

Are egg yolk antibodies an

In the search for alternatives to antibiotics for poultry we should not forget the ability of the chicken to produce antibodies against a number of diseases. Can these antibodies (in egg yolk) be administered to other birds? The following review of scientific work shows various opportunities.

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Antimicrobials have, for many years, been used in the poultry industry for growth promotion (sub-therapeutic doses), disease prevention (prophylactic doses), and for the treatment of infections. Years of research and practical experiences have shown that antimicrobial use has resulted in significant improvements in animal production performance and health status. Removal of these substances from poultry diets will therefore be a change with many consequences. On the contrary, there is increasing evidence concerning economical feasibility of incorporating antibiotics into poultry diets.

A group of researchers at the Johns Hopkins University (Baltimore, MD, USA) have recently investigated the economic effects of removing antibiotics used for growth promotion in commercial broiler chickens. This economic analysis - utilising large-scale empirical data collected by the US broiler industry - demonstrated that the use of antibiotic growth promoters in poultry production is associated with economic losses for producers. It was concluded that the weight gains that resulted from antibiotic growth promoters are not sufficient to offset the cost of the antibiotics.

Some of the antimicrobials have also been used for disease control in human medicine. Microbiological and clinical evidence is mounting to show that resistant bacteria may pass from animals to humans, resulting in infections that are more difficult to treat. This situation has put tremendous pressure on the poultry industry to withdraw or limit antibiotic use in animal feed and to look for viable alternatives.

Although the use of all sub-therapeutic antibiotics has been banned in the EU since January 2006, there are still many countries in different parts of the world that rely heavily on antibiotic use in feed. It is therefore difficult to assess the rate of success of current efforts in establishing practical approaches as viable alternatives for antimicrobials on a global basis.

The poultry industry must be able to

cope with consequences of antibiotic withdrawal and try to maintain or improve production performance, as well as address consumer demands. It seems that a single viable alternative to antimicrobials is unlikely to be implemented successfully. It has become increasingly clear that a multi-factorial approach will be needed to address the challenges of commercial poultry operations. Possible beneficial effects of an alternative observed under experimental conditions may not always be repeatable at the farm level. An effective alternative to in-feed antibiotics should: have a significant and sustainable beneficial impact on poultry production performance and health; be proven safe for both poultry and humans; be easy to apply and store, and provide a substantial return on investment.

Egg yolk antibodies

There is concern that antibiotic-resistance in bacteria may make commonly used antibiotics less effective. Oral immunotherapy (passive immunisation) with specific antibodies is a strategy that has been actively pursued in laboratory and clinical studies for the last two decades. Feeding of egg yolk antibodies to neutralise specific pathogens, especially enteric microorganisms, is a potential alternative to antibiotics.

In order to produce these antibodies, hens are exposed (usually injected) to particular antigens which induce immune responses, including the production of antibodies. Normally, these antibodies are then transferred to the egg yolk. Booster immunisation (second exposure) is usually given at a later time to ensure continued transfer of antibodies from hen to the egg yolk. These antibodies are then extracted from the egg yolk and processed to be administered directly to the animal or included in the feed.

In the last few years, several commercial sources of egg yolk antibodies have become available. Oral administration of these antibodies has met with some degree of success in prevention of viral and bacterial enteric infections in



Egg yolk from hens exposed to particular antigens contain high levels of antibodies which can be used for disease prevention.

humans, piglets, calves, fish and rabbits. It is proposed that the egg yolk antibodies may act against enteric pathogens by binding, immobilising and consequently reducing or inhibiting the growth, replication, or colony forming abilities of these pathogens.

Characteristics of egg yolk antibodies

Three immunoglobulin classes (IgA, IgM and IgY) have been shown to exist in chickens. Chicken IgG has been designated as IgY because it varies in several aspects from mammalian IgG. IgY is the main serum immunoglobulin in chickens. It is transported from the hen to the embryo via the egg yolk. The egg yolk thus contains high concentrations of IgY. Other Ig classes are present only in negligible amounts in the egg yolk. It has been shown that the presence of immunoglobulins in eggs is an

alternative to antibiotics?



example of passive immunity. This is because these antibodies originated from the hen and are used to protect the offspring from various infectious diseases after hatch. The acquisition of passive immunity in birds was first noted in 1893 when Klemperer demonstrated the transfer of immunity to tetanus toxin from hen to chick.

The amounts of IgG in yolk have been reported to be 20-25 mg/ml in the hen's egg. A laying hen can produce approximately 300 eggs annually and the volume of one egg yolk is approximately 15 ml. This could supply close to 100g of antibody per hen per year. It has been demonstrated that egg yolk weight and the percent of hen-day production in laying hens may affect efficiency of IgY production. The IgY concentration in the egg yolk is an important parameter for commercial production of antibodies. It has been shown that the IgY concentration varies among different lines and among individual hens. This suggests the possibility of

increasing IgY production by choosing high-producing lines and by genetic selection within the lines.

Egg yolk antibodies are stable over time; even when storing IgY for 5-10 yr at 4°C there is no significant loss in antibody activity. These antibodies also retain their activity after 6 months at room temperature or 1 month at 37°C.

Current challenges

Consumer and industry concerns about antibiotic-resistant bacteria and the desire to treat pathogens that do not respond to antibiotics have prompted research into the use of alternatives (e.g. egg yolk antibodies). Laying hens are highly cost-effective producers of antibodies in comparison with mammals. This is because the antibodies can be obtained non-invasively and are produced in high levels. It has shown that egg yolk-derived antibodies have biochemical advantages over mammalian antibodies.

Although beneficial effects of pathogen-specific egg yolk antibodies 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 the application of egg yolk antibodies in chickens is quite low compared to studies in other animal species.

There are still many obstacles which make oral administration of antibodies in commercial poultry a difficult goal to achieve. Like any other novel product, it may take a long time to get approval from regulatory authorities to use egg yolk antibodies in commercial farms. Finding an appropriate alternative to antibiotics is a high priority for the poultry industry. This lack of immediate availability may divert attention from antibodies to other more readily available alternatives. The production cost of high quality antibodies in a large scale is considerable and certainly higher than the cost of routine antibiotics. Cost-effectiveness is a very important determinant in implementation of a new strategy in the poultry industry worldwide.

Biological activity of antibodies in egg yolk is an important issue which needs to be addressed. It is well-known that immune responses of a hen to a specific bacterial or viral antigen could be quite variable after every challenge. Yolks of eggs from a hen or a group of hens (in the same or different flock) are pooled and IgY are extracted. In this way, it is not clear which bird or flock responds

better to antigenic stimulation.

Variability of IgY level in yolks could be an issue. Management inefficiency and environmental stressors may exacerbate this variability.

Another important concern is stability of these compounds in the gastrointestinal tract when they are fed to poultry. Orally-administered antibodies, like any other protein molecule, are susceptible to denaturation by the acidic pH of the stomach and degradation by proteases. But, it has been shown that a fraction of the administered dose retains some immunological activity against gastrointestinal tract infections. It has been demonstrated that antibody activity declined from proximal to distal regions of the intestine, but remained detectable in the caecum. This situation may influence the ability of egg yolk antibodies to prevent colonisation of specific pathogens in the lower parts of the intestinal tract. It might 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.

It seems that adding antibodies in the form of whole egg yolk powder to poultry feed may be the most practical way of inclusion. It is not known if egg yolk antibodies can tolerate heat-based feed processing techniques. There is, however, a possibility that antibodies can be sprayed on the feed (after processing) in order to minimise denaturation of these compounds.

Specificity of these antibodies is also important. Poultry are exposed to a wide variety of infectious agents in commercial operations. The poultry industry will benefit more from egg yolk antibodies if they are produced against a mixture of common disease-causing microorganisms. If this approach works well, it may help

(to some extent) to justify commercial application of these antibodies.

The industry has been dealing with the pros and cons of antibiotics for the last 50 years. It is quite simplistic to think that replacement of antibiotics by egg yolk antibodies or other alternatives can be easily done. Most commercially-available alternatives have been introduced to the industry within the past 10-15 years and there are still many unanswered questions about their practical applications and efficacy. Evidence suggests that a single strategy may not adequately replace antibiotics. The control or prevention of infectious diseases in the poultry industry involves numerous strategies. Traditionally, the use of antibiotics has been an important

aspect of that control; however, viable alternatives are becoming increasingly important. Intelligent use of the wide variety of available disease preventive measures may contribute to a more healthy state of the birds and improved economy of poultry production. Solid bio-security measures will remain as a very important part of disease prevention strategies.

IgY applications in poultry diseases

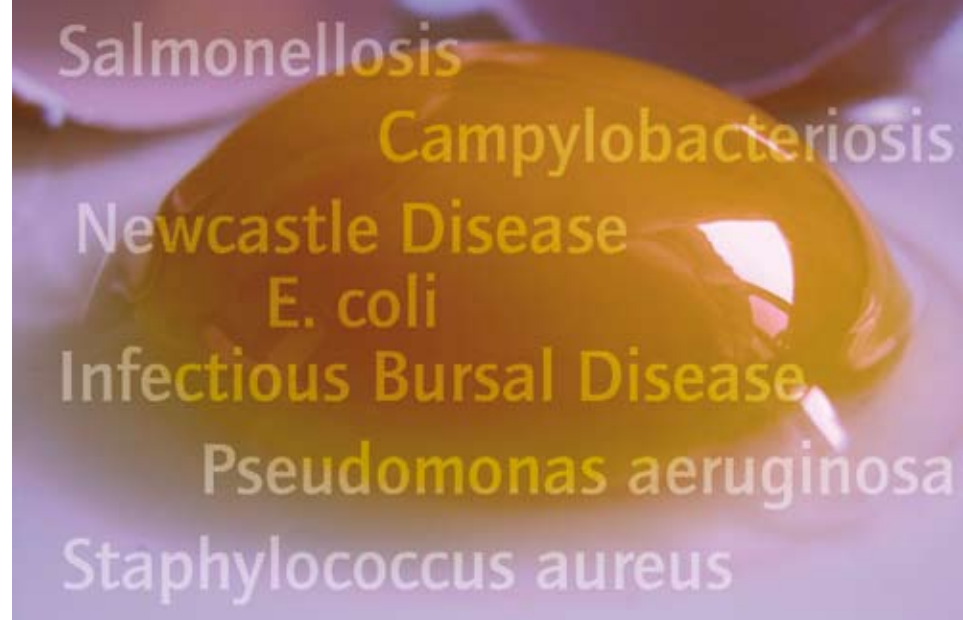
In order to better understand the important role that antibiotics have played in controlling or preventing these infections and, more importantly, how egg yolk antibodies might be used as an alternative to antibiotics, a number of poultry diseases are briefly discussed.

The main focus is directed to bacterial infections, but as viral infections (depending on the severity and presence of predisposing factors) may result in secondary bacterial infections in poultry flocks, the two most common viral diseases have also been discussed. It should also be mentioned that the reason for focusing on these specific diseases is due to the availability of peer-reviewed publications reporting possible beneficial effects of egg yolk antibodies in the prevention or control of these diseases.

Salmonellosis

Salmonella infections are responsible for a variety of acute and chronic diseases in poultry. Infected poultry are also among the most frequently implicated reservoirs of salmonella that can be transmitted through the food chain to humans. Resistance to multiple antibiotics has become prevalent among salmonella isolates from poultry farms all around the world.

The effectiveness of egg yolk antibodies for the reduction of intestinal colonisation by Salmonella spp. has been investigated. The binding activity of chicken egg yolk antibody (IgY) against *Salmonella enteritidis* or *Salmonella typhimurium* resulted in inhibition of bacterial growth *in vitro*. Microscopic observation revealed structural alterations of Salmonella surface by IgY. This finding may suggest that IgY can bind to Salmonella surface molecules and lead to the functional impairment of these components and inhibit bacterial growth. In another study, laying hens were vaccinated with *S. enteritidis* and the eggs were collected. Egg yolks were pooled, mixed in feed or prepared for oral gavage. The *in vitro* ability of these antibodies to inhibit pathogen attachment to rat epithelial cells and porcine mucin was demonstrated. In a



Antibodies found in egg yolk may provide protection against various diseases.

follow-up study, day-of-hatch broiler chicks were experimentally infected with *S. enteritidis*. Following the challenge, egg yolk antibodies were administered in the feed or via oral gavage. Despite measurable egg yolk antibody activity *in vitro*, it was not possible to demonstrate any significant reduction in intestinal colonisation by *S. enteritidis* in broiler chicks. It was concluded that although egg yolk antibodies show promise as a prophylactic strategy for the reduction of salmonella, more work is needed to understand the possible determinants that may result in effective antibody activity in broiler chickens, as well as the intestinal conditions that reduce their efficacy.

The use of whole egg powder (containing antibody) as a feed additive may be an alternative way to reduce the rate of Salmonella contamination of eggs. Research findings show that the rate of egg contamination could be reduced by oral administration of whole egg powder containing *S. enteritidis*-specific antibody.

It has also been shown that a combination of probiotics and egg yolk antibodies may be more effective in reducing the colonisation of *S. enteritidis* in poultry. Other trials demonstrated that the egg-derived anti-*S. enteritidis* antibody can prevent infection of ducklings if it is given at least 5 days before infection. It was also found that probiotics (with few exceptions) worked synergistically with orally-administered egg yolk antibodies to prevent infection. Based on these findings, it was suggested that egg-derived antibodies may serve as a means of preventing early Salmonella infection in ducklings.

In another study, different feed additives including *Lactobacillus* spp., organic acids, multiple probiotics, and egg powder containing antibody were evaluated for their efficacy in preventing gut colonisation and organ invasion in chicks infected with serotype *S. enteritidis* phage type 13a. None of the treatments prevented gut colonisation or organ invasion when compared to untreated controls. In all treatments, gut colonisation

and organ infection declined during the six-week experimental period. Although significant differences were more often seen with feed containing 5% egg powder compared to other treatments, it was concluded that none of the feed additives used in this study could be relied upon as the only measure for preventing *S. enteritidis* infection.

Egg yolk antibodies can also be used for diagnostic purposes for Salmonella spp. It has been shown that testing of egg yolk antibodies can provide a highly sensitive indication of prior exposure to *S. enteritidis* and should accordingly be useful for verifying the effectiveness of quality programmes designed to reduce the incidence of *S. enteritidis* infection in poultry.

Campylobacteriosis

Campylobacter *jejuni* is distributed widely among broiler, turkey and egg-producing flocks in all countries where commercial poultry industries operate. The isolation of antibiotic-resistant strains of Campylobacter spp. from commercial poultry represents an emerging food safety concern.

In a study conducted at the University of Saskatchewan, laying hens were vaccinated with *C. jejuni* and the eggs were collected. Egg yolks were pooled, mixed in feed or prepared for oral gavage. The *in vitro* ability of the pathogen-specific egg yolk antibodies to inhibit *C. jejuni* attachment to rat epithelial cells and porcine mucin was observed. In a

follow-up study, day-of-hatch broiler chicks were experimentally infected with *C. jejuni*, and following the challenge, egg yolk antibodies were administered in the feed or via oral gavage. Despite measurable egg yolk antibody activity *in vitro*, the researchers were unable to demonstrate any significant reduction in the intestinal colonisation by *C. jejuni* in chicks. It was concluded that although egg yolk antibodies show promise as a prophylactic strategy for the reduction of *C. jejuni*, more work is needed to understand the possible determinants which may result

in effective antibody activity in broiler chickens, as well as the intestinal conditions that reduce their efficacy.

Tsuokura *et al.* (1997) used egg yolk antibodies obtained from immunised hens for prophylactic and therapeutic applications in chickens infected with *C. jejuni*. In a prophylaxis experiment it was found that these antibodies caused a significant reduction (up to 99%) in the mean *C. jejuni* faecal count throughout the experiment. In a therapy trial, the therapeutic efficacy (antibodies were given after establishment of the infection) was demonstrated but at a lower rate of reduction (80-95%) compared to prophylactic trial.

Necrotic Enteritis

Necrotic enteritis, caused by *Clostridium perfringens*, has been reported from most areas of the world where poultry is produced. Toxins produced by this bacterium are responsible for intestinal mucosal necrosis, the characteristic lesion of necrotic enteritis. Necrotic enteritis has been controlled by adding antibiotics in the feed of poultry, but removal of in-feed antibiotics can influence the effectiveness of preventive strategies for this disease.

The use of specific egg yolk antibodies to reduce *C. perfringens* colonisation in the intestinal tract of broiler chickens has not been fully explored. In 2006, Wilkie *et al.* evaluated the ability of anti-*C. perfringens* egg yolk antibodies to reduce intestinal colonisation of *C. perfringens* in broiler chickens. Antibodies against *C. perfringens* were obtained from the eggs of laying hens hyperimmunised using a *C. perfringens* bacteria. Two feeding trials were performed to assess the efficacy of feed amended with the egg yolk antibodies in reducing the level of colonisation of *C. perfringens* in challenged birds. Antibody activity declined from proximal to distal regions of the intestine, but remained detectable in the cecum. In the first experiment, there was no significant reduction in the number of *C. perfringens* in the birds fed the diet amended with the anti-*C. perfringens* egg yolk antibody. In the second experiment, there was a significant decrease in *C. perfringens* intestinal populations 72 hours after treatment as assessed by culture-based enumeration, but there was no decrease as measured by quantitative PCR based on the *C. perfringens* phospholipase C gene. Intestinal lesion scores were higher in the birds that received the anti-*C. perfringens* egg yolk antibodies. It was concluded that the oral administration of egg yolk antibodies does not reduce intestinal *C. perfringens* in experimentally challenged birds. The finding of increased intestinal lesions in birds administered egg yolk antibodies indicate that the antibodies might in fact exacerbate necrotic enteritis, although the reasons for this observation are still unclear.

E. coli Infections

Escherichia coli infection is responsible for significant losses in the global poultry industry. Although the organism is a normal inhabitant of the intestinal tract of commercial poultry, toxigenic strains are responsible for both localised and systemic reactions. Antibiotics have always been one of the most effective options for controlling *E. coli*-associated problems in the poultry industry. It has been shown, however, that isolates of *E. coli* from poultry farms are frequently resistant to one or more antibiotics.

Egg-yolk antibodies induced by immunising hens with selected *E. coli* antigens were evaluated for their ability to protect broiler chickens against experimental respiratory tract infection and associated septicemia caused by avian pathogenic *E. coli*. Seven groups of broiler breeder hens were each vaccinated with *E. coli* antigens. Egg yolk antibodies, obtained from eggs produced by vaccinated broiler breeders, were injected intramuscularly into 11-day-old broiler chickens, which were challenged 3 days later with *E. coli* by the intra-air sac route. Mortality was recorded and surviving chickens were euthanised 1 week after the challenge and examined for macroscopic lesions. The results showed that passive antibodies were protective against *E. coli* infection.

In vitro studies have shown that egg yolk antibodies have inhibitory effects on *E. coli* O157:H7 growth. An investigation on the activity of chicken egg yolk antibody *in vitro* to determine the growth inhibitory effects of these antibodies on *E. coli* O157:H7 showed that the specific binding activity of egg yolk antibody against *E. coli* O157:H7 was high. The antibacterial function of egg yolk antibody appeared to result from the interaction of these antibodies with surface components of *E. coli* O157:H7.

Infectious Bursal Disease

Infectious bursal disease (IBD) is an acute, highly contagious viral infection of young chickens, which primarily targets lymphoid tissues. Immunisation of chickens is the principal method used for the control of IBD. The economic importance of the disease is more significant with the sub-clinical form, which usually results in immunosuppression. Immunosuppression can cause vaccination failures and susceptibility to other viral, parasitic and bacterial diseases including *E. coli* infections. Using antibiotics in affected flocks is the most readily available approach in dealing with IBD-induced secondary bacterial infections.

It has been shown that egg yolk can be considered as a source of antibodies for the control of IBD. In 2006, Malik *et al.* demonstrated that yolk from hyperimmunised hens can be used to control IBD in commercial laying hens. They vaccinated laying hens with IBD vaccines. The eggs were collected daily and antibodies were purified from the egg yolk.

Antibody titers were significantly higher in yolk than serum of the hens. Antibodies were lyophilised and stored at 4°C. Biological activity of these antibodies was not affected during 90 days of storage. IBD-infected laying hens then were injected with these antibodies, which induced 92% recovery (compared to control birds). This study indicates that the purified antibodies may be useful as a therapeutic agent to treat IBD-infected birds.

In-ovo inoculation of purified egg-yolk antibodies against IBD may be a good experimental model for maternally transmitted anti-IBD immunity. Concentrated egg yolk antibodies were inoculated via the intra-vitelline route into 7-day-old embryonated specific pathogen free (SPF) eggs. Although this had negative effects on hatchability, it resulted in hatching two series of SPF chicks with passive immunity against highly virulent IBD virus.

Newcastle Disease

Newcastle disease (ND) is a viral disease which can occur in different forms in susceptible commercial poultry. This disease is endemic in many countries in different parts of the world and can cause heavy economic losses in the poultry industry. Vaccination is the primary means to prevent this disease in endemic areas, but vaccines are not always successful and vaccinated flocks could still be affected. When a ND outbreak occurs in a commercial poultry flock, antibiotics may be administered to reduce the possibility of secondary bacterial infections, which are usually triggered by the disease.

It has been shown that egg yolk antibodies can be used for passive immunisation of chickens against ND. Trials have shown that subcutaneous administration of egg yolk containing high levels of antibody against ND protects 80% of the birds for the 4-week period of the study.

Mixed Infections

Many studies have shown that egg yolk antibodies obtained from hens immunised with a specific infectious agent is effective in protection against the disease caused by the pathogen. There is a possibility that these antibodies, if obtained from hens simultaneously immunised with various pathogens, could be effective against these pathogens. In 1996, Japanese researchers immunised hens with a mixture of 26 bacterial strains and investigated the effects of antibodies collected from eggs on *Pseudomonas aeruginosa*, *S. enteritidis* and *Staphylococcus aureus*. They demonstrated that egg yolk antibody from hens immunised with plural bacterial antigens could prevent multiple bacterial diseases by inhibiting processes such as growth, toxin production and adhesion to the intestinal epithelial cells. ■

References available on request