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Efficient production of chicken egg yolk antibodies against various infectious Diseases

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Abstract
Chickens have the potential to be used to complete the spectrum of animals used for antibody production. IgY technology is a fast developing field and we have tried to cover most of its aspects. IgY technology was that, once accepted and widely used, the technology will offer alternatives and solutions to science, to medicine and to society as a whole. In particular, because of the increasing resistance of microorganisms to antibiotics, research on all aspects related to the development of specific IgY against pathogenic microorganisms will have to be intensified. IgYs can be used both in veterinary medicine and in human medicine. This review offers summarized information about IgY technology and the use of these antibodies against various infectious diseases.

Keywords: IgY technology, antibody, microorganisms, medicine

Introduction
During the past 20 years, the use of chickens instead of mammals for antibody production has increased. A major advantage of using birds is that the antibodies can be harvested from the egg yolk instead of serum, thus making blood sampling obsolete. In addition, the antibody productivity of an egg-laying hen is much greater than that of a similar sized mammal (Hau & Hendriksen, 2005) [5]. 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. The production of antigen-specific antibodies in egg yolk also has significant implications for nutraceutical and functional food development.

Antibodies from eggs
Antibodies are produced by the immune system of an animal in a specific response to a challenge by an immunogen. Immunogens (antigens) are molecules which can induce a specific immune response and are usually foreign proteins or carbohydrates or sometimes lipids and nucleic acids. Antibodies are secreted from plasma cells which have differentiated from B lymphocytes after appropriate stimulation by the foreign immunogen. Chicken egg yolk antibody (IgY) has received much attention in recent years because it can be easily prepared in high concentration and is both affordable and safe (Gassmann *et al*., 1990) [3]. New vaccine technology has led to vaccines containing highly purified antigens with improved tolerability and safety profiles, but the immune response they induce is suboptimal without the help of adjuvants. Gottstein and Hemmeler, 1985 reported Chickens store high contents of IgY in the yolk and are considered to be efficient antibody producers.

IgY production
- **Immunization**

Specific IgY development and production can be achieved byimmunizing laying hens with the target antigen. However, the resulting immune response of the immunized hens cannot be very predictable. Mainly five factors influence this response: the antigen (dose and molecular weight), the type of adjuvant used, the route of application, the immunization frequency, and the interval between immunizations (Schade *et al*., 1996) [10].

- **Antigen**

The immune response is triggered by contact of the organism with antigen, which is a structure that is recognized by the immune system as foreign ("nonself"). The dose of antigen influences significantly the immune response and the antibody titre that is evoked. Too much or too little antigen may induce suppression, sensitization, tolerance or other unwanted immunomodulation found that the injection of antigen concentrations ranging between 10 µg and1 mg elicited good antibodies responses, and this was also reported by other researchers (Mahn, 1998) [7].

- **Adjuvant**

The induction of high and sustainable egg yolk antibody titre reclaims the use of adjuvant. There are more than 100 known adjuvants, which differ in their chemical characteristics, their efficacy in stimulating the immune system, and their secondary side-effects. Freund's complete adjuvant (FCA) remains the most effective adjuvant for antibodies production in laboratory animals. In mammals, the use of this adjuvant leads systematically to severe inflammation at the injection site. In birds, the use of FCA does not seem to result in the same severe lesions as in mammals. The results of Gassmann *et al.,* (1990) [3] suggest that chickens show higher resistance to tissue damaging potency of FCA than rabbits.

- **Route of application**

The most common route for antigen injection in hens for IgY production is the intramuscular route. Injection is usually performed in the breast muscle. Chicken can also be injected subcutaneously in the neck. With very young animals, it may be preferable to inject intramuscularly into the breast muscle, because subcutaneous injection is more difficult to perform and

can therefore cause more distress (Schade *et al.,* 1996) [10].

- **Immunization frequency**

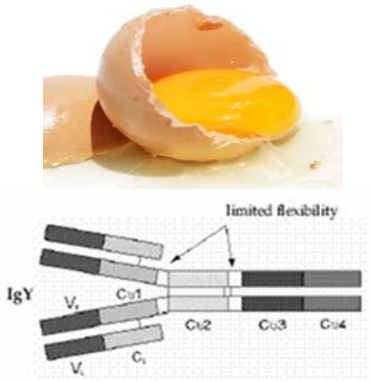
The total number of immunizations required depend on the type and dose of the antigen as well as the adjuvant employed. At least two immunizations have to be given. Yolk antibody titres should be checked 14 days after the last immunization. The success of an immunization protocol depends also on the interval between the first and second and subsequent immunizations. Often reported interval is two to four weeks (Tini *et al*., 2002) [13].

Importance of IgY

Over the past few years, we have successfully used the chicken egg yolk system to produce polyclonal antibodies to enamel proteins and other calcified tissue matrix proteins (Nanci *et al.,* 1996) [8]. Furthermore, the amount of antibodies produced from an egg is equivalent to that from 200 to 300 ml of mammalian blood, and the costs for animal care per unit production of antibodies are much lower in chicken than in mammals. However, the practical use of IgY in research and diagnostics is limited due to complex and time-consuming purification steps associated with the further purification of IgY (Akita and Nakai, 1992) [1].

Properties of IgY

Laying hens transfer large amounts of immunoglobulin from serum to egg yolk of their eggs, where it serves as a means of passively protecting the developing chicks (Kariyawasam *et al*., 2004) [6]. An average egg may contain 100~150 mg of yolk immunoglobulins (IgY), and substantial amounts of specific antibodies may be collected and purified from the eggs of immunized hens (Akita and Nakai, 1992) [1]. The availability of large amounts of relatively inexpensive IgY from egg yolks makes it feasible to use these antibodies for passive immunization by oral administration or injection (Carlander *et al*., 2000) [2].



Antibody stability

IgY is fairly heat stable and most antibody activity remain after 15 min at 70°C. Incubation of IgY at pH above 4 is well tolerated, but at pH 2 and 37°C the activity is rapidly decreased. The rapid activity loss is probably due to conformational changes, as the polypeptide is not broken down as observed by SDS-PAGE. The immunological activity of IgY is not affected by pasteurization at 60°C for 3.5 min. Addition of high concentrations of sucrose stabilizes IgY regarding heat denaturation, acid environment as well as high pressure. IgY fractions have been stored in 0.9% NaCl, 0.02% NaN_3 at +4°C for over 10 years without any significant loss of antibody titer. An egg can be stored in +4°C, with just a small loss of IgY activity for at least six months (Carlander, 2002).

Applications of IgY

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. IgY Abs are used successfully in immunohistochemistry for detection of antigens of viral, bacterial, plant and animal origin, and also to assess the incidence of intestinal parasites in domestic animals Schniering, (1995) [11] and the contamination of foods with toxins or drugs Pichler *et al.,* 1998. [9] During the past decade, IgY Abs have increasingly been used in therapy or prophylaxis of disease and also in the new context of socalled "functional food". Recent studies have compared the properties of specific IgY and IgG Abs originating from identically immunised animals. The results mostly confirm that IgY Abs can be used in the same way as IgG Abs, but additionally offer advantages in terms of specificity, cross-reactivity and/or sensitivity.

Powdered whole eggs or yolks have been used as an inexpensive alternative for the IgY treatment of enteric diseases in veterinary medicine. The most famous example of a successful therapeutic/prophylactic use of IgY is the treatment of calves and piglets with specific Abs against *Escherichia coli*, rotaviruses and coronavirus Mahn, (1998) [7]. Sunwoo *et al*. 2002 [12] were able to demonstrate *in vitro* a marked growth inhibiting effect of specific IgY on *E. coli* 0157:H7, and showed that the growth inhibition was actually caused by the binding of specific IgY to the bacterial surface antigens, which caused significant changes in the bacterial surface structure. Studies using both animal models and trials in field herds have been carried out. These studies confirmed that treatment of diarrhoea in calves and piglets with specific egg yolk Abs has achieved significant prophylactic and therapeutic benefits.

Conclusion

The significant potential of avian antibodies for further use in immunodiagnostics and identification of disease markers, immunotherapy and the treatment and prevention of disease is expected. IgY technology will allow for new potential applications of IgY in medicine, public health, veterinary medicine, and food safety. Since lot of benefits of IgY technology and its universal application in both research and medicine, it is expected that IgY will play an increasing role in research.

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