ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. LXIV (2) SECTIO DD 2009

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Antimicrobial defence mechanisms of chicken eggs and possibilities for their use in protecting human and animal health

Mechanizmy obrony przeciwzakaźnej jaja oraz możliwości ich wykorzystania w ochronie zdrowia ludzi i zwierząt

Summary. The chicken egg is an important source of nutrients. Furthermore, eggs have physical and biological defence mechanisms to protect the embryo against invasion and multiplication of micro-organisms. The egg shell and shell membranes physically obstruct microbial penetration of the egg, while the viscosity and pH of the egg white inhibit bacterial proliferation. The egg white also contains a number of proteins with demonstrated antimicrobial activities, such as bacterial cell lysis, metal binding, and vitamin binding, which are part of the natural defence system of the egg. Eggs are the subject of research aimed at identifying substances with biological functions beyond nutrition. Various biological functions of egg components, particularly antimicrobial activity, immunomodulating activity, and protease inhibition, underscore the importance of these substances in the prevention and treatment of human and animal diseases.

Key words: egg, antimicrobial defence system, lysozyme, immunoglobulin IgY, activities of biological substances

INTRODUCTION

The chicken egg is one the most valuable and most perfect foodstuffs of animal origin. An important source of nutrients, it contains proteins, lipids, vitamins, and minerals, which ensure the development of the living organism and provide a defence system against infection. Eggs have been shown to contain substances with various biological functions beyond their nutritive properties. A number of biological activities with novel applications are attributed to egg components, particularly antibacterial activity, immunomodulatory properties, antineoplastic activity, and protease inhibition. These functions have proven and potential applications in the prevention and treatment of human and animal diseases.

DEFENCE SYSTEMS OF THE EGG

The defence mechanism of the egg consists of a mechanical (physical) barrier and a biological barrier, comprised of a number of biologically active substances in the white and yolk and the pH of the white [Mayes and Takeballi 1983, Solomon *et al*. 1994, Wang and Slavik 1998, Sim *et al*. 2000, Theron 2003, Jones *et al*. 2004].

Mechanical defence

The mechanical barrier of the egg consists of the shell, together with the waxy membrane covering it known as the cuticle, and the shell membranes. The egg shell itself consists of two layers – the outer layer, called the spongy layer, and the inner, mammillary layer. The shell contains about 7,000 to 17,000 pores, with considerably more of these located at the blunt end [Solomon 1994]. The diameter of the shell also varies (10 to 60 μm). The outer mouth of the pores is blocked by the cuticle, preventing microbes from penetrating the egg. According to Vadehra *et al*. [1970], the cuticle functions most effectively within 96 hours after the egg is laid. It has been demonstrated that the cuticle can be damaged by enzymes of some bacteria, such as *Pseudomonas* or *Salmonella* [Board *et al*. 1979, Radkowski 1996]. Washing eggs can also damage this layer [Wang and Slavik 1998]. The shell membranes adhere to the inside of the shell [Mayes and Takeballi 1983]. These function basically like a filter, and are less permeable to bacteria than the shell itself. The speed with which bacteria penetrate the shell membranes varies depending on the type of microflora [Garibaldi and Stokes 1958, Radkowski 1996]. Bacteria introduced onto the shell membrane via the air chamber do not penetrate the white until after 4–8 days. Sometimes the shell membranes obstruct the micro-organisms for only 2–3 days, or even 1–2 days. Brown *et al.* [1965] demonstrated the presence of hydrolysis zones surrounding the bacteria located on the shell membranes. Enzymes produced by bacteria are thought to facilitate penetration of these membranes [Garibaldi and Stokes 1958, Hartung and Stadelman 1963, Brown *et al.* 1965].

After micro-organisms have overcome the defences of the cuticle, shell and shell membranes, their multiplication is inhibited by the structure of the albumen, which deprives them of easy access to nutrients [Yadev and Vadehra 1977, Brady *et al.* 2003]. The layer of thick albumen in the fresh egg makes it difficult for bacteria to move towards the yolk, while the chalaziferous layer, together with the chalazae and thick albumen, keep the yolk in a central position, significantly delaying infection [Board and Tranter 1995].

Biological defence

Defence mechanisms of the albumen

Changes occurring during egg storage, such as thinning of the albumen, or weakening of the chalazae and vitelline membrane, disrupt the mechanical defence system. For this reason, the initiative for battling micro-organisms is taken over by biologically active substances occurring in the egg white, the most important of which are lysozyme, conalbumin (ovotransferrin), avidin, cystatin, ovomucoid, and ovoinhibitor [Board *et al.* 1994].

The properties of lysozyme are best known. It was first described in the 1920s by Fleming, who determined that lysozyme also occurs in human saliva, tears, and milk.

According to Fenney and Allison [1969], the highest lysozyme content occurs in dry mass of chicken eggs (3.5–4%), and the lowest in the eggs of water fowl such as ducks (1.2%) and geese (0.6%). In some cases the lysozyme content in chicken eggs can be as high as about 8% [Sauter and Montoure 1972]. It occurs in two forms, of differing molecular mass and originating in different species. These are lysozyme c, or chicken lysozyme, occurring in Galliformes and ducks, and lysozyme g, characteristic of geese and wildfowl [Kowalska 1989, Trziszka 1994]. Lysozyme hydrolyzes β-bonds (1–4) between *N*-acetylomuramic acid and *N*-acetyloglucosamine of glycosides, which are a structural component of the bacterial cell wall. It exhibits the highest lytic activity against Gram positive bacteria, though the antibacterial effectiveness of chemically modified lysozyme against Gram negative bacteria has also been studied [Ibrahim *et al.* 1991, 1993, 1994]. Bacteria sensitive to lysozyme include *Salmonella, Brucella, Shigella, Pseudomonas, Ervinia, Escherichia, Listeria,* and *Staphylococcus aures* [Ibrahim *et al.* 1991]. Lysozyme is also thought to be capable of inactivating viruses by binding their DNA, forming insoluble complexes.

It is worth noting that lysozyme, as a substance with strong antibacterial and antiviral properties, has found increasingly wide application in the manufacturing, cosmetics, and pharmaceutical industries, as well as in medicine. Lysozyme is used as a biopreservative for meat, fish, milk, and products made with them, as well as vegetables, fruit, and baby food, which is highly significant, as lysozyme does not induce allergies [Banks *et al.* 1986, Hughey and Johnson 1987, Losso *et al.* 2000]. Due to the antibacterial properties of lysozyme, it has also been used in oral hygiene products such as toothpaste, mouthwash, and chewing gum, to protect against bacteria causing periodontitis and to prevent stomatitis [Sava 1996, Tenuovo 2002]. Oral and local application of lysozyme have also been shown to be effective in preventing and controlling viral skin infections, particularly those induced by herpesviruses and the chickenpox virus [Sava 1996]. Furthermore, Lee-Huang *et al.* [1999] demonstrated lysozyme activity against HIV-1.

Lysozyme has also found application as an immunomodulatory and immunostimulatory factor. Some sources suggest that antimicrobial activity of lysozyme may result from stimulation of the phagocytic function of macrophages, and the hydrolyzed products of peptidoglycan can act as an adjuvant or immunomodulator [Li-Chan and Nakai 1989]. Lysozyme has been found to improve the immune response in patients whose immunity has been reduced by chemotherapy. Moreover, following administration of lysozyme, improvement was observed in the condition of patients with chronic diseases such as chronic sinusitis in children with allergies and chronic bronchitis [Sava 1996].

Intensive research on the properties of lysozyme has shown that orally administered lysozyme exhibits antineoplastic activity, inhibiting tumour formation and growth both *in vitro* and *in vivo* [Sava 1989].

Like lysozyme, conalbumin content in egg white varies among different bird species. Chicken egg white contains 12–14% conalbumin, goose egg white 4%, and duck egg white 2% [Board *et al.* 1994, Węsierska 2006]. Conalbumin is capable of binding ions of iron and other metals such as copper, zinc and aluminium. It is thought to inhibit multiplication of bacteria by depriving them of the iron necessary for their growth [Bezkorovainy 1981]. Ovotransferrin has been shown to have a broad antibacterial spectrum, especially against *Pseudomonas* spp., *Escherichia coli, Streptococcus mutans* [Valenti *et al.* 1983], *Staphylococcus aureus, Bacillus cereus* [Abdallah and Chahine 1999] and

Salmonella Enteritidis [Baron *et al.* 2000]. It has also been found to exhibit antibacterial activity, penetrating the bacterial outer membrane, reaching the inner membrane, and causing selective permeation of ions and dissipation of electric potential [Aguilera *et al.* 2003]. Conalbumin has also been demonstrated to exhibit antiviral activity against Marek's disease virus in the fibroblasts of chicken embryos [Giansanti *et al.* 2002].

Xie *et al.* [2002] have reported that ovotransferrin can act as an immunomodulator, by modulating the functions of macrophages and heterophils *in vitro*. It has also been found to exert an immunomodulatory effect by inhibiting the proliferation of lymphocytes in the spleen of mice [Otani and Odashima 1997] and stimulating phagocytosis of mononuclear and polynuclear cells of peripheral blood in dogs [Hirota *et al.* 1995].

Avidin binds biotin with a high affinity. Biotin is essential to the growth of many micro-organisms, hence avidin is treated as a natural antibacterial agent [Korpela 1984]. Besides inactivating biotin, avidin can inhibit the growth of micro-organisms by binding with surface receptors of various Gram-negative and Gram-positive bacteria, such as *Escherichia coli* K-12, *Klebsiella pneumoniae, Serratia marcescens, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis* [Korpela *et al.* 1984, Banks *et al.* 1986].

Another group of biologically active substances with antimicrobial properties in egg white are protease inhibitors, of which the most important are cystatin, ovomucoid, and ovoinhibitor. Proteases play a key role in various physiological processes, such as intracellular protein degradation, bone remodelling, and antigen presentation. Their activity increases under pathological conditions such as inflammation or cancer metastasis. They also take part in pathogen invasion.

Cystatin exhibits strongly inhibits ficin and papain, as well as cathepsins, particularly B, C, H and L, and papain-like peptidases [Barret *et al.* 1986]. It was first isolated from chicken albumen and described by Fossum and Whitaker [1968]. Cystatin occurs in many tissues and systemic fluids in humans and animals. A particularly important function of cystatin involves extracellular and intracellular control of protein decomposition [Barret *et al.* 1986]. Studies also suggest that cystatin may be participate in inflammation and immune response via the cytokine network. Verdot *et al.* [1999] determined that cystatin in hens induces synthesis of TNFα and IL-10, thus increasing nitric oxide *in vitro*, using mouse peritoneal macrophages.

The antibacterial effect of ovomucoid consists in inactivation of proteolytic enzymes that play an essential role in bacterial multiplication. Ovomucoid inhibits various enzymes, depending on its source. In the albumen of chicken eggs it inhibits trypsin activity, turkey and duck egg ovomucoid inhibits trypsin and chymotrypsin, while pheasant ovomucoid inhibits only chymotrypsin. Ovomucoid has also been shown to be an allergy-inducing agent [Acker and Ternes 1994].

The activity of ovoinhibitor is similar to that of ovomucoid. It probably protects the egg against the development of moulds, which can penetrate it during storage or incubation [Tomimatsu *et al.* 1966, Acker and Ternes 1994].

Another antibacterial defence factor preventing development of bacteria is pH. The pH of chicken egg albumen immediately after laying ranges from 7.4 to 7.9 [Silversides and Scott 2001], close to the optimal value for the growth of many bacteria. In stored eggs, however, pH increases to 9 or even 9.6 [Silversides and Scott 2001, Węsierska 2006]. According to some sources, certain strains of bacteria do not grow or do not survive at pH 9.1 [Mayes and Takeballi 1983].

Defence mechanisms of the yolk

The vitelline membrane surrounding the yolk also has antibacterial properties. It not only constitutes a physical barrier to bacteria, but also contains biologically active compounds, such as lysozyme, conalbumin and ovomucin.

An important element of antimicrobial defence in the first period of the life of the chick is yolk antibodies (IgY). These are immunoglobulins transferred by the hen to the chick via the egg. The term IgY was introduced in order to distinguish avian IgG from mammalian IgG. Yolk antibodies have a different structure to mammalian IgG. Their upsilon (υ) heavy chains do not have a hinge region, but contain five domains – one variable (V) and four constant (Cυ1–Cυ4) [Sim *et al.* 2000]. Moreover, IgY immunoglobulins have a less stable molecular structure than mammalian IgG and a lower isoelectric point. They remain stable for long periods at low temperatures and during pasteurization [Jensenius *et al.* 1981, O'Farrelly *et al.* 1992].

Yolk immunoglobulins initiate the complement activation pathway in birds, thereby initiating the process of destroying pathogenic microbes and host cells infected by them. According to Sim *et al.* [2000], they can exert a kind of antibacterial activity against pathogenic microbes by binding and immobilizing them, thus restricting or inhibiting their growth, replication or colony-forming ability. Research conducted *in vitro* and *in vivo* has shown inhibition of infections by pathogens of the alimentary tract, such as human and bovine rotaviruses, bovine coronavirus, *Escherichia coli*, *Salmonella* spp., *Yersinia ruckeri*, *Edwardsiella tarda*, *Helicobacter pylori*, infectious bursal disease virus, *Staphylococcus aureus,* and *Pseudomonas aeruginosa* [Kovacs-Nolan and Mine 2004]. Oral application of anti-*P.aeruginosa* IgY antibodies has been found to prevent colonization of these bacteria in patients with fibrocystic changes in the lungs, suggesting an alternative to antibiotic therapy [Kollberg *et al.* 2003]. Horie *et al.* [2004] observed that consumption of yoghurt containing yolk immunoglobulins (IgY) against urease produced by *H. pylori* inhibited infection by this antigen.

Zhen *et al.* [2008] demonstrated that IgY immunoglobulins produced by hens immunized with *Escherichia coli* O111 inhibited growth of this strain, as well as five other strains of *E. Coli* that cause mastitis in cows. This suggested therapeutic possibilities involving the use of specific IgY antibodies. Almeida *et al.* [2008] determined that specific IgY antibodies against African snake venom obtained from the yolks of eggs from immunized hens, are highly effective at neutralizing the lethal venom. Moreover, IgY immunoglobulins obtained in this manner have been shown to be free of toxic products, pyrogens, and bacterial and fungal contamination. Another advantage of obtaining polyvalent IgY antibodies in this way is its low cost. The authors determined that 2 g of IgY antibodies from yolks (i.e. about 20 eggs/month/1 hen) is equivalent to the concentration of these immunoglobulins in 300 ml of serum or 600 ml of mammalian blood. For this reason they tried to perfect the procedure for wide-scale production of specific IgY immunoglobulins against *Bitis* spp. and *Naja* spp. snake venom for use in treatment of domestic animals and possibly humans bitten by poisonous African snakes.

The data gathered in this paper show that eggs contain many biologically active substances with potential or demonstrated therapeutic applications beyond their nutritive properties. As the content of eggs can be manipulated using various methods, particularly changes in diet or immunization, the concept has arisen of „the hen as a bioreactor" producing substances of medical significance. Hence continuation of research to deter-

mine new and innovative benefits of known biological functions of particular egg components can contribute to their wider use in treatment and prevention of chronic and infectious diseases in people and animals. Furthermore, the properties of biologically active substances in the egg may perhaps become an alternative to antibiotic therapy, limiting the phenomenon of drug-resistant pathogens.

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Streszczenie. Jajo kurze jest ważnym źródłem składników odżywczych. Dodatkowo jaja mają mechanizmy fizyczne i biologiczne obrony, chroniące zarodek przed inwazją i namnażaniem się mikroorganizmów. Skorupa i błony podskorupowe utrudniają mechaniczne wnikanie drobnoustrojów do wnętrza jaja, natomiast lepkość i pH białka ograniczają proliferację bakterii. Białko jaja zawiera także wiele protein z udowodnionymi czynnościami przeciwzakaźnymi, jak liza ściany komórkowej bakterii, wiązanie metali oraz wiązanie witamin, które stanowią część naturalnego systemu obrony jaja. Ponadto jaja poddawane są badaniom pod względem zawartości substancji o funkcjach biologicznych, poza właściwościami odżywczymi. Różne czynności biologiczne komponentów jaja, a zwłaszcza aktywność przeciwzakaźna, immunomodulująca, właściwości hamowania proteaz podkreślają znaczenie tych substancji w zapobieganiu i leczeniu chorób ludzi i zwierząt.

Słowa kluczowe: jajo, system obrony przeciwbakteryjnej, lizozym, immunoglobulina IgY, czynności substancji biologicznych