doi:10.1017/S0043933910000048

Application of egg yolk antibodies as replacement for antibiotics in poultry

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The ban of sub-therapeutic antibiotic use in the European Union countries and elsewhere, and recent moves toward removal or reduced use of these compounds in other countries has put pressure on the poultry industry to look for viable alternatives. Available data suggest that specific egg yolk antibodies (EYA) have beneficial effects in prevention or treatment of bacterial and viral infections in humans and different animal species. The number of studies conducted in chickens, however, is quite low compared to other species and this may be a limiting factor in the current use of this technology in the poultry industry. The objectives of this review paper are to discuss how EYA are produced and work, to present examples of their applications in different pathological conditions in chickens, and to address challenges that this technology is currently facing.

Keywords: egg yolk antibodies; IgY; infectious diseases; antibody responses; antigens; feed; poultry

Introduction

Antimicrobials have been used in the poultry industry for growth promotion (sub-therapeutic doses), disease prevention (prophylactic doses), and treatment of infections (therapeutic doses; World Health Organization, 1997). Phasing out of these substances from poultry diets is a change with many consequences (Bedford, 2000). Years of research and practical experience have shown that antimicrobial use has resulted in significant improvements in animal production performance and health status (Coates *et al.*, 1955; Miles *et al.*, 2006). There is, however, increasing evidence concerning the economic feasibility of inclusion of antibiotic growth promoters (AGP) into poultry diets. A group of researchers at the Johns Hopkins University have recently investigated the economic effects of removing antibiotics used for growth promotion in commercial broiler chickens (Graham *et al.*, 2007). The use of AGP in poultry production may actually be associated with economic losses to producers. They concluded that the

Portions of the content of this paper were published in a trade magazine, World Poultry 2007 23(5): 22-25

© World's Poultry Science Association 2010
World's Poultry Science Journal, Vol. 66, March 2010
Received for publication January 23, 2009
Accepted for publication July 3, 2009

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weight gains that resulted from these antibiotics were not sufficient to offset the cost of their incorporation into poultry diets.

Certain types of antimicrobials have also been used for disease control in human medicine. There is increasing microbiological and clinical evidence that resistant bacteria may pass from animals to humans, resulting in infections that are more difficult to treat (World Health Organization, 1997; Gorbach, 2001). Consumer and industry concerns regarding antibiotic-resistant bacteria and the need to treat diseases caused by pathogens that do not respond to antibiotics, has put tremendous pressure on the poultry industry to withdraw or limit use of AGP in poultry feed and to look for viable alternatives (Karlsson *et al.*, 2004). It is unlikely that a single viable alternative to antimicrobials will be implemented successfully. An effective alternative to in-feed antibiotics should have a significant and sustainable beneficial impact on production performance and health, be safe for both poultry and humans, be easy to apply and store and provide a substantial return on investment (Collett, 2004). Although several review papers have been published on egg yolk antibodies (Schade *et al.*, 1996; Carlander *et al.*, 1999, 2000; Karlsson *et al.*, 2004), there is a need to focus on the roles that these antibodies may play in prevention and control of poultry diseases.

Characteristics of Egg Yolk Antibodies (EYA)

The presence of immunoglobulins in eggs is an example of passive immunity because these antibodies are derived from the dam and protect the offspring from various infectious diseases after hatch (Hatta *et al.*, 1997). The acquisition of passive immunity in birds was first noted in 1893 when Klemperer showed the transfer of immunity to tetanus toxin from hen to chick (Rose and Orlans, 1981). Three immunoglobulin classes (IgA, IgM, and IgY) are present in chickens (Karlsson *et al.*, 2004). Leslie and Clem (1969) proposed that chicken IgG be designated as IgY, as it is different from mammalian IgG and forms the main immunoglobulin in chickens (Leslie and Clem 1969; Leslie *et al.*, 1971a;b). IgY is transported from the hen to the embryo via the egg yolk, which, as a result contains high concentrations of this antibody (Hamal *et al.*, 2006). Other immunoglobulin classes are present in negligible amounts in the egg yolk (Carlander *et al.*, 1999) and IgY is not present in the egg white (Rose *et al.*, 1974). The amount of IgY deposited in the egg is directly influenced by the circulating levels in the dam (Hamal *et al.*, 2006; Kitaguchi *et al.*, 2008). As stated earlier, higher levels of antibodies are usually found in egg yolk than serum, although published data in this area of research are not consistent (Rose *et al.*, 1974; Kariyawasam *et al.*, 2004; Malik *et al.*, 2006).

A laying hen can produce approximately 300 eggs annually, and each egg yolk volume is approximately 15 ml (Wilkie, 2006). The amounts of IgY in yolk are 20-25 mg/ml (Rose and Orlans, 1981), which would supply over 100 g of antibody per hen per year. There are several reports in the literature indicating that IgY levels in the egg yolk are not always consistent and may vary within and between bird populations. Carlander *et al.* (2001) demonstrated that there was day to day variation in concentration of IgY in eggs produced by individual laying hens although this variability was smaller than what was seen among hens. In another study, it was found that the IgY concentration varies significantly among different genetic lines. Great variations were also observed among individual hens within each strain (Carlander *et al.*, 2003). Production parameters may play a role in this regard. Li *et al.* (1998) demonstrated that egg yolk weight and the percent hen-day production in laying hens may influence efficiency of IgY production. They compared two lines of laying hens and found that the total content of yolk IgY in

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the line with higher rate of egg production and larger egg size was greater, although there was no significant difference in the activity of IgY produced by two strains of laying hens. The above-mentioned information indicates that it is possible to increase IgY production by genetic selection within high-producing lines (Carlander *et al.*, 2003). This could be an important step for large-scale production of egg yolk antibodies (EYA).

Egg yolk antibodies

Laying hens are efficient producers of antibodies compared to mammals as EYA can be obtained non-invasively (blood collection is not needed), are produced in high amounts, and isolation process of these antibodies from the yolk is efficient and economical (Schade *et al.*, 1996; Karlsson *et al.*, 2004). Higher levels of antibodies are usually found in egg yolk than serum of hens following immunisation against a specific pathogen (Rose *et al.*, 1974, Kariyawasam *et al.*, 2004; Malik *et al.*, 2006). In order to produce EYA, hens are exposed (usually through intramuscular or subcutaneous injection) to an antigen which induces immune responses including production of specific antibodies to those antigens. These antibodies are then naturally transferred to the egg yolk (Loeken and Roth, 1983; Sunwoo *et al.*, 1996). Booster immunisations are usually given at later time on a regular basis to ensure continued transfer of antibodies from hen to the egg yolk (Sunwoo *et al.*, 1996).

These antibodies are subsequently extracted from the egg yolk. The isolation process consists of separation of the yolk from the white and then antibodies (present in the yolk) are purified to remove all other materials. There are various methodologies for harvesting of IgY from the yolk, which generally have different rates of recovery (Akita and Nakai, 1992; Ko and Ahn, 2007; DeMeulenaer and Huyghebaert, 2001). After extraction of antibodies from the yolk, they are purified and processed to be administered directly to the animal or incorporated into the feed.

Adding antibodies in the form of whole egg yolk powder to poultry feed is currently the most common method of application. Thermal destruction of IgY may occur at temperatures higher than 75°C, with antigen binding activity of IgY being decreased with increasing temperature and heating time (Chang *et al.*, 1999). However, antibodies might be sprayed on the feed after processing in order to minimise denaturation of these compounds.

EYA can be administered *in ovo* but there are some concerns that injection of these antibodies may cause significant mortality and reduced hatchability. It may also affect absorption of yolk sac after hatch and subsequent growth rate of *in-ovo* injected chicks (Eterradossi *et al.*, 1997). These drawbacks may limit this type of application of EYA at commercial level.

Administering EYA has met with some degree of success in prevention or treatment of viral and bacterial infections in humans (Sarker *et al.*, 2001; Amaral *et al.*, 2002; Shin *et al.*, 2002; Karlsson *et al.*, 2004; Nilsson *et al.*, 2008), pigs (Yokoyama *et al.*, 1992; Yokoyama *et al.*, 1997; Kweon *et al.*, 2000; Owusu-Asiedu *et al.*, 2003; Girard *et al.*, 2006), calves (Özpinar *et al.*, 1996; Yokoyama *et al.*, 1998), dairy cows (Zhen *et al.*, 2008), fish (Lee *et al.*, 2000) and rabbits (O'Farrely *et al.*, 1992). EYA have also been used for diagnostic purposes in humans and animals through laboratory assays such as ELISA (Piela *et al.*, 1984; Gottstein and Hemmeler, 1985; Gast *et al.*, 1997; Holt *et al.*, 2000; Hagan *et al.*, 2004; Malmarugan *et al.*, 2005; Thomas *et al.*, 2006; Young *et al.*, 2007; Cray and Villar; 2008).

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EYA and poultry infectious diseases

SALMONELLOSIS

Lee *et al.* (2002) observed that the binding activity of IgY against *Salmonella enteritidis* or *S. typhimurium* resulted in inhibition of bacterial growth *in vitro*. This finding suggests that IgY can bind to *Salmonella* surface components and results in structural and functional changes of these molecules which may subsequently inhibit bacterial growth.

In a further study conducted by Wilkie (2006), anti-*S. enteritidis* EYA were administered to broilers through feed or oral gavage. These antibodies were able to inhibit pathogen attachment to rat epithelial cells and porcine mucin *in vitro*. In a follow-up study, EYA were orally administered to day-of-hatch broiler chicks which had been infected with *S. enteritidis*, however no significant reduction in intestinal colonisation was observed. It was concluded that more work is needed to understand the factors influencing antibody activity in broilers and the intestinal conditions that can antibody efficiency before EYA can be considered a prophylactic strategy to reduce salmonellosis.

The use of whole egg powder containing antibodies as a feed additive may be an alternative way to reduce the rate of *Salmonella* contamination of eggs. Gurtler *et al.* (2004) investigated the protective effect of orally-administered egg powder containing antibodies against *S. enteritidis* in laying hens. The rate of egg contamination was reduced from 29.4% to 13.3% by oral administration of whole egg powder containing *S. enteritidis*-specific antibodies.

A combination of probiotics and EYA might be more effective in reducing the colonisation of *S. enteritidis* in poultry (Fulton *et al.*, 2002). These researchers found out that egg-derived anti-*S. enteritidis* antibody prevented infection of ducklings when given at least 5 d before experimental infection. It was observed that probiotics (*Lactobacillus acidophilus, L. bulgaricum, L. reuteri, and Bifidobacterium bifidum*) worked synergistically with orally-administered EYA to prevent infection. This synergism might be due to prevention of *S. enteritidis* from colonising the intestine through competitive exclusion or via the antimicrobial effect of bacteriocin secreted by probiotic bacteria (Fulton *et al.*, 2002). A similar synergism between EYA and probiotics was reported by Tellez *et al.* (2001).

Different feed additives including *Lactobacillus spp.*, organic acids, multiple probiotics, or egg powder containing antibodies were evaluated for their efficacy in preventing gut colonisation and organ invasion in chicks infected with serotype *S. enteritidis* phage type 13a (Opitz *et al.*, 1993). Gut colonisation and organ infection declined during the six-week experimental period in all treatments, but none of the feed additives proved to be effective as a single measure for preventing *S. enteritidis* infection in chickens.

CAMPYLOBACTERIOSIS

Anti-*Campylobacter jejuni* EYA were capable of significant reduction in colonisation by *C. jejuni* in rat epithelial cells and porcine mucin *in vitro*, there was no effect in day-of-hatch broiler chicks (Wilkie, 2006). It was suggested that work is still needed to better identify prophylactic effects that these antibodies might have *in vivo*. Tsubokura *et al.* (1997) used EYA obtained from immunised hens for prophylactic and therapeutic applications in chickens infected with *C. jejuni*. In a prophylaxis experiment, these antibodies caused a significant reduction (up to 99%) in the *C. jejuni* faecal count throughout the experiment. In a further trial, the therapeutic efficacy (antibodies given after establishment of the infection) was demonstrated, but at a lower rate of reduction

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(80-95%) compared to prophylactic trial. It was suggested that the main mechanism of action was prevention of attachment of bacteria to the intestinal wall which led to reduced colonisation.

NECROTIC ENTERITIS

Laying hens hyperimmunised using *Clostridium perfringens* inoculation resulted in production of antibodies against *C. perfringens*, indicating possibility for EYA to be used to prevent clostridial diseases in poultry. However, more work is needed in this area. Feeding trials were performed to assess the efficacy of feed amended with the EYA in reducing the level of colonization of *C. perfringens* in challenged birds. It was observed that the oral administration of EYA did not reduce intestinal *C. perfringens* in experimentally-challenged birds. Surprisingly, intestinal lesion scores were higher in the birds that received the anti-*C. perfringens* EYA. This finding is an indication that the antibodies might in fact worsen necrotic enteritis although the reasons for this are unclear.

E.COLI INFECTIONS

EYA induced by immunising hens with selected *Escherichia coli* antigens were evaluated for their ability to protect broiler chickens against experimental respiratory tract infection and associated septicaemia caused by avian pathogenic *E. coli* (Kariyawasam *et al.*, 2004). Seven groups of broiler breeder hens were each vaccinated with *E. coli* antigens and EYA were obtained from eggs produced by the vaccinated hens. This was injected intramuscularly into 11-day-old broiler chickens, which were challenged three days later with *E. coli* by the intra-air sac route. One-day-old chicks which received IgY intramuscularly developed protective antibodies in serum that were significantly higher compared to the control group for up to 21 days after administration of the IgY. These protective antibodies were still detectable at day 28 when the experiment was terminated.

Sunwoo *et al.* (2002) investigated the activity of chicken EYA *in vitro* to determine the growth inhibitory effects of these antibodies on *E. coli* O157:H7. It was found that the growth inhibitory effect of IgY was high, dose-dependent and could be enhanced by higher concentrations of IgY. The mechanism of growth inhibition by antibodies is not fully understood but it appears to be related to interaction of these antibodies with surface components (*i.e.* outer membrane protein, lipopolysaccharide, flagella, and fimbriae) of *E. coli* O157:H7. This may impair biological functions of these essential surface components (Sunwoo *et al.*, 2002).

INFECTIOUS BURSAL DISEASE (IBD)

Malik *et al.* (2006) investigated the efficacy of EYA, obtained from laying hens immunized with IBD vaccines, in controlling of IBD in commercial laying hens. Antibody titres were significantly higher in yolk than serum of the hens. IBD-infected birds (commercial broilers, layers, broiler breeders, and indigenous birds) injected with these antibodies showed a 92% recovery rate, as compared to a 10% rate for saline-injected controls.

In-ovo inoculation of purified anti-IBD EYA may be a good experimental model for maternally transmitted anti-IBD immunity (Eterradossi *et al.*, 1997). Concentrated EYA were inoculated via the intra-vitelline route into 7-day-old embryonated specific pathogen-free (SPF) eggs. This resulted in hatching two series of SPF chicks with passive immunity against highly virulent IBD virus. The protective immunity produced as result of this method of application was comparable with those reported for non *in-ovo* injected chicks.

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NEWCASTLE DISEASE (NDV)

Wills and Lunginbuhl (1963) found that sub-cutaneous administration of egg yolk containing high levels of antibodies protected 80% of the birds against virulent NDV for the four week duration of the study. It was suggested that this approach can also be used for other poultry infectious diseases. Although there is a relative lack of recent studies in this area, other older studies reported similar results (Box *et al.*, 1969; Stedman *et al.*, 1969).

MIXED INFECTIONS

EYA obtained from hens immunised with a specific infectious agent can provide protection against the disease caused by that pathogen. Simultaneous immunisation of hens with a mixture of pathogens may result in production of EYA effective against each of these pathogens (Sugita-Konishi *et al.*, 1996). These researchers immunised hens with a mixture of twenty-six strains of bacteria and investigated the effects of antibodies collected from eggs on three infectious strains of bacteria (*Pseudomonas aeruginosa*, *S. enteritidis* and *Staphylococcus aureus*), representing opportunistic, invasive, and toxin-producing bacteria, respectively. The EYA produced by these hens prevented multiple bacterial diseases (produced by the above-mentioned strains) by inhibiting processes such as bacterial growth, toxin production or adhesion to cultured human intestinal epithelial cells (Caco 2).

Current challenges

Although beneficial effects of pathogen-specific EYA in animals have been known for about 20 years, results of experimental application of these antibodies to poultry have not always been consistent. Another interesting point is that the quantity of studies concerning possible beneficial effects of EYA in chickens is quite low compared to studies in human and other animal species. This may be a limiting factor in the application of this technology in the poultry industry. In addition, it has been suggested that technical difficulties including bird housing and problems associated with antibody extraction might have limited wider use of IgY in the industry (Schade *et al.*, 1996).

There are still many obstacles which make administration of EYA to commercial poultry a difficult goal to achieve. Like any other novel product, it may take a long time to get approval from regulatory authorities in order to use EYA on commercial poultry farms. Finding suitable alternatives to AGP is a high priority for the industry. This lack of immediate availability appears to have diverted attention from EYA to other more readily available alternatives such as organic acids, prebiotics and probiotics.

The dose of antigen in a given vaccine, route of vaccine administration (intramuscular vs. subcutaneous), frequency of vaccination (initial and boosters) and type of adjuvant present in the vaccine can all affect antibody production in the egg yolk. Adjuvants are different in efficacy and this difference can influence antibody titre in serum and subsequently in the egg yolk (Erhard *et al.*, 2000; Levesque *et al.*, 2007). Chang *et al.* (1999) demonstrated that specificity of IgY of intramuscular treated hens was nearly ten times higher than those antibodies produced by subcutaneous route.

Another important consideration is the way that chickens generally respond to a treatment protocol in commercial operations. When a disease outbreak occurs, responses to treatment may vary within and between flocks. The same rule may apply when EYA are used for prophylactic or therapeutic purposes which may not be in agreement with what has been seen under experimental conditions. Variation in

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response to treatment is, to some extent, related to the virulence of disease-causing agent. The rate of transfer of antibodies against specific pathogens to the egg yolk is significantly different and some part of this variation might be due to genetic differences among chicken lines (Abdel-Moneim and Abdel-Gawad, 2006; Hamal *et al.*, 2006; Gharaibeh *et al.*, 2008).

Poultry are exposed a wide variety of infectious agents in commercial farms. The poultry industry could benefit more from EYA if they are produced against a variety of common disease-causing microorganisms (Sugita-Konishi *et al.*, 1996). If this approach works well, it may help, to some extent, to justify commercial application of these antibodies.

There is no consensus on what extraction method is most appropriate to use to obtain EYA (Schade *et al.*, 1996). Differences in methods can affect yield, purity, stability, and efficacy of EYA harvested from the egg yolk (Sunwoo *et al.*, 2002; Karlsson *et al.*, 2004; Ko and Ahn, 2007; Kitaguchi *et al.*, 2008).

Storage stability of EYA over time is a major concern, although literature in this area, including means of storage, is scattered and inconsistent. Larsson *et al.* (1993) stored IgY for five to ten years at 4°C without any significant loss in antibody activity. These antibodies also retained their activity after six months at room temperature or one month at 37°C. In another study, purified antibodies stored at 24°C, 4°C, or -20°C lost their precipitating ability after seven days, 56 days, and 70 days, respectively. Lyophilised antibodies did not lose their precipitating ability until after 90 days, suggesting that lyophilisation may be the most effective mean of storage (Malik *et al.*, 2006). Nilsson and Larsson (2007) showed that IgY preparations are very stable when freeze-dried in the presence of disaccharides (which are used as a stabilising adjuvant). It was suggested that this may be an efficient way to simplify storage and transport requirements of antibodies for diagnostic and therapeutic applications. Shimizu *et al.* (1994) reported that sugars, especially a non-reducing sugar such as sucrose had a stabilising effect on IgY under various physical and chemical conditions.

Orally-administered antibodies, like any other protein molecule, are susceptible to denaturation by the acidic pH of the proventriculus and gizzard and degradation by proteases, however a fraction of the administered dose retains some immunological activity against gastrointestinal tract infections (Reilly *et al.*, 1997). Activity of orally-administered antibodies decline from proximal to distal regions of the intestine but remained detectable in the caecum (Wilkie *et al.*, 2006), which may influence the ability of EYA to prevent colonisation of specific pathogens in the lower parts of the intestinal tract. It may be possible to develop a protease-resistant oral dosage form of IgY in order to increase the fraction of immunoreactive antibody delivered locally in the gastrointestinal tract (Reilly *et al.*, 1997). Viscosity and passage rate of the intestinal tract, in relation to dietary composition, can influence the stability of EYA.

Although there have been no studies conducted to provide estimates on the cost of EYA use in poultry production, it appears that production cost of high quality EYA in a large scale, commercial application is substantial and certainly higher than the cost of routine antibiotics (Casadevall and Scharff, 1994). Cost-effectiveness is a very important determinant in making a shift or implementing a new strategy in the poultry industry. It has been suggested that constant administration of EYA would be necessary for prophylaxis or therapy in viral or bacterial infections (Wilkie *et al.,* 2006). This could be a restrictive factor in terms of economical feasibility of their applications.

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Conclusions

Specific EYA have beneficial effects in prevention or treatment of bacterial and viral infections in humans and animals. Available literature indicates that this technology has the potential to be used as a disease control strategy in the poultry industry. However, there are still many obstacles remaining, which make commercial production and administration of EYA a difficult goal to achieve.

References

ABDEL-MONEIM, A.S. and ABDEL-GAWAD, M.M.A. (2006) Genetic variations in maternal transfer and immune responsiveness to infectious bursal disease virus. *Veterinary Microbiology* **114**: 16-24.

AKITA, E.M. and NAKAI, S. (1992) Immunoglobulins from egg yolk: Isolation and purification. *Journal of Food Science* **57**: 629-634.

AMARAL, J.A., TINO DE FRANCO, M., CARNEIRO-SAMPAIO, M.M. and CARBONARE, S. (2002) Anti-enteropahtogenic *Escherichia coli* immunoglobulin Y isolated from eggs laid by immunized Leghorn chickens. *Research in Veterinary Science* **72**: 229-234.

BEDFORD, M. (2000) Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimize subsequent problems. *World's Poultry Science Journal* **56**: 347-365.

BOX, P.G., STEDMAN, R.A. and SINGLETON, L. (1969) Newcastle disease. I. The use of egg yolk derived antibody for passive immunization of chickens. *Journal of Comparative Pathology* **79**: 495-506.

CARLANDER, D., KOLLBERG, H., WEJAKER, P.E. and LARSSON, A. (2000) Peroral immunotherapy with yolk antibodies for the prevention and treatment of enteric infections. *Immunology Research* **21**: 1-6.

CARLANDER, D., STALBERG, J. and LARSSON, A. (1999) Chicken antibodies: A clinical chemistry perspective. *Upsala Journal of Medical Science*s **104**: 179-189.

CARLANDER, D., WILHELMSON, M. and LARSSON, A. (2001) Limited day to day variation of IgY levels in eggs from individual laying hens. *Food and Agricultural Immunology* **13**: 87-92.

CARLANDER, D., WILHELMSON, M. and LARSSON, A. (2003) Immunoglobulin Y levels in egg yolk from three chicken genotypes. *Food and Agricultural Immunology* **15**: 35-40.

CASADEVALL, A. and SCHARFF, M.D. (1994) Serum therapy revisited: animal models of infection and development of passive antibody therapy. *Antimicrobial Agents and Chemotherapy* **38**: 1695-1702.

CHANG H.M., OU-YANG, R.F., CHEN, Y.T. and CHEN, C.C. (1999) Productivity and some properties of immunoglobulin specific against Streptococcus mutants serotype c in chicken egg yolk (IgY). *Journal of Agricultural and Food Chemistry* **47**: 61-66.

COATES, M.E., DAVIES, M.K. and KON, S.K. (1955) The effect of antibiotics on the intestine of the chick. *British Journal of Nutrition* **9**: 110-119.

COLLETT, S.R. (2004) Controlling gastrointestinal disease to improve absorptive membrane integrity and optimize digestion efficiency, in: TUCKER, L.A. & TAYLOR-PICKARD, J.A. (Eds) *Interfacing Immunity, Gut Health and Performance,* pp. 77-91 (Nottingham, Nottingham University Press).

CRAY, C. and VILLAR, D. (2008) Cross-reactivity of anti-chicken IgY antibody with immunoglobulins of exotic avian species. *Veterinary Clinical Pathology* **37**: 328-331.

DEMEULENAER, B. and HUYGHEBAERT, A. (2001) Isolation and Purification of Chicken Egg Yolk Immunoglobulins: A Review. *Food and Agricultural Immunology* **13**: 275-288.

ERHARD, M.H., SCHMIDT, P., ZINSMEISTER, P., HOFMANN, A. and MÜNSTER. U. *et al.* (2000) Adjuvant effects of various lipopeptides and interferon-γ on the humoral immune response of chickens. *Poultry Science* **79**: 1264-1270.

ETERRADOSSI, N., TOQUIN, D., ABBASSI, H., RIVALLAN, G., COTTEE, J.P. and GUITTET, M. (1997) Passive protection of specific pathogen free chicks against infectious bursal disease by in-ovo injection of semi-purified egg-yolk antiviral immunoglobulins. *Journal of Veterinary Medicine* **B44**: 371-383.

FULTON, R.M., NERSESSIAN, B.N. and REED, W.M. (2002) Prevention of Salmonella enteritidis infection in commercial ducklings by oral chicken egg-derived antibody alone or in combination with probiotics. *Poultry Science* **81**: 34-40.

GAST, R.K., PORTER JR, R.E. and HOLT, P.S. (1997) Assessing the sensitivity of egg yolk antibody testing for detecting Salmonella enteritidis infections in laying hens. *Poultry Science* **76**: 798-801.

GHARAIBEH, S., MAHMOUD, K. and AL-NATOUR, M. (2008) Field evaluation of maternal antibody transfer to a group of pathogens in meat-type chickens. *Poultry Science* **87**: 1550-1555.

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GIRARD, F., BATISSON, I., MARTINE, G., BRETON, C., HAREL, J. and FAIRBROTHER, J.M. (2006) Use of virulence factor-specific egg yolk-derived immunoglobulins as a promising alternative to antibiotics for prevention of attaching and effacing Escherichia coli infections. *FEMS Immunology and Medical Microbiology* **46**: 340-350.

GOTTSTEIN, B. and HEMMELER, E. (1985) Egg yolk immunoglobulin Y as an alternative antibody in the serology of echinococcosis. *Zeitschrift for Parasitenkunde* **71**: 273-276.

GORBACH, S.L. (2001) Antimicrobial use in animal feed-time to stop. *New England Journal of Medicine* **345**: 1202-1203.

GRAHAM, J.P., BOLAND, J.J. and SIRBERGELD, E. (2007) Growth promoting antibiotics in food animal production: An Economic Analysis. *Public Health Reports* **122**: 79-87.

GURTLER, M., METHNER, U., KOBILKE, H. and FEHLHABER, K. (2004) Effect of orally administered egg yolk antibodies on Salmonella enteritidis contamination of hen's eggs. *Journal of Veterinary Medicine B Infectious Disease Veterinary Public Health* **51**: 129-134.

HAGAN, J.C., ASHTON, N.J., BRADBURY, J.M. and MORGAN, K.L. (2004) Evaluation of an egg yolk enzyme-linked immunosorbent assay antibody test and its use to assess the prevalence of Mycoplasma synoviae in UK laying hens. *Avian Pathology* **33**: 93-97.

HAMAL, K.R., BURGESS, S.C., PEVZNER, I.Y. and ERF, G.F. (2006) Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. *Poultry Science* **85**: 1364-1372.

HATTA, H., OZEKI, M. and TSUDA, K. (1997) Egg yolk antibody Ig Y and its application, in: YAMAMOTO, T., JUNEJA, L.R., HATTA, H. & KIM, M. (Eds) *Hen Eggs: Their Basic and Applied Science*, pp. 151-178 (Florida, CRC Press).

HOLT, P.S., STONE, H.D., GAST, R.K. and GREENE, C.R. (2000) Application of the agar gel precipitin test to detect antibodies to Salmonella enterica serovar enteritidis in serum and egg yolks from infected hens. *Poultry Science* **79**: 1246-1250.

KARIYAWASAM, S., WILKIE, B.N. and GYLES, C.L. (2004) Resistance of broiler chickens to Escherichia coli respiratory tract infection induced by passively transferred egg-yolk antibodies. *Veterinary Microbiology* **98**: 273-284.

KARLSSON, M., KOLLBERG, H. and LARSSON, A. (2004) Chicken IgY: utilizing the evolutionary advantage. *World's Poultry Science Journal* **60**: 341-348.

KITAGUCHI, K., MINOURA, M., NORITAKE, M., MIZUTANI, M., KINOSHITA, K., HORIO, F. and MURAI, A. (2008) Determination of immunoglobulin Y concentration in yolk extract prepared by water dilution method: Comparisons among three strains of chickens. *The Journal of Poultry Science* **45**: 82-87.

KO, K.Y. and AHN, D.U. (2007) Preparation of immunoglobulin Y from egg yolk using ammonium sulfate precipitation and Ion exchange chromatography. *Poultry Science* **86**: 400-407.

KWEON, C.H., KWON, B.J., WOO, S.R., KIM, J.M., WOO, G.H., SON, D.H., HUR, W. and LEE, Y. S. (2000) Immunoprophylactic effect of chicken egg yolk immunoglobulin (Ig Y) against porcine epidemic diarrhea virus (PEDV) in piglets. *Journal of Veterinary Medical Sciences* **62**: 961-964.

LARSSON, A., BALOW, R.M., LINDAHL, T.L. and FORSBERG, P.O. (1993) Chicken antibodies: taking advantage of evolution--a review. *Poultry Science* **72**: 1807-1812.

LEE, E.N., SUNWOO, H.H., MENNINEN, K. and SIM, J.S. (2002) In vitro studies of chicken egg yolk antibody (IgY) against Salmonella enteritidis and Salmonella typhimurium. *Poultry Science* **81**: 632-641.

LEE, S.B., MINE, Y. and STEVENSO, R.M. (2000) Effects of hen egg yolk immunoglobulin in passive protection of rainbow trout against Yersinia ruckeri. *Journal of Agricultural and Food Chemistry* **48**: 110-115.

LESLIE, G.A. and CLEM, L.W. (1969) Phylogeny of immunoglobulin structure and function. III. Immunoglobulins of the chicken. *The Journal of Experimental Medicine* **130**: 1337-1352.

LESLIE, G.A., WILSON, H.R. and CLEM, L.W. (1971a) Studies on the secretory immunologic system of fowl. I. Presence of immunoglobulins in chicken secretions. *Journal of Immunology* **106**: 1441-1446.

LESLIE, G.A., CRANDALL, R.B. and CRANDALL, C.A. (1971b) Studies on the secretory immunologic system of fowl. II. Immunoglobulin-producing cells associated with mucous membranes. *Immunology* **21**: 983-987.

LEVESQUE, S., MARTINEZ, G. and FAIRBROTHER, J.M. (2007) Improvement of adjuvant systems to obtain a cost-effective production of high levels of specific IgY. *Poultry Science* **86**: 630-635.

LI, X., NAKANO, T., SUNWOO, H.H., PAEK, B.H., CHAE, H.S. and SIM, J.S. (1998) Effects of egg and yolk weights on yolk antibody (IgY) production in laying chickens. *Poultry Science* **77**: 266-270.

LOEKEN, M.R. and ROTH, T.F. (1983) Analysis of maternal IgG subpopulations which are transported into the chicken oocyte. *Immunology* **49**: 21-28.

MALIK, M.W., AYUB, N. and QURESHI, I.Z. (2006) Passive immunization using purified IgYs against infectious bursal disease of chickens in Pakistan. *Journal of Veterinary Science* **7**: 43-46.

MALMARUGAN, S., RAMAN, M., JAISREE, S. and ELANTHALIR, P. (2005) Egg immunoglobulins - an alternative source of antibody for diagnosis of infectious bursal disease. *Veterinarski Arhiv* **75**: 49-56.

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MILES, R.D., BUTCHER, G.D., HENRY, P.R. and LITTELL, R.C. (2006) Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. *Poultry Science* **85**: 476-485.

NILSSON, E., LARSSON, A., OLESEN, H.V., WEJAKER, P. and KOLLBERG, H. (2008) Good effect of IgY against Pseudomonas aeruginosa infections in cystic fibrosis patients. *Pediatric Pulmonology* **43**: 892-899.

NILSSON, E. and LARSSON, A. (2007) Stability of chicken IgY antibodies freeze-dried in the presence of lactose, sucrose and threalose. *The Journal of Poultry Science* **44**: 58-62.

O'FARRELLY, C., BRANTON, D. and WANKE, C.A. (1992) Oral ingestion of egg yolk immunoglobulin from hens immunized with an enterotoxigenic Escherichia coli strain prevents diarrhea in rabbits challenged with the same strain. *Infection and Immunity* **60**: 2593-2597.

OPITZ, H.M., El-BEGEARMI, M., FLEGG, P. and BEANE, D. (1993) Effectiveness of five feed additives in chicks infected with Salmonella enteritidis phage type 13a. *Journal of Applied Poultry Research* **2**: 147-153.

OWUSU-ASIEDU, A., NYACHOTI, C.M., BAIDOO, S.K., MARQUARDT, R.R. and YANG, X. (2003) Response of early-weaned pigs to an enterotoxigenic Escherichia coli (K88) challenge when fed diets containing spray-dried porcine plasma or pea protein isolate plus egg yolk antibody. *Journal of Animal Science* **81**: 1781-1789.

ÖZPINAR, H., ERHARD, M.H., AYTUG, N., ÖZPINAR, A., BAKLACI, C. *et al.* (1996) Dose-dependent effects of specific egg-yolk antibodies on diarrhea of newborn calves. *Preventive Veterinary Medicine* **27**: 67-73.

PIELA, T.H., GULKA, C.M., YATES, V.J. and CHANG, P.W. (1984) Use of egg yolk in serological tests (ELISA and HI) to detect antibody to Newcastle disease, infectious bronchitis, and Mycoplasma gallisepticum. *Avian Diseases* **28**: 877-883.

REILLY, R.M., DOMINGO, R. and SANDHU, J. (1997) Oral delivery of antibodies. Future pharmacokinetic trends. *Clinical Pharmacokinetics* **32**: 313-323.

ROSE, M.E., ORLANS, E. and BUTTRESS, N. (1974) Immunoglobulin classes on the hen's egg: their segregation in yolk and white. *European Journal of Immunology* **4**: 521-523.

ROSE, M.E. and ORLANS, E. (1981) Immunoglobulins in the egg, embryo and young chick. *Developmental and Comparative Immunology* **5**: 15-20.

SARKER, S.A., CASSWALL, T.H., JUNEJA, L.R., HOQ, E., HOSSAIN, I., FUCHS, G.J. and HAMMARSTROM, L. (2001) Randomized, placebo-controlled, clinical trial of hyperimmunized chicken egg yolk immunoglobulin in children with rotavirus diarrhea. *Journal of Pediatric Gastroenterology and Nutrition* **32**: 19-25.

SCHADE, R., STAAK, C., HENDRIKSEN, C., ERHARS, M., HUGL, H. *et al.* (1996) The production of avian (egg yolk) antibodies: IgY. *ATLA* **24**: 925-934.

SHIMIZU, M., NAGASHIMA, H., HASHIMOTO, K. and SUZUKI, T. (1994) Egg yolk antibody (IgY) stability in aqueous solution with high sugar concentrations. *Journal of Food Science* **59**: 763-765.

SHIN, J.H., YANG, M., NAM, S.W., KIM, J.T., MYUNG, N.H., BANG, W.G. and Roe, I.H. (2002) Use of egg yolk-derived immunoglobulin as an alternative to antibiotic treatment for control of Helicobacter pylori infection. *Clinical and Diagnostic Laboratory Immunology* **9**: 1061-1066.

STEDMAN, R.A., SINGLETON, L. and BOX, P.G. (1969) Purification of Newcastle disease virus antibody from the egg yolk of the hen. *Journal of Comparative Pathology* **79**: 507-516.

SUGITA-KONISHI, Y., SHIBATA, K., YUN, S.S., HARA-KUDO, Y., YAMAGUCHI, K. and KUMAGAI, S. (1996) Immune functions of immunoglobulin Y isolated from egg yolk of hens immunized with various infectious bacteria. *Bioscience, Biotechnology and Biochemistry* **60**: 886-888.

SUNWOO, H.H., LEE, E.N., MENNINEN, K., SURESH, M.R. and SIM, J.S. (2002) Growth inhibitory effect of chicken egg yolk antibody (IgY) on Escherichia coli O157: H7. *Journal of Food Science* **67**: 1486-1494.

SUNWOO, H.H., NAKANO, T., DIXON, W.T. and SIM, J.S. (1996) Immune responses in chickens against lipopolysaccharide of Escherichia coli and Salmonella typhimurium. *Poultry Science* **75**: 342-345.

TELLEZ, G., PETRONE, V.M., ESCORCIA, M., MORISHITA, T.Y., COBB, C.W., VILLASENOR, L. and PROMSOPONE, B. (2001) Evaluation of avian-specific probiotic and Salmonella enteritidis-, Salmonella typhimurium-, and Salmonella Heidelberg-specific antibodies on cecal colonization and organ invasion of Salmonella enteritidis in broilers. *Journal of Food Protection* **64**: 287-291.

THOMAS, E., BOUMA, A., VAN EERDEN, E., LANDMAN, W.J.M., VAN KNAPEN, F., STEGEMAN, A. and BERGWERFF, A.A. (2006) Detection of egg yolk antibodies reflecting Salmonella enteritidis infections using a surface plasmon resonance biosensor. *Journal of Immunological Methods* **315**: 68-74.

TSUBOKURA, K., BERNDTSON, E., BOGSTEDT, A., KAIJSER, B., KIM, M., OZEKI, M. and L. HAMMARSTROM, L. (1997) Oral administration of antibodies as prophylaxis and therapy in Campylobacter jejuni-infected chickens. *Clinical and Experimental Immunology* **108**: 451-455.

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WILKIE, D.C. (2006) Non-antibiotic approaches to control pathogens in the gastrointestinal tract of the broiler chicken. *Ph.D. Thesis*, University of Saskatchewan.

WILKIE, D.C., VAN KESSEL, A.G., DUMONNCEAUX, T.J. and DREW, M.D. (2006) The effect of hen-egg antibodies on Clostridium perfringens colonization in the gastrointestinal tract of broiler chickens. *Preventive Veterinary Medicine* **74**: 279-292.

WILLS, F.K. and LUGINBUHL, R.E. (1963) The use of egg yolk for passive immunization of chickens against Newcastle disease. *Avian Diseases*. **7**: 5-12.

WORLD HEALTH ORGANIZATION (1997) The medical impact of antimicrobial use in food animals. Proceedings of WHO meeting, Berlin. pp. 1-21.

YOKOYAMA, H., HASHI, T., UMEDA, K., ICATLO JR, F.C., KUROKI, M., IKEMORI, Y. and KODAMA, Y. (1997) Effect of oral egg antibody in experimental F18+ Escherichia coli infection in weaned pigs. *Journal of Veterinary Medical Science* **59**: 917-921.

YOKOYAMA, H., PERALTA, R.C., DIAZ, R., SENDO, S., IKEMORI, Y. and KODAMA, Y. (1992) Passive protective effect of chicken egg yolk immunoglobulins against experimental enterotoxigenic Escherichia coli infection in neonatal piglets. *Infection and Immunity* **60**: 998-1007.

YOKOYAMA, H., PERALTA, R.C., UMEDA, K., HASHI, T., ICATLO JR, F.C., KUROKI, M., IKEMORI, Y. and KODOMA, Y. (1998) Prevention of fatal salmonellosis in neonatal calves, using orally administered chicken egg yolk Salmonella-specific antibodies. *American Journal of Veterinary Research* **59**: 416-420.

YOUNG, C.A., SILVERSIDES, F.G. and JONES, S.R.M. (2007) Chicken-derived IgY recognizes developing and mature stages of Loma salmonae (Microsporidia) in Pacific salmon, Oncorhynchus spp. *Aquaculture* **273**: 398-404.

ZHEN, Y.H., JIN, L.J., GUO, J., LI, X.Y., LU, Y.N., CHEN, J. and XU, Y.P. (2008) Characterization of specific egg yolk immunoglobulin (IgY) against mastitis-causing Escherichia coli. *Veterinary Microbiology* **130**: 126-133.

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