



Uses of Immunoglobulins as an Antimicrobials Alternative in Veterinary Medicine

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ABSTRACT

As a result of increasing the resistance to antimicrobials in the field of veterinary medicine that reflects on human health, there is a great demand to use some drug alternatives. The application of avian immunoglobulins (IgY) is regarded as an important alternative strategy. The IgYs have been produced by several techniques and applied for animals using different methods. In addition, egg yolk IgYs have many advantages over blood type ones. There are many uses of IgYs in veterinary medicine. They have been used for the prophylaxis and treatment of different infections especially the enteric ones in cattle, pigs, rabbits, dogs, rats, mice, and fish species. Moreover, several studies showed the importance of IgY for competing for the *in vivo* enteric pathogens in poultry and the *in vitro* foodborne pathogen. Therefore, it is important to put a spotlight on applications of egg yolk immunoglobulins IgY in veterinary medicine to overcome the problems of antimicrobials' resistance as well as the tissue residues that adversely affect human health.

Keywords: Advantages, Animals, Poultry, Production, Yolk antibodies

INTRODUCTION

The incidence of antibiotic-resistant bacteria (Wierup, 2000), and the demand to control organisms that do not respond to antimicrobials have increased in recent years (Carlander et al., 2000). Therefore, it is of utmost importance to conduct studies related to the use of specific immunoglobulins as an alternative to antimicrobial chemotherapy for infection treatment. Oral passive immunotherapy using specific antibodies is considered a new strategy actively pursued in different clinical studies for the last two decades (Leiva et al., 2020). Immunoglobulins can be defined as glycoproteins secreted by plasma cells after exposure to the specific antigen, and considered as a major component of humoral immunity (Tizard, 2002).

Immunoglobulin Y (IgY) acts a similar biological character as mammalian IgG, being regarded as the major antibodies against different infectious agents. Accordingly, both IgY and IgG have been used as synonyms, but IgY has become globally accepted relying on its unique features and its origin from the yolk of avian species (Tizard, 2002). The IgY antibodies were found to be transferred from the blood to the egg yolk of chickens for the first time (Klemperer, 1893). A protein of IgY that is known as gamma globulin has been detected in a gamma-livetin fraction of yolk (Williams, 1962). Later on, Leslie and Clem (1969) detected IgY in other parts of the body as duodenum, trachea, and seminal plasma. The concentration of IgY in the blood is 5-6 mg/ml while in the yolk is 10-25 mg/ml (Leslie and Martin, 1973; Rose et al., 1974). It is well known that immunoglobulins IgY, IgM, and IgA exist in chicken eggs (Leslie and Clem, 1969). Both IgM and IgA are present in egg white and secreted by the oviduct's mucosa (Rose et al., 1974). However, IgY immunoglobulins are found abundantly in the egg yolk (20 mg of IgY /1 ml, Yegani and Korver, 2010). Although immunoglobulin class IgY is present in a higher concentration in chickens (5-15mg/ml), other classes as IgM and IgA are also present at lower concentrations (1-3mg/ml and 0.3-0.5mg/ml; respectively) (Leslie and Martin, 1973; Kowalczyk et al., 1985). Immunoglobulins IgY represents about 75% of the total immunoglobulins in poultry. Egg yolk contains over 100 mg of IgY/egg (Mine and Kovacs-Nolan, 2002; Criste et al., 2020).

Oral immunotherapy using specific IgY antibodies has had an increasing interest in the last decade for the treatment of localized infections (Reilly et al., 1997). The transfer of pathogen-specific immunoglobulins via eggs from hens to their chicks, and their role in the protection of newly hatched chickens from the pathogens have been reported (Hamal et al., 2006; Liou et al., 2010). It has been documented that IgY could be used successfully for scientific, diagnostic, prophylactic, and treatment purposes, as well as preparation of immunochemical reagents, and the formulation of food due to their stability under processing conditions (Raj et al., 2004; Schade and Terzolo, 2006). Immunized chickens with a specific antigen produce antigen-specific IgY which is very important for prophylaxis and control of several diseases especially the enteric ones (Hatta et al., 1993; Hatta et al., 1997; Kovacs-Nolan and Mine, 2012; de Faria et al., 2019).

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Therefore, the present review article focused on the production and advantages of using IgY as a source of specific antibodies. In addition, the article investigated the role of IgY in the prophylaxis and treatment of different animals, poultry, and foodborne pathogens in humans.

Production of IgY

First, the production of IgY was a problem. Warr et al. (1995) termed the production and use of IgY antibodies as “IgY Technology”. In 1996, the European Centre for the Validation of Alternative Methods (ECVAM) recommended the use of yolk antibodies instead of mammalian antibodies for animal welfare (Schade et al., 1996). The egg yolk contains only class IgY antibodies that can be easily extracted from the yolk by simple precipitation techniques (Gassmann et al., 1990). The antibodies produced in chickens can recognize different epitopes, compared to the antibodies of mammals, and this gives access to a different antibody range than mammalian antibodies (Carlander et al., 1999).

The transfer of IgY from the ovaries to the embryos takes nearly 3-6 days (Patterson et al., 1962). The levels of IgY antibodies in the egg transferred to the offspring were directly related to the circulating levels of IgY in their hens (Al-Natour et al., 2004). The selective transfer of IgY from the hen’s serum to the membrane of the yolk sac occurs through specific receptors (Tressler and Roth, 1987). The crystalizable constant Fc and hinge regions of the antibody molecule region are required for this transfer (Morrison et al., 2001). In addition, the CH2-CH3 domain is detected by the receptor responsible for IgY transport. During the formation of eggs, IgYs corresponding to IgG in mammals are concentrated in the yolk, whereas IgM and IgA are present in the egg white (Morrison et al., 2001). After hatching, the yolk sac is considered as a good source of a passive humoral immune response as IgY is circulating in the blood, while IgM and IgA are passing from eggs white to the gastrointestinal tract for localized immunity. Furthermore, protein integrity of the yolk sac is very important for normal absorption of the yolk sac contents, and the transfer of IgY into the chicks’ circulation (Ulmer-Franco, 2012).

Different forms of IgY could be used in feed as whole eggs powder, whole yolks powder, water-soluble fraction powder, or purified IgY material. Laying hens should receive either oil emulsion or lipopeptide adjuvanted IgY as primary and booster immunization doses with four to six weeks interval (Schade et al., 1996). However, once the titers of antibodies begin to decline, it is a must to use another booster immunization dose during the laying time. The IgY secreted by B-cells could be detected in chickens’ blood in the first week after hatching, and then it gradually declined to start the active immunity (Hamal et al., 2006). Therefore, detection of the protective levels of humoral immunity in chickens depends mainly on the concentration of circulating-IgY in post-hatch. There is a strong positive relationship between the withdrawal of circulating-IgY from the chickens’ body and their activity levels during the first week of post-hatching, signifying the vital role of IgY in the activation of the chickens’ initial immunity (Rehan et al., 2019). The IgY remains in the chickens’ blood till the beginning of the second week of age (Smith and Beal, 2008). Accordingly, the adaptive immunity develops during the second week of life, and the early humoral protection in the chickens depends mostly upon the maternal transfer (Hamal et al., 2006). After immunization by a specific antigen, it takes about five to six days for IgY to transfer from the blood, and reach the egg yolk (Smith and Beal, 2008). The concentration of IgY in the yolk is relative to its concentration in the blood (Hamal et al., 2006). Among different avian species as well as the same lines of species, the amount of IgY is greatly variable (Carlander, 2002).

Advantages of using IgY

Contrary to antimicrobials, IgY antibodies have no side effects, including resistance to diseases or toxic residues, and they are environmentally friendly (Coleman, 1999). As an alternative to antibiotics, passive immunization using IgY antibodies is used to control many infectious diseases (Mine and Kovacs-Nolan, 2002; Schade et al., 2005; Xu et al., 2011). The production of IgY antibodies in hens is much less invasive and stressful, requiring only the collection of eggs rather than the bleeding of the animal (Schade et al., 1991). Besides, rapid induction of a considerable amount of antibodies with a relatively low cost, highly specific and immunogenic, and can be stored at 4° C for at least one year in eggs (Rose et al., 1974; Larsson et al., 1991). Production of IgY provides a more hygienic, cost-efficient, convenient, and abundant source of immunoglobulins when compared with the methods used for obtaining antibodies from mammals (Gassmann et al., 1990; Carlander et al., 2000). It has been documented that over 100 mg of IgY can be obtained from one egg indicating high yolk IgY concentrations (Akita and Nakai, 1992). Amro et al. (2018) demonstrated that IgY antibodies could be used as an alternative to mammalian ones, and it is preferable to immunize chickens before laying to avoid the stress of handling that adversely affects egg production.

Immunoglobulins IgY which reacted with rheumatoid factor or human anti-mouse IgG don’t interact with the complement or Fc receptors of antibodies (Larsson et al., 1991). So, IgY is an excellent antibody for immunodiagnostic assays that involved mammalian sera. Moreover, they can poorly cross-react with mammalian IgG due to immunological differences. Chickens’ IgY antibodies have advantages over mammalian types that they can detect various epitopes and giving access to a wide range of antibody repertoires (Carlander et al., 1999).

It is well known that ingested IgY antibodies (like other proteins) can be degraded by the action of acidity and proteolytic enzymes in the gastrointestinal tract. Therefore, the inclusion of IgY in the feed in the form of whole egg yolk powder may be a protective and economic method (Jaradat and Marquardt, 2000). In addition, microencapsulation has been found to be another efficient protective method (Chang et al., 2002; Cho et al., 2005; Kovacs-Nolan and Mine, 2005) but it is expensive to perform.

Applications of IgY

IgY and different animal species

There are different uses of IgY, especially in veterinary medicine. Whole eggs or yolk powders have been used as an alternative for the IgY treatment, especially for enteric diseases in various animal species. The mode of action of these IgY has been hypothesized before. This mechanism depends mainly on binding of immunoglobulins to specific bacterial surface epitopes, such as outer membrane protein, lipopolysaccharide, flagella, and fimbriae. After binding, impairment of biological functions of these epitopes may lead to the inhibition of the bacterial growth (Sim et al., 2000) as well as adhesion to the intestinal cells (Yokoyama et al., 1998). Accordingly, IgY could prevent intestinal bacterial adhesion (Girard et al., 2006; Chalghoumi et al., 2009b), and inhibit epithelial cells' invasion (Sugita-Konishi et al., 2002).

IgY antibodies have been used in calves for the prevention of bovine rotavirus (Kuroki et al., 1994; Özpınar et al., 1996; Vega et al., 2011), bovine coronavirus (Ikemori et al., 1997), *Salmonella Typhimurium* (*S. Typhimurium*), or *S. Dublin* (Yokoyama et al., 1998), *Yersinia ruckeri*, *Edwardsiella*, *Staphylococci*, *Pseudomonas* spp. (Mine and Kovacs-Nolan, 2002), and K99-piliated enterotoxigenic *Escherichia coli* (ETEC) (Ikemori et al., 1992).

In pigs, different studies have been performed to prevent diarrhea caused by different strains of *E. coli*, especially in young piglets (Wiedemann et al., 1991; Yokoyama et al., 1992; Yokoyama et al., 1993; Imberechts et al., 1997; Zuniga et al., 1997; Marquardt et al., 1999). Moreover, there was an increase in the growth performance of piglets fed on IgY (Owusu-Asiedu et al., 2003). Similarly, in rabbits, IgY antibodies have been used against diarrhea caused by ETEC infection (O'Farrelly et al., 1992). Pokorova et al. (2000) used IgY for the protection of dogs from canine parvovirus. In rodents, IgY has been used for the prevention of dental caries caused by *Streptococcus mutans* (Hamada et al., 1991), and for deactivation of urease of *Helicobacter pylori* (*H. pylori*) in rats (Chang et al., 2002), as well as prevention of gastritis in mice (Mony et al., 2019).

Oral administration of egg yolk plasma derived from sialyoligosaccharides, and their derivatives are valuable for the prophylaxis of *Salmonella* infection in mice (Sugita-Konishi et al., 2002). Lipopolysaccharides elicited a strong immunogenic reaction with the production of a large quantity of specific IgY, so they are potentially applied for inhibition of *Salmonella* adhesion and prevention of salmonellosis (Sunwoo et al., 1996; Mine, 1997). Mice challenged with *S. Enteritidis*, and treated with anti- *S. Enteritidis* flagella 14 IgY showed a survival rate of 77.8%, compared to 32% in mice fed normal egg yolk IgY (Peralta et al., 1994). In fish, aquarium treatment with anti-*Edwardsiella tarda* IgY succeeded in the protection of Japanese infected eels (Hatta et al., 1994). Li et al. (2016) showed that egg yolk antibody (IgY) has a protective effect against experimental *Vibrio splendidus* infection in the sea cucumber.

IgY in poultry

It has been demonstrated that newly hatched chicks could rely on IgY present in yolk as a source of acquired immunity till complete development of their immune systems occurs (Schade et al., 2005). Maternally derived antibodies are the primary sources of antigen-specific protection in young chickens as they are very susceptible to several pathogens during the first weeks of life due to their undeveloped immune system. Egg yolk IgY immunoglobulins have been used to neutralize specific organisms, especially enteric pathogens. Tamilzarasan et al. (2009) and Diraviyam et al. (2011) concluded that purified chicken immunoglobulins can be used for passive immunization and protection of young chickens against enteric infections. It has been regarded that IgY are effective substitutes to antimicrobials as they can bind with pathogens and inhibit their growth, multiplication and colony-forming abilities (Yegani and Korver, 2007).

Specific IgY antibodies significantly prevented and treated poultry from many bacterial pathogens, such as ETEC (Jin et al., 1998; Karamzadeh- Dehaghani et al., 2020), *S. Typhimurium* (Kassaify and Mine, 2004b; Chalghoumi et al., 2009a), *S. Enteritidis* (Lee et al., 2002; Chalghoumi et al., 2009a), *Campylobacter jejuni* (*C. Jejuni*) (Kassaify and Mine, 2004b; Vandeputte et al., 2019), and *Gallibacterium anatis* (Zhang et al., 2019) as well as infections with infectious bursal disease virus (El Khashab et al., 1995; Eterradossi et al., 1997; Malik et al., 2006; Yousif et al., 2006).

To reduce *S. Enteritidis* shedding in layers chickens, egg yolk powder containing anti-*S. Enteritidis* antibodies has been given to them (Kassaify and Mine, 2004a). The results revealed that oral treatments with powders in concentrations of 15% (wt/wt) for 28 days after experimental infection with *S. Enteritidis* induced a rapid reduction as well as a complete elimination of the organisms in the droppings after two weeks of treatments. Moderate to the high percentage of sero-positivity for *Salmonella*-specific IgY in hens immunized with polyvalent *Salmonella* bacteria has been detected (Agrawal et al., 2016).

Tsubokura et al. (1997) used egg yolk immunoglobulin IgY from immunized hens for the prevention and treatment of chickens infected with *C. jejuni*. In a preventive study, 14-days old chickens were orally inoculated with 0.5 g of anti-

C. jejuni IgY preparation while in the therapeutic trial, *C. jejuni*-infected chickens were given 0.2 g of IgY four days after infection. The reduction in bacterial shedding in the droppings was 99% and 80-95% in the preventive and therapeutic studies, respectively. Vandeputte et al. (2019) concluded that yolk IgY revealed a strong reactivity to *C. jejuni* and *C. coli* clonal complexes which reflected the passive immunization of bacterin-derived IgY to control *Campylobacter* colonization in poultry.

Broiler chickens treated with hen egg antibody showed a reduction of both *C. jejuni* and *S. Enteritidis* intestinal colonization (Wilkie, 2006). Experimentally infected chickens with *S. Enteritidis* or *C. jejuni* and treated either orally or in feed with egg yolks powders containing anti-*S. Enteritidis* or anti-*C. jejuni* IgY showed measurable IgY activity without significant reduction in the intestinal bacterial colonization. Khalf et al. (2016) demonstrated that oral administration of 40, 20, 10, and 5 IU/ml of IgY/bird after experimental infection with *Clostridium perfringens* type A, resulted in protective rates of 96%, 88%, 80%, and 60%, respectively. In addition, broilers sera of passively immunized chickens revealed antibody titers of 1, 2, and 1.5 IU in the first, second, and third days after immunization, respectively. Brady et al. (2002) detected that fractionated lipoprotein egg yolk from non-immunized hens has an *in vitro* antibacterial activity against some *Streptococcus* strains. Lillehoj and Sasai, (1994) and Kim et al. (2001) produced monoclonal IgY against *Eimeria* spp. causing avian coccidiosis.

Foodborne pathogens

The protective role of yolk fraction against some foodborne pathogens was identified *in vitro* (Kassaify et al., 2005). The inhibitory activities of specific IgY on the growth of *Salmonella* spp. (Lee et al., 2002; Chalghoumi et al., 2009b), *E. coli* (Sunwoo et al., 2002; Amaral et al., 2008), and *Candida albicans* (Wang et al., 2008) have been studied previously *in vitro*. Anti-*E. coli* O78:K80 IgY immunoglobulins revealed a reduction of *in vitro* growth of *E. coli* by 1.18 log colony-forming unit /ml (Mahdavi et al., 2010). It has been documented that IgY immunoglobulins can inhibit bacterial growth and biofilm creation *in vitro* through binding to the bacterial pathogen (Pereira et al., 2019).

CONCLUSION

Treatment with IgY is considered as an effective and safe alternative to the traditional treatment with antimicrobial agents. The effects of using IgY for controlling different pathogens in various animal species have been done with very successful results. Therefore, it is crucial to increase the uses of IgY in the field of veterinary medicine to counteract these significantly important pathogens, to overcome the antimicrobial resistance problem as well as to reduce the level of tissue residues that can affect on the human's health.

DECLARATIONS

Competing interests

The author has no conflict of interest.

Authors' contributions

Wafaa Abd El-Ghany collected all the data, wrote and revised the manuscript.

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