similarly, it has been observed that the activity of IgY is irreversibly lost when the pH drops to 3.5 (Lösch et al., 1986; Schmidt et al., 1989; Hatta et al., 1993; Lee et al., 2002). It is reported that the faster decrease in activity of IgY compared to mammalian IgGs may be due to conformational changes that affect the antigen binding site (Shimizu et al., 1993). Reports suggest that the acid-induced inactivation of IgY can be accelerated by complex carbohydrates, sugars, or stabilizers such as sorbitol (Shimizu et al., 1994; Lee et al., 2002). It has been reported that under alkaline conditions, the conditioning of IgY antibodies is stable up to pH 11 but drops significantly at pH 12 and above (Lösch et al., 1986; Shimizu et al., 1992). The effectiveness of IgY, when taken orally, depends on its ability to resist enzymatic degradation in the stomach and bowel. Researchs indicates that while IgY antibodies show some resistance to trypsin and chymotrypsin, they are mainly susceptible to pepsin digestion, especially in acidic environments (Shimizu et al., 1988; Otani et al., 1991; Reilly et al., 1997; Jaradat and Marquardt, 2000). The proteolytic activity of the pepsin enzyme on IgY ends at pH 5. In their study, Schmidt et al. (1989) reported that trypsin had a more substantial inactivating effect on IgY antibodies than chymotrypsin. Furthermore, in the same study, in vitro, incubation of egg yolk, egg suspensions, and isolated IgY antibodies with trypsin and chymotrypsin for 3 hours was reported to result in complete inactivation of IgY antibodies by trypsin and significant inactivation by chymotrypsin. However, an increase in antibody activity of egg yolk and egg suspensions has been reported, attributed to other protein components in the egg acting as a buffer. Therefore, the stability of IgY in the intestine depends on various factors, including age, health status, nutritional factors, stomach pH, and enzyme levels.

Research on the stability of egg yolk antibodies has mostly been conducted in laboratory settings. However, several studies have shown that orally administered antibodies maintain their biologically active form after passing through the intestine (Reilly et al., 1997; Carlander et al., 2000; Berghman et al., 2005). Conducting additional in vivo studies to demonstrate the stability of IgY in feed, egg yolk, and whole egg matrices is crucial for the potential commercial use of IgY.

Mechanism of effect

The mechanism behind orally administered egg yolk antibodies has yet to be fully understood. However, this mechanism is known to be largely the result of antigen-antibody interaction (Rahman et al., 2013). Various theories have been suggested to explain the protective effect of IgY. The primary mechanism involves antibodies binding to specific antigens on pathogens, disrupting their biological functions or ability to proliferate. IgY antibodies bind to bacterial antigenic structures, including flagella, fimbria, outer membrane proteins, and lipopolysaccharides, preventing their adherence to the intestinal wall (Peralta et al., 1994; Tsubokura et al., 1997; Jin et al., 1998; Yokoyama et al., 1998). IgY inhibits the adherence of pathogens to intestinal cells. This reduces the proliferation and colonization of pathogens within the cells (Jin et al., 1998; Sugita-Konishi et al., 2000; Lee et al., 2002; Sunwoo et al., 2002; Girard et al., 2006). Additionally, IgY antibodies

2002; Girard et al., 2006). Additionally, IgY antibodies have other mechanisms of action on antigens, such as bacterial agglutination resulting in immobilization and death, neutralization of toxins (Wang et al., 2011), and inhibition of enzyme activity (Rahman et al., 2013). It has been reported that binding specific IgY to bacteria can alter cellular signaling events, thereby reducing toxin production and release (Xu et al., 2011).

Production of IgY

When producing egg yolk antibodies, it is possible to use various immunization protocols and recommendations. However, it has been reported that antibody titers can be affected by various factors, including the type of antigen, route of administration, type of adjuvant used, frequency of immunization, age, strain, and stage of animal development (Schade et al., 2005; Chalghoumi et al., 2009). Specific protocols have been implemented to obtain IgY by controlling these variables (Schade et al., 2005; Michael et al., 2010). Agent-specific IgY can be produced in chickens using a variety of antigenic structures, including complex antigens like bacteria and parasites, as well as simpler antigens such as proteins, peptides, polysaccharides, and nucleic acids (Chalghoumi et al., 2009; Michael et al., 2010). The data showed that chickens generally produced specific antibodies after immunization with protein antigens. However, it is reported that the lowest antigenic molecular weight required to obtain a sufficient immune response is similar in mammals and chickens and is approximately in the range of 5 to 10 kDa (Schade et al., 2000).

Additionally, adjuvants are required to trigger the formation of high antibody titers. Among the adjuvants used for this purpose, Freund's Complete Adjuvant (FCA) is considered the "gold standard" and is the most used adjuvant for immunization purposes (Schade et al., 2000). However, although FCA has the strongest effect on the formation of high levels of antibody titers, it can cause severe inflammation in the areas where it is applied (Chalghoumi et al., 2009). Some studies have shown that chickens better tolerate immunization with Freund's Complete Adjuvant (FCA) than mammals and does not cause tissue injury in chickens (Gassmann et al., 1990; Bollen et al., 1996). However, other studies contradict these results (Wanke et al., 1996; Olbrich et al., 2002).

Freund's Incomplete Adjuvant (FIA), which does not include mycobacteria, is one of the best alternatives to FCA (Schade et al., 2000). For immunization applications, it is recommended to use FCA for the first immunization and FIA for subsequent immunizations to prevent inflammation in the injection area (Reddy et al., 2013; Łupicka-Słowik et al., 2014). In young chickens, antigens are usually administered intramuscularly (i.m.) into the breast muscle to produce egg yolk antibodies. Subcutaneous injection through the neck can cause unnecessary stress to the animal. Injection in the leg muscle can cause lameness and should be avoided (Schade et al., 1996). Alternatively, antigens can be administered orally for a non-invasive approach (Thibodeau et al., 2017).

The number of required immunizations varies depending on the adjuvant, antigen characteristics, and application dose. To achieve the desired level of antibodies, it is recommended to administer at least two immunizations with an interval of four or six weeks before the chickens enter the ovulation period. After the final immunization, IgY titers should be measured 14 days later (Pereira et al., 2019). If the antibody titres are below the desired level, increasing the frequency of immunization during the ovulation period is recommended (Schade et al., 1996). The titers of IgY increase from the 14th day following the immunization (Sui et al., 2011; Wen et al., 2012) or from the fifth week after antigen inoculation (Grzywa et al., 2014). After a gradual increase, the antibody titer reaches its peak and stabilizes. It then gradually decreases (Wen et al., 2012). However, it has been reported that egg yolk antibodies can be kept at high titers for more than 150 days with booster immunization applications (Meenatchisundaram et al., 2011).

The process of extraction IgY involves removing the lipids and extracting the antibodies to produce a water -soluble form. Various methods exist for extracting IgY from egg yolk, including the polyethylene glycol precipitation Methot, water dilution method, ammonium or sodium sulfate precipitation methot, dextran sulfate precipitation, pre-cooled propane and acetone method and water dilution, ultrafiltration method. Polson's polyethylene glycol precipitation method (Palson et al., 1980) is widely accepted among these methods (Schade et al., 2000). However, the appropriate method depends on the purpose, cost, and available technology. It may be necessary to purify to varying degrees (Chalghoumi et al., 2009). In research, IgY production is primarily carried out in chickens. This process can also be applied to other birds, such as geese (Fink et al., 2017) and quails (Najdi et al., 2016), using a similar immunization protocol for chickens.

Immunotherapeutic applications of IgY

Polyclonal egg yolk antibodies, produced specifically

against contagious diseases, can reduce the likelihood of microbial resistance. For this reason, specific IgY antibodies are a suitable option for antimicrobial use in both animal and human health, particularly against resistant bacteria (Rahman et al., 2013). The antibacterial effects of IgY against gastrointestinal agents have been extensively studied. Orally administered IgY has been shown to protect against gastrointestinal pathogens such as human (HRV) and bovine Rotaviruses, Salmonella spp., and enterotoxigenic Escherichia coli (Karlsson et al., 2004). HRV is a leading cause of acute gastroenteritis in children, resulting in over one million deaths per year on average. A study on mice infected with Rotavirus found that IgY derived from eggs inoculated with three different serotypes (mouse, monkey, and human) effectively relieving diarrhea (Yolken et al., 1988; Hatta et al., 1993).

Several studies have been conducted on the protective effect of IgYs against the influenza virus. In a study conducted in Vietnam, yolk antibodies against the avian influenza A (H5N1) virus were isolated from eggs sold in markets. The study's results showed that administering IgY against H5N1 intranasally to mice before and after infection with H5N1 and H5N2 prevented the onset of the disease (Nguyen et al., 2010). Wen et al. (2012) investigated the protective effect of IgY against Influenza B virus. The results showed that intranasal administration of anti-Influenza B IgY prevented the development of influenza in mice before exposure to the virus.

Additionally, it reported that it alleviated the disease in mice treated after infection. In addition, IgYs have been produced that are effective against Newcastle disease virus (NDV), Infectious Bursal Disease virus (IBDV), Influenza, and Reovirus, which cause infection in poultry (Aizenshtein et al., 2016). These studies demonstrate that egg yolk antibodies offer protection against viral agents, and passive immunization with IgY may be possible.

Enterotoxigenic Escherichia coli (ETEC) remains a significant health concern due to diarrhea. ETEC is a common cause of enteric Colibacillosis in newborn piglets, calves, children, and travelers to developing regions. It causes an average of one million deaths per year (Mine et al., 2002). A study examined the protective effect of anti-ETEC IgY against ETEC infections in piglets and calves. It was found to offer a prophylactic and therapeutic approach that can control diarrhea caused by infection in both. The oral administration of anti-ETEC IgY has been successful in preventing gastrointestinal infections in animals (Ikemori et al., 1992; Yokoyama et al., 1992).

Salmonella spp. is a common cause of foodborne illness. The Salmonella agent possesses several surface components, including outer membrane proteins associated with virulence, flagella, lipopolysaccharides, and, in certain strains, fimbrial antigens (Mine and Kovacs-Nolan, 2002). A study was conducted to control Salmonellosis by investigating the protective effect of specific egg yolk antibodies produced against the agent's outer membrane proteins, lipopolysaccharides, or flagellar antigens in calves and mice. The research report indicates that chickens treated with anti-Salmonella IgY antibodies had a higher survival rate (Yokoyama et al., 1998). Additionally, it has been reported that IgY inhibits the adhesion of Salmonella Enteritidis to human intestinal cells in vitro (Sugita-Konishi et al., 1996).

Additionally, administering egg yolk antibodies against Clostridium difficile spores orally has been observed to delay the onset of diarrhea and reduce its recurrence in mice treated before infection (Pizarro-Guajardo et al., 2017). The therapeutic effect of IgY against Helicobacter pylori has also been extensively researched. It has been reported that anti- H. pylori egg yolk antibodies inhibit the growth of H. pylori in vitro and reduce gastric inflammation in mice (Malekshahi et al., 2011).

The protective effect of egg yolk antibodies against pathogens that cause respiratory tract infections has also been studied. It has been determined that IgYs produced specifically for the purpose of combating Mycobacterium tuberculosis cause dose-dependent proliferation of peripheral blood mononuclear cells in rats. RT-PCR analysis revealed increased mRNA amounts of interleukin-2 (IL-2) and interferon-gamma (IFN-ɣ). The results suggest that the anti-M.tuberculosis IgY has significant potential as an immunotherapeutic that stimulates the immune response (Sudjarwo et al., 2017). Additionally, specific IgYs produced against multidrug-resistant strains of Acinetobacter baumannii have been reported to halt bacterial growth in mice in laboratory environments, significantly reduce the death rate of infected mice, and alleviate lung inflammation (Shi et al., 2017).

Additionally, the antibacterial effect of IgY against infections in aquatic creatures has also been investigated. IgY was produced against Vibrio spp., the leading cause of death of shrimps (Litopenaeus vannamei). It has been reported that using IgY in powder form, which contains anti-Vibrio IgY, significantly reduces the mortality rate of shrimps infected with V. harveyi and V. parahaemolyticus (Gao et al., 2016).

Another study investigating the effectiveness of IgY

produced against Propionibacterium acne bacteria reported that specific antibodies could be a potential alternative to antibiotics in the treatment of acne. (Revathy et al., 2014). Moreover, it examined the protective effect of egg yolk antibodies against Pseudomonas aeruginosa, which causes opportunistic infections and develops resistance to many antimicrobials. The research findings indicate that anti-P. aeruginosa IgY can enhance the cellular immune response, suggesting its potential use for prophylaxis in cystic fibrosis patients (Thomsen et al., 2016). IgY antibody purification has been evaluated for use in anti-venom serotherapy, specifically for Bothrops sp. venom. Anti-venom egg yolk antibodies have been produced and reported to effectively neutralize the venom with minimal side effects in mice (Araújo et al., 2010). In another study, anti-venom IgY was produced against the venom of Bothrops atox, also known as the Peruvian snake. The anti-venom IgY has been reported to demonstrate significant cross-reactivity with the venom of Bothrops brazili (Mendoza et al., 2012).

Conclusions

It is clear that the immunotherapeutic and immunoprophylactic use of IgY antibodies, as described above, to control various infectious diseases is beneficial. IgY antibodies, which can be produced and used similarly to mammalian IgG antibodies, are considered a valuable alternative therapeutic tool for treating many diseases in humans and domestic animals. It may also provide a new avenue for producing antidotes against natural toxins and serve as a diagnostic tool. Additionally, passive immunization with agent-specific IgY is a safer and non-toxic alternative to antibiotics and preserves fan welfare. In the future, IgY technology is expected to have increased benefits in both research and universal applications in medicine. However, this technology also has limitations. The principle of passive immunization provides short-term protection, which requires continuous application of antibodies in adequate doses. This can be costly for large-scale production. Therefore, new methods must be developed to produce high-quality antibodies economically and efficiently.

Moreover, precautions should be taken to increase the stability of egg yolk antibodies in the intestines and at high temperatures. Further researchs should be conducted on selecting adjuvants and immunogens to immunize chickens. This will increase the capacity to produce specific IgY antibodies against multiple pathogens simultaneously. Additionally, the use of new immunogens should be considered, as it can reduce the need for purification procedures and protein expression.

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