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The Use of Egg Yolk Antibodies for Food Protection and Immunity

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ABSTRACT

The chicken immune system has been studied for many years. These studies have contributed substantially to our understanding of the fundamental concepts of immunology and the development of different immunoglobulin classes. It is thus surprising that only a small fraction of the antibodies presently used in laboratories are of avian origin. Laying hen produces more yolk antibodies than rabbit at the same time. Animal care costs are lower in chickens compared to rabbits. Chicken antibodies offer many advantages to the traditional mammalian antibodies when used for the detection of mammalian antigen. Chicken antibodies can also be used to avoid interference in immunological assays caused by the human complement system, rheumatoid factors, human anti-mouse IgG antibodies or human and bacterial Fc-receptors.

Introduction

Immunity is the state of the multicellular organisms to cope with the exogenous and endogenous infectious threats either by body natural response via humoral cells or by the acquired / induced via vaccination and antigen exposures

There are basically two types of immunity which involves innate and adaptive immune response. Innate immunity is a sort of body natural immediate immune response in accordance to the attacking pathogen. Innate response is produced by skin, cilia, body hairs, and secretions like mucous, bile, gastric acid, saliva, tears and sweat, and some responses like inflammation and complement system (Kaur and Secord, 2019; Riera Romo et al., 2016). Inflammation increases the blood flow to flush the immune cells on the specific area (Singh et al., 2019). Complement system enhances the ability of the antibodies and phagocytes to neutralize and disable the pathogen from binding to the organ cell receptors (Duffy et al., 2014).

Adaptive immunity is of two types; Humoral and cellular immunity (Mitsuyama, 2008). Cellular immunity is mediated by the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to antigen (Ma et al., 2013).

The transfer of the ready mate

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antibodies to the non-immunized organism either as preventive or curative is the passive immunity, for example, colostrum feeding to newly born calves (Kovacs-Nolan and Mine, 2012). The neo-natal baby feed and colostrum feeding to the calves, and egg yolk antibodies are examples of natural passive immunity (Chase et al., 2008). An injection of the antisera i.e. a suspension of the antibodies particles as it is done in foot and mouth disease in animals (Sharma et al., 2017), and injection of snake venom following a bite (Pradhan et al., 2007), and ingestion of egg yolk antibodies that are produced by immunizing the layers against specific antigens are the examples of the artificially induced passive immunity (Müller et al., 2015).

Egg Yolk Antibodies

A scientist in 1893 named as Klemperer reported first time that antibodies are produced inside the egg yolk of the eggs laid by the immunized hens and it can transfer specific antibody to whom who eats it (Klemperer, 1893). After a long time, when the animal welfare became a serious threat for the researchers, they came into field to work on the Klemperer view. In 1959, Russell and Burch published an article about "The Principles of Humane Experimental Technique" on the IgY (Russell and Burch, 1959). After constant 20 years of the researches about the IgY, some commercial reagents about the Ig, purification kits, IgY-standards and labeled antibodies for example fluorescein isothiocyanate and peroxidase, and alkaline phosphatase was introduced in 1980 (Schade and Hlinak, 1996). After Dr. Claus Staak in 1995 introduced IgY technology, it was accepted internationally for passive immunization of the animals (Schade et al., 1996).

Similar to the mammalian antibodies, IgY consists of two light and two heavy chains. The heavy chain consists of one variable and four constant domains while the IgG have three constant domains in heavy chains. Mass spectrometric molecular weight of the IgY is 167,250 Da (IgY light chain MW= 18660, heavy chain MW= 65105 Da, Fab fragment MW= 45,359 Da) while of IgG is 1600,000 Da (Sun et al., 2001).

There is one variable and one constant domain of the light chain (Shimizu et al., 1992). The Fc part of the IgY contains two carbohydrate side chains while the IgG have only one CHO side chain (Cser et al., 1982).

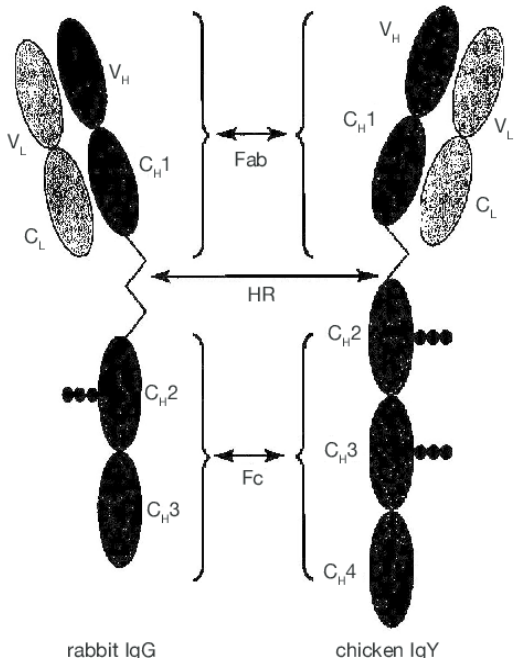

Table 1. Antibody sources in egg yolk and blood serum (Rudolf et al., 2009)

Parameters	*IgY*	*IgG*
Source of antibody	Egg yolk	Blood serum
Antibody amount	100–150 mg/egg (5–7 eggs per week)	200 mg/bleed (40 ml blood)
Amount of specific antibody	2–10%	5%
Interference with mammalian IgG	No	Yes
Interference with rheumatoid factors	No	Yes
Activation of mammalian complement	No	Yes
Protein A/G binding	No	Yes

IgY hydrophobic moiety is the Fc part of the antibody and it is bigger in size than the Fc part of the IgG. Hence IgY is more hydrophobic than IgG. This sort of property makes it easy for stable absorption on the latex particles and coated to the latex microspheres maintains its binding activity. The isoelectric point of the IgY ranges from 5.7 to 7.6 while that of the IgG is 6.1 and 8.5 (Dávalos-Pantoja et al., 2000).

The practice of IgY orally demands the analysis of the temperature and pH stability in gut as well as the resistance to the proteolysis as it passes from stomach to the intestines (Wei et al., 2019).

IgY is cost effective and does not activate the complement system. High titers of specific IgY are obtained from immunized hen eggs without pain and bleeding for a long time.

IgY production processes as following (Pauly et al., 2011);

a) Immunization of the chicken i.e. the layers

b) Egg collection

c) Extraction of the antibodies

d) Purification of the antibodies

The factors affecting immunization intensity of the chicken as following (Pauly et al., 2009);

a) The antigen dose

b) The adjuvant

c) The application route

d) Environmental factors

e) Breed and age

For each antigen the optimal dose should be tested experimentally for fruitful immunization because the dose may vary according to the molecular structure of the antigen. Antigen dose has direct effect on the antibody titer after the immunization (Tini et al., 2002).

The first antigen inoculation was done in 1893 as intra peritoneal by Klemperer (1893). Comparative analysis of intramuscular and subcutaneous route for antibody titer and it was reported that subcutaneous produce more antibodies than intramuscular (Hodek and Stiborova, 2003). The intravenous application is also can be done (Müller et al., 2015). Antigen could also be given in drinking water orally by diluting at appropriate concentration (Araújo et al., 2010).

In early embryonic development, parent birds transfer the antibodies to

their newly hatched chicks (Ward, 2004). IgY is transferred from the mother blood to the egg yolk through the oocyte cytoplasmic membrane receptors during its development to the complete egg (Nimmerjahn and Ravetch, 2007) and it ranges from the 50 to 100 mg/egg. IgY secretion in the blood stream of the newly hatched chicks start after the 6 day of the hatching (Rose et al., 1974) and the process of antibody secretion in the blood stream and the sequestration in the oocyte continue throughout the productive life of the hens at an equilibrium rate (Dias da Silva and Tambourgi, 2010).

Using Egg Yolk Antibodies

Many researches reported about the uses of the egg yolk antibodies for decades. They are important in the fields of biomedical sciences, laboratory purposes, aqua farming, human medicines and veterinary medicines to enhance the immune system (Greunke et al., 2008).

It is recognized universally that any pathogen specific antibodies can be obtained in large quantities and more rapidly production from the eggs laid by the hyper-immunized hens as compared to the other mammalian IgG. It is also recognized that there is no need to slaughter the animal or bleed the animal to collect the antibodies; the antibodies can be stored for a long period of time at 4 C as compared to the IgG (Pauly et al., 2009).

Neonatal calf diarrhea is caused by Bovine Rota Vira (BRV) and is a common diarrheal disease in cattle (Lee et al., 1995) and for the passive immunization of the calves' anti-BRV IgY is developed (Yoo et al., 1997). Monoclonal antibodies developed against the VP8 antigens on the surface of the rotavirus are found to neutralize the virus in vivo and protect the mice against infection passively (Kim et al., 2017). In a study conducted on the mice, IgY showed a preventive response against murine rotavirus infection (Vega et al., 2011) and bovine rotavirus and human rotavirus induced gastroenteritis (Thu et al., 2017). Anti-Bovine Rotavirus IgY were given to infected calves orally in the powdered egg form and it controlled the diarrhea and hyperthermia (Vega and Parreño, 2014). Egg yolk antibodies are also reported to have good effects in poultry disease causing viruses. IgY were developed against the NDV virus, IBDV (Bursa disease virus) influenza and reo-virus and passive immunization was performed of the broilers and layers (Boudaoud and Mamache, 2012). The efficacy of Anti-IBD IgY was checked in commercial laying hens, broilers commercial and breeders and 92% recovery rate was reported as compared to the 10% rate for control birds (Malik et al., 2006).

Egg yolk antibodies are prepared against *Vibrio parahaemolyticus* were prepared by immunizing the hens with outer membrane proteins of the bacteria and these were proved to be very effective for the growth inhibition of the *Vibrio parahaemolyticus* in experimentally infected shrimps. The increased the activity of superoxide dismutase (SOD) in muscles of the infected shrimps helps to mediate tissue health by destroying the potentially

damaging reactive oxygen species (Hu et al., 2019).

IgY antibodies are successfully used to develop IgY-based ELISA to quantify the pregnancy associated protein having molecular Weight 29KDa (Kilo Delton) which is known as early pregnancy factor in sows as early as 72 hours after insemination. So for early verification/ diagnosis of pregnancy IgY technology made its use in sows. This pregnancy protein is separated from the serum and it is used for ELISA and it serves as an antigen (Grosso et al., 2015).

Trypnosama cruzi is a flagellated protozoon which belongs to the *Trypanosomatidae* family and cause Chagas disease (Elmayan et al., 2019). There is no effective vaccine and treatment for this disease. The IgY anti-*T. cruzi* characterization was performed using polyacrylamide gel electrophoresis (SDS-PAGE), western-blot and enzyme-linked immune-sorbent assay (ELISA) (Grando et al., 2017).IgY have been reported to be used for the identifying some of the harmful substances i.e toxins and antibiotic residues in the consumer food products.

The influence of chitosan coating augmented with IgY produced against suspension of psychrophilic bacteria, *Pseudomonas fluorescens* and *Shewanella putrefaciens* was seen on microbial and sensory quality of rainbow trout fillet during refrigeration (4 ± 1 °C) and it increased the shelf life of the stored food products (Ehsani et al., 2019).

The antibacterial activity of the specific IgY against *Pseudomonas fluorescens* and *Shewanella putrefaciens* was confirmed by chemical analysis (pH, total volatile base nitrogen and 2-thiobarbituric acid value) and sensory evaluation, and the shelf life of the refrigerated Paralichthys olivaceus samples at (4 ± 1 °C) and shelf life was extended approximately from 9 days to 12-15 days in the presence of the specific IgY (Xu et al., 2012).

A potential use of IgY, egg yolk antibodies, are for the prophylaxis of the dengue fever. Anti-DENV2 IgY were obtained from the goose and used in mice orally and in vitro. The result showed that the antibodies neutralized the virus in vitro and in vivo without adherence to myeloid tissue receptors (Fink et al., 2017).

For some oral infections, IgY has been used as a therapeutic agent. For example, specific IgY against *Prevotella intermedia* inhibited bacterial growth in vitro in a liquid medium and also show therapeutic activity in rats with induced gingivitis due to P. intermedia and histo-pathalogical examinations showed no abnormal sins in the gums of the rats (Hou et al., 2014).

IgY were prepared against *Fusobacterium necleatum* and therapeutic effect was seen in vitro in liquid medium and in vivo in mice and result showed that the antibodies reduced the growth and biofilm formation in vitro, and reduced the bone loss in mice with periodontal disease (Xu et al., 2012).

Efficacy of anti-Propionibacterium IgY was seen in a trial. This is a bacterium that causes acne on skin. The antibody inhibited the bacterial growth and biofilm formation in vitro (Revathy et al., 2014).

Conclusion

According to the above-mentioned information, there is no doubt that the egg yolk antibodies are very beneficial for the animal and human health by passive immunization. Not only egg yolk antibodies made its emissive use in diagnostics both for animal and human diseases but also very important for the detection of the undesired particles in the food products, hence, very useful for the food safety.

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