

# Egg antibodies

*A deeper understanding of the localised intestinal immune response to a variety of pathogens has led to the development of specific antibodies for feed applications. Of these, hyper-immunised eggs have emerged as the most successful, not least because of their low processing requirements and extensive availability.*

By John Twigge

Imparting passive immune protection through oral administration of antibodies is neither a new nor a novel concept. This is a naturally occurring process in all mammalian species, in which man has experimented for centuries. In the past researchers have studied oral administration of antibodies harvested from various sources such as colostrum, milk, eggs and plasma, and investigated their applications in livestock production (Mitchell *et al.* 1967; Petersen and Campbell, 1955). Although results have generally been positive, the techniques used were financially infeasible to implement such a process on a commercial scale with the exception of one possible antibody source, the egg. Moreover, additional studies showed that eggs could be produced to contain enhanced concentrations of specific antibodies of interest (Larsson *et al.* 1993). The implication of such capabilities has never been more important than now. In recent years, consumers have placed enormous pressure on livestock producers to reduce or eliminate the subtherapeutic use of antibiotics in animal feeds. This was (and in many countries still is) the primary means of maintaining health and maximising production. The most prevalent use had been in nursery livestock where scours remains the single largest health problem and chief cause of mortality for all livestock species. In the US alone, scours is one of the major causes of death for preweaned calves and pigs (Lay *et al.* 2001, NAHMS, 1996). Producers must now accept the challenge to manage scours, improve production, and concurrently address consumer demands. This elevates the impact that natural alternatives to antibiotics could have on the livestock industry, placing products such as egg antibodies into a new spotlight. Indeed, in the last few years several commercial sources of egg antibodies have become available to the livestock

market. What has made these products worth taking note of is that they have been altered to contain higher levels of antibodies to specific bacteria and viruses commonly responsible for nursery scours. Industry acceptance and use of these products is just becoming established, but many producers still have questions concerning the implications and value of egg antibodies in today's livestock production.

## Antibodies in intestinal health

The function and actions of antibodies in the immune system are numerous and varied. They play a critical role in the intestine, in maintaining intestinal and systemic health. The primary role of antibodies in the intestine is to prevent access for pathogens such as viruses, parasites and bacteria to the luminal surface.

In order to understand the role of antibodies in maintaining health and defending against infection, we must first understand how bacteria convey pathogenicity. Bacterial infection is the result of a combination of colonisation, invasion and the subsequent release of toxins. Colonisation is the process in which bacteria attach to the surface cells of the intestine and begin to multiply. This is initiated by adhesion of the organism to a specific receptor site. Adhesion is not a random act; it is a specific interaction between a particular epithelial cell receptor and a specific portion of the bacterium known as adhesin. Adhesin sites are specific to the organism, and can be a portion of the fimbriae/pilli protein projection of the bacterium cell, or a polysaccharide portion of the cell wall itself. Fimbriae or pilli adhesins are specialised structures that are antigenically distinct from the common bacterial fimbriae/pilli. These adhesin pilli are called colonisation-factor antigens and play a role in host specificity, a common example of which are *E. coli* serotypes K88, K99, and 987P



(Siegfried and Kmetova, 1997). These structures are highly antigenic and commonly used to distinguish various subspecies or serotypes within a bacterial family. The shape of bacterial adhesins correspond to a

# s in the spotlight



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specific glycoprotein or glycolipid receptors of the epithelial cell. The simple process of receptor ligand binding results in dramatic and damaging effects on the epithelial cell structure. For example, adherence of

enteropathogenic *E. coli* physically damages the microvilli structure, and subsequent colonisation will result in lesions along portions of the villi (Pearson and Logan, 1978). Not only does this result in nutrient malab-

sorption, but it also stimulates accelerated renewal of the surrounding enterocytes causing further reduction in nutrient absorption and manifestation of inflammatory responses. Additionally, colonisation can alter the normal transport system of the cellular membrane by interfering with normal cellular signal transduction. This causes inhibition of fluid uptake and stimulation of net fluid secretion resulting in osmotic or watery diarrhoea (Fisher and Martinez, 1975).

## Invasion of the pathogens

Bacteria may also express pathogenicity through actual invasion of the epithelial cells. Some gram negative *E. coli*, and almost all species of *Salmonella* have the ability to invade their host cell once colonisation occurs, resulting in local and possibly systemic infection. After adherence, these bacteria release chemicals called invasins, proteins that stimulate engulfment of the bacteria by host cells (Horwitz, 1982). This is a complex process with salmonella infection that results in an event known as ruffling whereby the epithelial cell wall ruffles up and around the organism and engulfs it in a manner similar to phagocytosis (Strauss, 1999). Inside the cell, the salmonella is able to escape phagocytic degradation by modifying the vacuole they are contained in through the release of several factors which prevent or inhibit the recruitment of lysosomal proteases. Once bacteria have invaded the cell and escaped immune destruction they have the ability to replicate within the host cell, release toxic chemicals, cause widespread cellular destruction, and have access to the circulatory system. As a result, enteroinvasive bacteria can cause systemic infection, and far more severe forms of diarrhoea.

The final form of pathogenicity involves the release of enterotoxins and their effects on the host system. There are two types of enterotoxins, endo- and exotoxins. Typically associated with gram-negative bacteria, endotoxins are heat stable lipopolysaccharides of very low molecular weight (10 – 20 kDa) (Holmgren, 1985). Exotoxins are heat labile protein structures of a larger size (50 – 1000 kDa). Endotoxins are part of the outer cell wall of gram-negative bacteria whereas exotoxins are small fragments of the fimbriae or pilli. Both are highly antigenic and typically released during reproduction, death and disintegration of the bacterial cell. The types and actions of enterotoxins are many and varied. Infection may be initiated by binding to epithelial cell receptors,



permeating the epithelial barrier, or being released inside the epithelial cell after bacterial invasion. The host's reactions to enterotoxins can be as varied as the enterotoxins themselves, including diarrhoea, fever, elevation in white blood cell counts, shock and death (Patrick and Larkin, 1995).

### Antibody function

There are several subtypes of antibodies-immunoglobulins (Ig) G, A, M, D and E. The most important regarding intestinal defence are IgG and IgA. Their primary function is the prevention of bacterial adherence. The *fab* portion of antibodies recognises antigenic epitopes, for bacteria, these epitopes are adhesins. The simple act of binding adhesin prevents bacteria from binding to the epithelial receptors, preventing bacterial colonisation and invasion (Acres, 1983). A second and equally important function is the trapping of soluble antigens. Through the process of agglutination, IgG and to a lesser extent, IgA will also form a complex network of antibodies and endotoxins bound together. This network can grow to such a size that it becomes insoluble and can no longer penetrate the epithelial barrier (Kilian and Russell, 1994). IgA and IgG also play other roles in host immunity.

Immunoglobulin A is most intimately associated with gut immunity. Specialised lymphoid tissue of the GI tract known as Peyer's patches are responsible for sampling antigens passing through the intestinal lumen and, if needed, triggering the process which bathes the mucosal surface in IgA. Mucosal B cells which produce IgA do so by creating IgA polymers with a secretory protein attached to ensure efficient external transportation to the intestinal lumen. Immunoglobulin A is the first line of defence for the intestine, but not the only one. Bacteria and their associated toxins that are able to bypass the barrier through invasion will eventually encounter serum-derived IgG antibodies. When IgG binds with an antigen, the most characteristic response is the activation of the complement system and inflammatory mediators. Pooling of these mediators in the lamina propria will result in a temporary leakage of IgG into the intestinal lumen. Here IgG has the same opportunity as IgA in prevention of adherence and binding of toxins (Brandtzaeg, 1998).

### Conferring protection

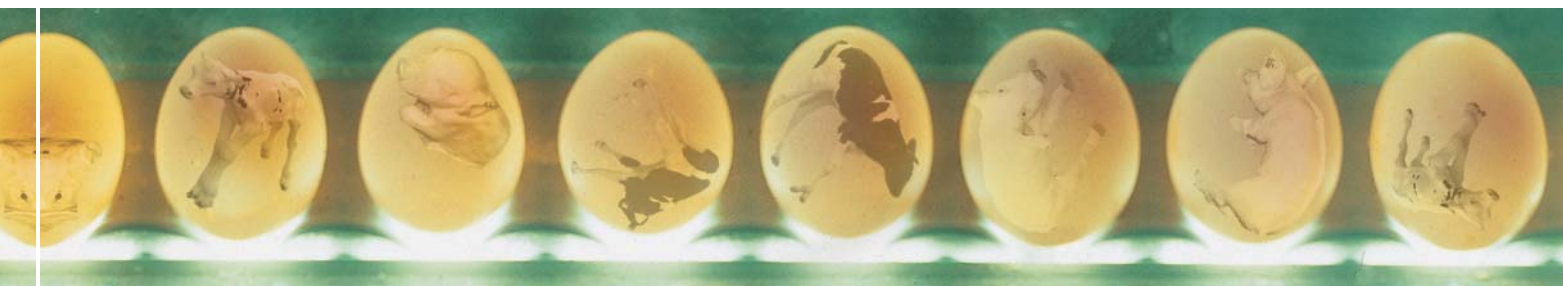
Antibodies are incredibly efficient in their role of immune protection, however, they have to be present to be functional, and during certain stages of life, this is not always the case. Livestock such as ruminants and pigs have a disadvantage over other species of animals. The unique placental structure for these animals does not allow the transfer of maternal immunoglobulins (Ig) to the foetus. Consequently, immediately following birth, circulating levels of the important antibodies such as IgG, IgA, and IgM are practically immeasurable. This leaves the newborn particularly vulnerable to its immediate environment, until it has the opportunity to acquire antibodies from its mother's colostrum. Quality colostrum regardless of the species derived is rich in IgG comprising almost 80% of all antibodies present, however the overall concentration and ratio to other Ig's rapidly declines. Furthermore, maternal protection is short lived, as the average half-life of colostrum Ig is only about 10-20 days (Frenyo *et al.* 1981). Additionally, maturity of the Ig producing cells of the newborn does not start until approximately 1 month of age and adult levels are not achieved until around 6 months of age. Likewise, intestinal development requires several months for full development and prior to that IgA cells are either absent or very rare (Allen and Porter, 1977). Thus, both cattle and pigs face a critical period in their lives from birth to about 35 days of age. They do not have the ability to manufacture adult quantities of their own Ig, and passive acquisition of maternal Ig is often insufficient or not available at all. If the quantities of immunoglobulins necessary to achieve immune protection are not present, the result is illness and early mortality, most commonly reflected as scours in pre-weaned calves and piglets.

### Application of exogenous antibodies

The production and collection of antibodies from one individual to infer immunity to another has been studied for centuries. Therapeutic use of antibodies includes intravenous and oral applications. Oral application has primarily been focused on prophylaxis and treatment of enteric infections, typically using exogenous antibodies from serum, milk and eggs. Milk and colostrum quickly became preferred to serum, as it is easier to collect, requires no

invasive procedures and is of less concern for transmitting infectious diseases. Additionally, it is relatively easy to expose the host to a vaccine or infection, and then collect relatively large pools of polyclonal antibodies to those vaccines in a short period of time. Colostrum is essential for imparting immune protection to the neonate, so it did not take long for people to realise the potential of this medium. Successful manipulation of antibody populations was achieved through intravenous or intramuscular injection, or even localised infusion of antigen to the udder itself. Some of the earliest experiments in this field were conducted by Peterson's group at the University of Minnesota. They showed that production of specific antibodies could be achieved by infusion of various antigens directly into the milk channels of the udder (Petersen and Campbell, 1955). They experimented with many diverse types of antigens, including killed bacteria, viruses, parasites and even simple protein antigens. Not only were high levels of specific antibodies produced, but these could also be used efficaciously across species to impart immune protection. Antibody expression in colostrum and milk reservoirs has since been fine-tuned, and the focus shifted to pooling and concentrating specific antibodies of interest. Furthermore, with





increased understanding of enteropathogens, the antigens used for vaccination became more specialised and species-specific. Several researchers reported that vaccination of the dam with *E. coli* K99 pilli antigens not only resulted in increased antibody levels (hyperimmune) to *E. coli* K99 in the colostrum, but also this colostrum was able to better protect their calves from artificially induced *E. coli* diarrhoea. Combs *et al.* (1993) reported that colostrum-deprived calves receiving concentrated colostrum antibodies to *E. coli* K-99 were able to withstand an artificial challenge with *E. coli* K-99. Calves receiving colostrum antibody preparations had significantly reduced scours and overall mortality in comparison to control calves. Similar results were shown when performing similar trials with hyperimmunised colostrum antibodies to coronavirus. Cross-species protection of colostrum-derived antibodies has also been demonstrated. Hyperimmune bovine derived colostrum antibodies to rotavirus have been shown to confer protection to other species including pigs (Bridger and Brown, 1981) and humans (Hipert *et al.* 1987).

### The ideal medium

Manipulating the antibody profiles of colostrum and milk is much easier and less invasive than serum, however, it is still not an ideal medium. The overall antibody concentrations of milk are quite low in comparison to colostrum, so extensive purification and concentration protocols have had to be used. Colostrum contains about 150 times higher antibody levels than milk but its availability is low and short-lived. Eggs on the other hand are easily available in large quantities. The antibody concentration of eggs is higher than that of milk or plasma and when comparing on a per body weight basis, eggs have about 20 times higher antibody concentrations than colostrum (Kuhlmann *et al.*, 1988). Chickens are easier to house and feed as well as manipulate with various vaccination profiles. It was not long before researchers demonstrated that laying hens could be vaccinated to produce antibodies to species-specific pathogens, and that these antibodies would be quickly and efficiently transferred to the egg from the serum. Kuhlmann *et al.* (1988) were among



the first to show that vaccination of hens against pilli antigens of *E. coli* K88, K99, 987P and F41 resulted in a rapid increase of serum IgG levels, followed by a proportionate increase in egg yolk IgG concentrations to these antigens. Both serum and egg increases reached a plateau that was maintained for more than 100 days post immunisation. The economy of an egg antibody production scheme in comparison to colostrum or milk was quickly recognised. Follow up studies showed that lyophilised yolk from eggs produced from vaccinated hens could be given orally to pigs to reduce *E. coli*-associated scours and mortality. Many reports show that feeding hyperimmunised eggs produced from vaccinating hens with *E. coli*, to pigs could reduce or eliminate *E. coli* associated diarrhoea and mortality (Erhard *et al.*, 1996, Jungling *et al.*, 1991). Additional reports soon followed for prophylactic and therapeutic treatment of livestock enteric infections. Wiedemann *et al.* (1991) demonstrated that administering egg antibodies cured 92% of 299 diarrhoea affected pigs and Zuniga *et al.* (1997) reported that pigs challenged with *E. coli* F18 were fully protected when fed egg antibodies for the F18 fimbriae. Further trials showed that immune protection from a variety of bacterial and viral challenges could be imparted not only in pigs but also in calves, mice and humans (Kuroki *et al.* 1994; Bartz *et al.* 1980; Yolken *et al.* 1988).

### Mode of action revealed

Soon attention turned towards the mechanism of action. It was surmised that since the antibodies were not absorbed through the GI tract in older animals, the antibodies were imparting a localised form of protection. *In vitro* trials were initially conducted to examine immunoglobulin-bacteria interactions with enterocytes. Yokoyama *et al.* (1992) isolated epithelial cells from newborn piglets deprived of colostrum, and co-incubated these cells with *E. coli* K88, K99 or 987P at 37°C for 30 minutes. After incubation, they separated out whole epithelial cells and with a light microscope counted the number of individual bacteria adhered to each cell. This process was then repeated by co-incubating egg antibodies for K88, K99 and 987P with fresh cells along with the bacteria. They found that adherence was reduced from 18.5 to 2.3 cells for *E. coli* K88, from 13.5 to 2.4 cells for K99 and from 11.7

to 2.3 for cells for 987P. Likewise, Imberechts *et al.* (1997) reported that egg antibodies to *E. coli* F18ab fimbriae were able to inhibit attachment of F18ab to intestinal mucosa *in vitro*. Jin *et al.* (1998) performed *in vitro* trials using immobilised epithelial cells from 14-day old healthy piglets. Co-incubating egg *E. coli* K88 antibodies with H<sup>3</sup>-labeled K88 bacteria, and adding epithelial cells to the mixture, they demonstrated 97% inhibition of adhesion. When epithelial cells were incubated with H<sup>3</sup> *E. coli* K88 before adding egg antibodies there was no significant reduction in adhesion of bacterial to epithelial cells. This was to be expected because although antibodies can prevent adhesion they are not able to displace bacteria that are already bound to epithelial receptors. Clearly, these studies demonstrate that hyperimmunised egg antibodies impart local anti-colonisation immunity to intestinal cells just as endogenous antibodies.

### The final challenge

Although there have been many positive trials using egg antibodies, the degree of protection afforded has been quite variable if at all. One factor that may play a role in this variability is dose. Many researchers have found a definite dose dependant effect of antibodies on the survivability of animals challenged with enteric pathogens. Yokoyama *et al.* (1992) reported that pigs given egg antibody titre levels of 625 and 2500 had 100% recovery compared with death rates of 86, 100 and 80% for pigs challenged with K88, K99 and 987P, respectively. Zuniga *et al.* (1997) reported that a daily intake of 5.5 g of egg antibody was required for protection from *E. coli* F18 challenge whereas 3.5 g per head per day was insufficient for homologous protection. Variability may also be attributed to the nature of the challenge. There is no means of controlling a bacterial or viral challenge introduced to a group of animals, resulting in variability among and between treatment groups, with further interference from extraneous environmental factors. Despite the variability in animal response, there remains overwhelming evidence for the effectiveness of exogenous antibodies in the prevention and treatment of enteric diseases. ●