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REVIEWS

Advances in the Value of Eggs and Egg Components for Human Health

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The avian egg is an important source of nutrients, containing all of the proteins, lipids, vitamins, minerals, and growth factors required by the developing embryo, as well as a number of defense factors to protect against bacterial and viral infection. Moreover, eggs are now understood to contain substances with biological functions beyond basic nutrition, and extensive research has been undertaken to identify and characterize these biologically active components. This review mainly focused on biological activities of proteins and peptides derived from egg components. Several biological activities have now been associated with egg components, including novel antimicrobial activities, antiadhesive properties, immunomodulatory, anticancer, and antihypertensive activities, antioxidant properties, protease inhibitors, nutrient bioavailability, and functional lipids, highlighting the importance of egg and egg components in human health and in disease prevention and treatment. Continued research to identify new and existing biological functions of hen egg components will help to define new methods to further improve the value of eggs as a source of numerous biologically active compounds with specific benefits for human and animal health and secure their role in the therapy and prevention of chronic and infectious disease.

Keywords: Avian eggs; egg white; yolk; bioactive proteins and peptides; human health; functional foods; nutraceuticals; chronic diseases

1. INTRODUCTION

Eggs consist of approximately 9.5% eggshell (including shell membrane), 63% albumen, and 27.5% yolk (*1*) (**Table 1**). The main components are water (75%), proteins (12%), and lipids (12%), as well as carbohydrates and minerals (*2*, *3*). The proteins are distributed throughout the egg, with the majority found in the egg yolk and egg white, and a small proportion in the eggshell and shell membrane (*4*, *5*). The lipids are found almost exclusively in the egg yolk, mainly in the form of lipoproteins (*2*, *4*). Several minerals have also been found in eggs, most of them in the eggshell. Carbohydrates are a minor egg component, present throughout the egg, as both free and conjugated forms, attached to proteins and lipids (*4*).

Many diverse biological functions have been attributed to egg components and will be discussed here.

2. EGG WHITE

The egg white, or albumen, makes up ∼60% of the total egg weight (*3*), of which water and protein are the major constituents (*3*, *4*). The egg white proteins include ovalbumin, which is the major protein, followed by ovotransferrin and ovomucoid. Other egg white proteins include ovomucin, which is responsible for the viscosity of the albumen, lysozyme, avidin, cystatin, ovoinhibitor, and ovomacroglobulin (ovostatin) (*4*). The biological activities of egg white proteins are summarized in **Table 2**.

2.1. Antimicrobial Activity. Eggs possess physical and biological defense systems to protect the embryo against the invasion and multiplication of microorganisms. The eggshell and shell membrane physically obstruct invading organisms, and differing viscosities and pH values in the egg white inhibit bacterial proliferation (*6*). Egg white also contains a number of proteins with demonstrated antimicrobial activities, which act as part of the natural defense system of the egg. These antimicrobial effects may be attributed to several mechanisms, including bacterial cell lysis, metal binding, and vitamin binding (*7*).

Lysozyme, which exerts bacteriolytic activity by hydrolyzing the $\beta(1-4)$ linkage between *N*-acetylmuraminic acid and *N*-acetylglucosamine of peptidoglycan, the structural component of bacterial cell walls (*8*), has been studied at length and has been applied as a natural food preservative (*9*, *10*). It is most effective against Gram-positive bacteria such as *Bacillus stearo-*

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Table 1. Chemical Composition of Hen Eggs [Adapted from Li-Chan et al. (*3*) and Mine (*5*)]

constituent	% (w/v)	major components (relative %, w/w)
egg shell	9.5 (including shell membrane)	inorganic salts (91.87) calcium carbonate (98.4) magnesium carbonate (0.8) tricalcium phosphate (0.8) proteins (6.4) water (1.7) lipids (0.03)
egg white	63.0	proteins (9.7–10.6): ovalbumin (54) ovotransferrin (12.0) ovomucoid (11) ovomucin (3.5) lysozyme (3.4) G2 globulin (4.0?) G3 globulin (4.0?) ovoinhibitor (1.5) ovoglycoprotein (1.0) ovoflavoprotein (0.8) ovomacroglobulin (ovostatin) (0.5) cystatin (0.05) avidin (0.05) lipids (0.03) carbohydrates (0.4–0.9) ash (0.5–0.6)
egg yolk	27.5	proteins (15.7–16.6): spovitellenin (I–VI) (37.3) lipovitellin apoproteins (40.0) α-lipovitellin β-lipovitellin livetins (9.3) α-livetin (serum albumin) β-livetin (α2-glycoprotein) γ-livetin (γ-globulin) phosvitin (13.4) biotin-binding protein (trace) lipids (32.0–35.0) triglycerol (66) phosphatidylcholine (PC) (24) phosphatidylethanolamine (PE) (2.8) lysophosphatidylcholine (LPC) (0.6) sphingomyelin (0.6) cholesterol (5.0) others (1.0) carbohydrates (0.2–1.0) ash (1.1)

thermophilus, *Clostridium tyrobutyricum*, and *Clostridium thermosaccharolyticum*, but this spectrum of activity can be broadened to include other spoilage and pathogenic organisms when used in conjunction with compounds such as EDTA, organic acids, or nicin (*10*). The chemical modification of lysozyme, to increase its antimicrobial activities against Gram-negative bacteria, has been examined (*11*). It was found that by equipping the lysozyme molecule with a hydrophobic moiety, through fatty acylation (*12*, *13*), or by the genetic fusion of hydrophobic peptides to the C terminus of lysozyme (*14*−*16*), the bactericidal activity of lysozyme against the Gram-negative bacteria *Escherichia coli* was enhanced, likely by mediating its interaction and insertion into the bacterial membrane. Likewise, lysozyme−polysaccharide conjugates have also demonstrated enhanced antimicrobial activity against Gram-negative bacteria (*17*−*19*). Ibrahim et al. (*20*) also found that when perillaldehyde, a naturally occurring phenolic aldehyde, was coupled to lysozyme, antibacterial activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli*) bacteria was markedly enhanced.

Studies have suggested that lysozyme may possess further antibacterial activity independent of its catalytic functions (*21*).

Table 2. Biological Activities of Egg White Proteins

protein	biological activity	refs
ovalbumin	antibacterial activity	*30*
	antihypertensive activity of ovalbumin-derived peptides	*77–83*
	immunomodulating activity	*51–56*
ovotransferrin	antimicrobial activity	*31–35*, *37*
	antibacterial activity of ovotransferrin peptide (OTAP-92)	*36*
	immunomodulating activity	*60–62*
ovomucoid	serine protease inhibitor	*115*, *120*
	immunomodulating activity	*57*
	drug delivery	*116–119*
	biospecific ligand	*131*
ovomucin	antimicrobial activity	*42–46*
	antiadhesive properties	*48*
	antitumor activity	*74*, *75*
lysozyme	antibacterial activity	*9–26*
	antiviral activity	*27*, *28*
	immunomodulating and immunostimulating activity	*7*, *27*, *58*, *59*
	antitumor activity	*66–73*
ovoinhibitor	serine protease inhibitor	*100*
	antiviral activity	*101*
ovomacroglobulin	serine, cysteine, thiol, and metallo protease inhibitor	*91*, *92*, *114*
	antimicrobial activity	*92–99*
cystatin	cysteine protease inhibitor	*3*
	antimicrobial activity	*87–90*, *101–103*
	antitumor activity	*109–113*
	immunomodulating activity	*63–65*
	inhibition of bone degradation	*121*
avidin	antibacterial activity	*40*
	biospecific ligand and drug delivery	*125–130*

Enzymatic hydrolysis of lysozyme has been found to enhance its activity, by exposing antibacterial portions of the protein (*21*, *22*), and producing peptides with antibacterial activity. Peptides corresponding to amino acid residues 98−112 (*23*), 98−108, and 15−21 (*24*) possessed antimicrobial activity against *E. coli* and *S. aureus*, and synthetic bactericidal lysozyme polypeptides were found to not only damage bacterial outer membranes but also to inhibit DNA and RNA synthesis (*23*, *25*). They were also found to prevent antibiotic-induced bacteriolysis and subsequent endotoxin release while retaining antibiotic efficacy, suggesting their use for the prevention of endotoxemia in Gram-negative sepsis with the treatment of antibiotics (*26*).

The antibacterial properties of lysozyme have led to its use in oral health care products, such as toothpaste, mouthwash, and chewing gum, to protect against periodontis-causing bacteria and prevent infections in the oral mucosa (*27*, *28*).

Lysozyme has also demonstrated antiviral activity. Oral and topical applications of lysozyme were found to be effective in preventing and controlling several viral skin infections, including herpes simplex and chicken pox (*27*), and Lee-Huang et al. (*29*) found that chicken lysozyme also possessed activity against HIV type 1.

Peptides produced by the enzymatic digestion of ovalbumin, and their synthetic counterparts, were found to be strongly active against *Bacillus subtilus* and to a lesser extent against *E. coli*, *Bordetella bronchiseptica*, *Pseudomonas aeruginosa*, and *Serratia marcescens*, as well as *Candida albicans* (*30*).

Ovotransferrin, a member of the transferrin family, a group of iron-binding proteins widely distributed in various biological fluids, has the capacity to reversibly bind iron (*31*). It is

suggested to function as an iron scavenger, preventing iron use by microorganisms, and as an iron delivery agent (*32*). Ovotransferrin has demonstrated antibacterial activity against a wide spectrum of bacteria, including *Pseudomonas* spp., *E. coli*, *Streptococcus mutans* (*33*), *S. aureus*, *Bacillus cereus* (*32*), and *Salmonella enteritidis* (*34*). It has also been found to exert antibacterial activity by permeating bacterial outer membranes, reaching the inner membrane and causing the selective permeation of ions and dissipation of electrical potential (*35*). A 92-amino acid ovotransferrin peptide, OTAP-92, was found to be capable of killing Gram-negative bacteria by crossing the bacterial outer membrane by self-promoted uptake, damaging the cytoplasmic membrane (*36*). It has also shown antiviral activity against Marek's disease virus in chicken embryo fibroblasts (*37*).

Avidin, which possesses the unique ability to specifically bind the water-soluble vitamin biotin, has been found to inhibit the growth of biotin-requiring bacteria and yeasts (*38*, *39*). The antimicrobial activity has also been attributed to its ability to bind to various Gram-negative and Gram-positive bacteria, including *E. coli* K-12, *Klebsiella pneumoniae*, *S. marcescens*, *P. aeruginosa*, *S. aureus*, and *Staphylococcus epidermis* (*40*).

Ovomucin and ovomucin-derived peptides, besides their physical functions such as maintaining the structure and viscosity of the egg white albumen and thus preventing the spread of microorganisms (*41*), have demonstrated antiviral activity against Newcastle disease virus, bovine rotavirus, and human influenza virus in vitro (*42*−*46*).

2.2. Antiadhesive Properties. The adhesion of microorganisms to host tissues is the first step in the infection process, in many cases mediated by an interaction between components on the surface of the microorganism and carbohydrates on the mucosal surface of the host (*47*). It has been suggested, then, that oligosaccharides and glycoconjugates, such as analogues of carbohydrates on the mucosal surface, would competitively inhibit microorganism−carbohydrate adhesion on intestinal cells, thereby preventing microbial infection (*48*).

Much of the research involving the antiadhesive properties of eggs has focused on egg yolk components and will be discussed later. Kobayashi et al. (*48*) found, however, that enzymatic digestion of the egg white protein ovomucin, which is highly glycosylated, produced ovomucin glycopeptides (OGP), which possessed *E. coli* O157:H7-specific binding sites consisting of sialic acid. On the basis of these findings, it was suggested that OGP may be protective against *E. coli* O157:H7 infection in vivo and is a potentially novel probe for the detection of the bacteria.

2.3. Immunomodulating activity. The immune system responds to antigenic stimulation with a complex array of molecular events, involving antigen-presenting cells, B-cells, T-cells, and phagocytes. Cytokines play a significant role in regulating such immune responses (*49*). Cytokines and growth factors mediate a wide range of physiological processes, including hematopoiesis, immune responses, wound healing, and general tissue maintenance. They are concomitantly involved in the pathology of a wide range of diseases and have potential use in replacement and immunomodulatory therapy (*50*).

Several egg white proteins and peptides have demonstrated immunomodulating activity. Ovalbumin has been found to induce the release of tumor necrosis factor (TNF) α in a dose-dependent manner in vitro, when modified with dicarbonyl methylglyoxyl (*51*), and immunogenic ovalbumin peptides have been used to enhance immune responses for cancer immunotherapy (*52*−*54*). Tezuka and Yoshikawa (*55*) found that the phagocytic activity of macrophages was increased by the addition of ovalbumin peptides, OA 77−84 and OA 126−134, derived from peptic and chymotryptic digestions, respectively. Likewise, ovomucin peptides have shown macrophage-stimulating activity in vitro (*56*). Synthetic ovomucoid peptides have also demonstrated immunomodulating activity, inducing T-cell secretion of cytokines interleukin (IL)-4, IL-10, IL-13, interferon (IFN) γ, and IL-6 (*57*).

Lysozyme has been shown to act as an immune-modulating and immune-stimulating agent. When combined with immunotherapy, lysozyme was effective in improving chronic sinusitis (*58*) and in normalizing humoral and cellular responses in patients with chronic bronchitis (*27*). Lysozyme was also found to enhance antibody production in hybridomas and lymphocytes, being termed an immunoglobulin production stimulating factor (*59*), and to regulate and restore the immune responses in immune-depressed patients undergoing anticancer treatments (*27*). Furthermore, it has been suggested that the antibacterial activity of lysozyme might also occur via stimulation of the macrophage phagocytic function, and the hydrolysis products of peptidoglycan may act as an adjuvant or immunomodulator (*7*).

Ovotransferrin is an acute phase protein in chickens, the serum levels of which increase in inflammation and infections (*60*). Xie et al. (*60*) demonstrated that ovotransferrin can act as an immunomodulator, modulating macrophage and heterophil functions in vitro. Further immunomodulating effects of ovotransferrin have also been shown, including the inhibition of proliferation of mouse spleen lymphocytes (*61*) and the enhanced phagocytic response of peripheral blood mononuclear cells and polymorphonuclear cells in dogs (*62*).

Finally, research has suggested that cystatins may also be involved in inflammation and immune responses through the cytokine network, through mechanisms unrelated to the known protease inhibitory regions of the molecule (*63*). Verdot et al. (*64*, *65*) found that chicken cystatin induced the synthesis of TNF-α and IL-10, resulting in an up-regulation of nitric oxide in vitro using mouse peritoneal macrophages. Cystatin was also found to up-regulate the production of IL-6 by human gingival fibroblast cells and murine splenocytes and the IL-8 production of gingival fibroblasts (*63*).

2.4. Anticancer Activity. Lysozyme has been studied extensively as an anticancer agent and has been shown to inhibit tumor formation and growth, both in vitro and in vivo when administered orally, in a number of experimental tumors, including lung carcinoma (*66*−*71*). It was also found to enhance the efficacy of chemotherapy treatments (*72*, *73*) and to have a preventative effect when administered to normal mice (*69*). Evidence suggests that the anticancer effects of orally administered lysozyme may rely heavily upon the host-mediated immune response, including activation of the spleen and macrophages (*68*); however, more recent data indicate that lysozyme may also exert action on the tumor cells themselves (*69*, *71*).

Pronase-prepared glycopeptides of ovomucin have also demonstrated antitumor effects in a double-grafted tumor system in mice (*74*), suggested to be related to the antiangiogenic activity of ovomucin, inhibiting tumor growth (*75*).

2.5. Antihypertensive Activity. It has been reported that certain egg white-derived peptides can play a role in controlling the development of hypertension by exerting vasorelaxing effects (*76*). A vasorelaxing peptide, ovokinin (OA 358−365), was isolated by the peptic digestion of ovalbumin (*77*). Ovokinin (2−7), a peptide produced by chymotrypsin digestion and

corresponding to OA 359−364, was also found to possess vasorelaxing activity (*78*). Both peptides were found to significantly lower the systolic blood pressure in spontaneously hypertensive rats, in a dose-dependent manner, when administered orally (*79*). The replacement of amino acids in the ovokinin (2−7) peptide has resulted in enhanced antihypertensive activity, with the most potent derivative resulting in a 100-fold more potent antihypertensive activity (*80*, *81*). Two angiotensin I converting enzyme (ACE)-inhibitory peptides were also identified in ovalbumin by peptic (OA 183−184) and tryptic (OA 200−218) digestions (*82*).

Miguel et al. (*83*) examined peptides with ACE-inhibitory properties produced by enzymatic hydrolysis of crude egg white, which were mainly derived from ovalbumin. Among these peptides, two novel peptides with potent ACE-inhibitory activity were found, with amino acid sequences Arg-Ala-Asp-His-Pro-Phe-Leu and Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu.

2.6. Antioxidant Properties. Reactive oxygen species and other free radicals cause oxidative damage to DNA, proteins, and other macromolecules such as lipids. They have also been implicated in a number of multifactorial degenerative diseases including diabetes, cancer, and cardiovascular disease (*84*).

Davalos et al. (*76*) reported that the enzymatic hydrolysis of crude egg white proteins with pepsin resulted in the production of peptides with strong antioxidant activities. The peptide Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu, which was shown previously to possess ACE-inhibitory activity, also exhibited a high radical-scavenging activity. These results would suggest that the combined antioxidant and ACE-inhibitory properties of egg white hydrolysates, or the corresponding peptides, would make a useful multifunctional preparation for the control of cardiovascular diseases, in particular, hypertension. The antioxidant effects of ovalbumin were also found to be enhanced by glycosylation, via the covalent attachment of galactomannan, suggested to be a result of an increase in lipid affinity (*19*).

2.7. Protease Inhibition. Proteases play key roles in several physiological processes, including intracellular protein degradation, bone remodeling, and antigen presentation, and their activities are increased in pathophysiological conditions such as cancer metastasis and inflammation. They are also required for invasion by microorganisms (*85*). Therefore, protease inhibitors represent an important class of compounds of therapeutic significance. Four protease inhibitors have been identified in egg white: cystatin, ovomucoid, ovomacroglobulin (also known as ovostatin), and ovoinhibitor (*7*).

Microbial proteases are involved in the mechanism of penetration of tissues by bacteria, in the proteolytic cleavage of precursor proteins for virus replication, and in the facilitation of host invasion by parasites (*86*). Egg white cystatin, a type 2 cystatin that inhibits most cysteine proteases, including ficin, papain, and cathepsins B, C, H, and L (*3*), has been shown to possess antimicrobial activity, preventing the growth of group A streptococcus (*87*), *Salmonella typhimurium* (*88*), and the periodontis-causing *Porphyromonas gingivalis* (*89, 90*).

Ovomacroglobulin possesses broad-spectrum inhibitory activity against various types of proteases, including serine proteases, cysteine proteases, thiol proteases, and metalloproteases (*91, 92*), and has demonstrated antimicrobial activity against *S. marcescens* and *P. aeruginosa*, both in vitro (*92*−*95*) and in vivo. It was found to reduce corneal destruction in an experimental keratitis model in rabbits and to accelerate wound healing (*95*−*97*). Ovomacroglobulin was also found to suppress *P. aeruginosa* and *Vibrio vulnificus* septicemia due to the inhibition of kinin-generating proteases (*98, 99*).

Both cystatin and ovoinhibitor, a serine protease inhibitor (*100*), have been found to prevent rotavirus infection in mice (*101*), and cystatin has been found to inhibit the action of poliovirus protease, effectively inhibiting virus replication in vitro (*102, 103*). As well, cystatin was capable of greatly reducing parasite numbers in a mouse model of visceral leishmaniasis (*104*).

Several proteolytic enzymes, including cysteine proteases, are believed to play an important role in cancer invasion and metastasis (*105*). Increased levels of cysteine proteases, and concomitant decreases in cystatin, have been observed in various cancers (*106, 107*). Cystatins inhibited the tumor-associated activity of intracellular cysteine proteases and have been suggested as potential anticancer drugs (*108*). Cystatin inhibited tumor invasion in ras-transformed breast epithelial cells (*109*) and was found to reduce the activity of the key proteolytic enzymes responsible for the growth of gastric cancer in vitro (*110*). Multifunctional inhibitors, composed of chicken cystatin in conjunction with other protease inhibitors, have been suggested for the therapy of solid tumors (*111*−*113*). Similarly, ovomacroglobulin was found to suppress metalloproteases and vascular permeability in skin tissues, which play a role in tumor metastasis (*114*).

Ovomucoid, a serine protease inhibitor (*115*), has been shown to be particularly useful for the oral delivery of protein/peptide therapeutics, the application of which is often limited due to extensive proteolytic degradation in the gastrointestinal tract (*116*). Because ovomucoid inhibits digestive enzymes, such as trypsin, α-chymotrypsin, and elastase, it has been found to improve the oral delivery of insulin (*117*−*119*) and has been examined for co-administration with calcitonin, a polypeptide associated with calcium homeostasis and bone remodeling, which is often used in the management of osteoporosis (*116*). In addition, ovomucoid has been used as a model for the design of therapeutic inhibitory peptides (*120*).

Proteolytic enzymes, in particular cysteine proteases, are involved in bone resorption, and egg white cystatin has been found to inhibit bone matrix degradation and calcium release, in vitro (*121*). Furthermore, proteases have also been implicated as contributors in several other important diseases, including viral diseases, such as HIV (*122*), and Alzheimer's (*123, 124*), suggesting an important role for egg white-derived protease inhibitors in human health.

2.8. Biospecific Ligand. Avidin has been used in cancer treatment, to localize and image cancer cells and to pretarget drugs to tumors. Because of its tight biotin binding and signal amplification due to the tetrameric structure, it leads to the accumulation of higher effective doses and increased persistence of biotinylated anticancer drugs, as compared to other immunotherapeutic procedures (*125*). Tumor pretargeting with avidin has also been found to be effective in increasing the uptake of TNF α conjugated to biotin in vitro, improving the antitumor activity of TNF (*126*−*128*). Yao et al. (*129*) found that radiolabeled avidin also bound to lectins expressed on the surface of tumor cells and localized highly and rapidly in various types of tumors in mice, thereby reducing radioactivity accumulation in other organs. The utilization of avidin for drug delivery through the blood−brain barrier has also been demonstrated, facilitating delivery of therapeutics to the brain (*130*). Ovomucoid has been found to promote the targeting of drugs to the blood, by acting as a biospecific ligand to lectins on the walls of the gastrointestinal tract (*131*).

Table 3. Biological Activities of Egg Yolk and Yolk Components

component	biological activity	refs
egg yolk	antiadhesive	*150*, *155–157*
immunoglobulin Y	antimicrobial activity	*133–135*, *139–146*
phosvitin	antibacterial activity	*148*, *149*
	antioxidant activity	*162*, *163*
	enhancement of calcium solubility	*149*, *164*, *165*
sialyloligosaccharides and sialylglycopeptides	antiadhesive properties	*151–154*
yolk lipids	antioxidant activity	*158–160*
lipoproteins	antibacterial activity	*6*, *147*
fatty acids	antibacterial activity	*6*
phospholipids	role in brain development and function	*172–175*, *179*
	reduction of cholesterol levels	*170*, *172*
cholesterol	component of cell membranes	*166–168*

3. EGG YOLK

The major constituents of egg yolk are proteins and lipids, present mainly in the form of lipoproteins, and can be separated into a granule fraction and plasma fraction (*4*). The granules contain α- and β-lipovitellins (high-density lipoproteins), phosvitin, and low-density lipoproteins (*132*). The plasma can be divided into the low-density lipoprotein fraction and the water-soluble fraction, which contains livetins, which are lipid-free globular proteins, among them γ-livetin, also referred to as immunoglobulin Y (*3*). Egg yolk also contains minerals and carbohydrates, most of which are oligosaccharides bound to protein, as well as pigment (*4*). The biological activities of egg yolk components are summarized in **Table 3**.

3.1. Antimicrobial activity. Although the egg white is the main line of defense against invading microorganisms, a number of egg yolk components have also demonstrated antimicrobial activity. One of the most extensively studied is immunoglobulin (Ig) Y, which has been reviewed in detail elsewhere (*133*−*135*). IgY is the functional equivalent of IgG, the major serum antibody in mammals (*136*). It is transferred from the hen to the developing embryo, to give acquired immunity to the chick (*133*, *137*). Specific IgY can be produced by immunization of chickens with the target antigen and then purified from the egg yolk (*136*, *138*). It has been suggested that the antibodies may exert a sort of antimicrobial activity against pathogenic organisms by binding, immobilizing, and consequently reducing or inhibiting their growth, replication, or colony-forming abilities (*133*).

IgY has been produced against a number of bacteria and viruses and has been shown to bind to and inhibit the infection and disease symptoms, in vitro and in vivo, of gastrointestinal pathogens such as human and bovine rotavirus, bovine coronavirus, *E. coli*, *Salmonella* spp., *Yersinia ruckeri*, *Edwardsiella tarda*, *Helicobacter pylori*, porcine epidemic diarrhea virus, and infectious bursal disease virus, as well as *S. aureus* and *P. aeruginosa* (*134*). IgY against *S. mutans* has been shown to prevent adhesion of the bacteria in vitro and in vivo and to reduce dental caries development in an animal model (*139*−*143*). In human studies, orally administered anti-*P. aeruginosa* IgY was found to prevent *P. aeruginosa* colonization in the lungs of cystic fibrosis patients, indicating its use as an alternative to antibiotic treatment (*144*, *145*), and the suppression of *H. pylori* infection in humans was observed following the consumption of a yogurt beverage fortified with IgY against *H. pylori* urease enzyme (*146*).

Brady et al. (*147*) found that egg yolk lipoproteins were also important for lipid-mediated antimicrobial activity. The authors reported that a water-soluble protein fraction of egg yolk was capable of inhibiting the growth of *Streptococcus* spp. and suggested that it was likely attributable to lipoproteins (LDL) present in the water-soluble fraction. They found that the antimicrobial activity increased upon treatment with digestive enzymes, indicating that the molecule may require degradation to release the maximal levels of the active component. It was also found that lipids and lipoproteins, as well as their component fatty acids, oleic and linoleic acid, when extracted from egg yolk, exerted antimicrobial effects against *Streptococcus* (*6*).

Finally, egg yolk phosvitin, an iron-binding phosphoprotein, has also demonstrated antibacterial activity against *E. coli* under thermal stress, causing disruption of cells and DNA leakage, suggested to be a result of the synergistic effect of its high metal-chelating ability and high surface activity (*148*, *149*).

3.2. Antiadhesive Properties. As previously mentioned, microbial infection is initiated by invasion of the epithelial layer of the gastrointestinal tract. Attachment is required for the microorganism to invade the intestine and to initiate infection. Blocking of this attachment in the gastrointestinal tract represents a potential strategy for disease prevention and has recently become a target for prevention therapy (*150*).

Several studies have demonstrated that oligosaccharides in foods such as milk and eggs have an inhibitory effect on the binding of bacteria and viruses to host cells (*151*). The use of natural antimicrobials is a particularly attractive approach, due to the increasing prevalence of microorganisms resistant to, or untreatable by, traditional antibiotic therapy (*48*).

Koketsu et al. (*152*) examined the antiadhesive effect of egg yolk sialyloligosaccharides on viral infection. They found that the oligosaccharide-enriched fraction of egg yolk and the sialyloligosaccharide fraction inhibited rotavirus infection in vitro in a dose-dependent manner, suggesting that sialic acid plays an important role in the binding and inhibition of the virus. They also found that egg yolk sialyloligosaccharides significantly decreased the incidence of rotavirus diarrhea in vivo, in mice (*152*) and in rats (*153*).

More recently, the effects of egg yolk and its components on bacterial adhesion have also been studied. On the basis of the observations that milk-derived oligosaccharides were capable of preventing the binding of bacteria and toxins to intestinal cells, Sugita-Konishi et al. (*154*) examined the effects of egg yolk sialyloligosaccharides and their derivatives, asialo-yolk-derived sialyloligosaccharides and sialylglycopeptide, on the binding of *Salmonella enteritidis* and *E. coli*. These compounds inhibited the binding of both organisms to human intestinal cells in vitro and prevented *Salmonella* infection when orally administered to mice. The effect of the sialyloligosaccharides and their derivatives on macrophage activation was also examined, and the results suggested that these yolk compounds exerted their antibacterial action by preventing bacterial adhesion and entry through the intestine, rather than via immunomodulatory effects (*154*). Similar results were observed using sialylglycopeptides from egg yolk, conjugated to nondigestive polysaccharides to increase retention time in the gut (*151*).

Deignan et al. (*150*) examined the antiadhesive effects of egg yolk on *S. typhimurium*. They found that egg yolk from both immunized and nonimmunized hens similarly inhibited *S. typhimurium* binding to intestinal epithelial cells in vitro and concluded that egg yolk may contain some antiadhesive factors beyond IgY, which could prevent bacterial adhesion. The authors went on to suggest that this could be due to nonspecific mechanical inhibition by the egg yolk or that some component

of the yolk might alter bacterial electrostatic charge or hydrophobicity, reported to be important factors in the interaction of bacteria with host cells (*150*).

Nonimmunized egg yolk powder was also found to eliminate and prevent the intestinal colonization of *S. enteritidis* in vivo, in laying hens, when administered at a concentration of 5% in normal feed (*155*), and eliminated or significantly reduced colonization of *S. typhimurium*, *Campylobacter jejuni*, and *E. coli* O157:H7, at concentrations of 7.5 and 10% (*156*). Furthermore, these pathogens were absent or significantly reduced in internal organs, indicating that the egg yolk supplement inhibited colonization and invasion. The low intrinsic concentrations of sialyloligosaccharide components in egg yolk may suggest that the observed effects were due to the presence of a yet unidentified antiadhesive factor of egg yolk (*155*). Egg yolk proteins also significantly inhibited the ability of fluorescent *S. mutans* to adhere to hydroxylapatite, an in vitro model surface used to mimic adhesion of oral bacteria to saliva-coated surfaces (*157*).

3.3. Antioxidant Properties. The antioxidant effects of egg yolk lipids and phospholipids are well-known (*158*−*160*) and have been examined for the prevention of unsaturated fatty acid oxidation. Phosvitin has also been recognized as an egg yolk antioxidant component, acting as an antioxidant by chelating iron ions (*161*). Conjugation of phosvitin with galactomannan was found to significantly increase its antioxidant activity (*162*). Recently, phosvitin and its enzymatic digests were found to protect against iron-catalyzed hydroxyl radical formation and to protect DNA against oxidative damage induced by Fe(II) and peroxide, suggesting that phosvitin may be useful for the prevention of iron-mediated oxidative stress-related diseases, such as colorectal cancer (*163*).

3.4. Nutrient Bioavailability. In general, phosvitin has been considered to have no real nutritional value, due to low iron bioavailability and resistance to proteolytic enzymes. However, phosvitin phosphopeptides, derived by tryptic hydrolysis following partial alkaline dephosphorylation, have been found to enhance calcium-binding capacity and inhibit the formation of insoluble calcium phosphates (*164*, *165*). Furthermore, phosvitin peptides demonstrated better calcium-solubilizing activity than commercial casein phosphopeptides (*149*, *164*).

3.5. Health and Development. Dry egg yolk contains approximately 60% lipids, of which around 65% is triglyceride, 28% is phospholipid, and 5% is cholesterol (*3*). Egg yolk lipids have been found to possess numerous nutritional and health benefits.

Cholesterol, an important component in cell membranes, is required for the growth of infants and is a precursor of bile acids, sex hormones, and cortex hormones (*166*). The supplementation of infant formulas with egg yolk lipids has been suggested to more closely resemble the mother's milk, and it has been found that while providing essential nutrients, the yolk lipids did not result in an increase in plasma cholesterol, indicating that it could safely be included in the infant diet (*167*). The administration of an egg yolk-derived lipid mixture to elderly individuals, formulated for in vivo rectification of rigidified cell membranes, to restore proper physiological function, also resulted in an increase in lymphocyte responsiveness (*168*).

Phospholipids are lipids that contain phosphate and have a glycerol-phosphate backbone. They make up ∼31% of the egg yolk lipids (*166*). Feeding infant formula containing egg phospholipids was found to reduce the incidence of necrotizing enterocolitis, suggesting that one or more of the compounds of egg phospholipids may enhance the immature intestinal functions of infants (*169*).

Egg yolk phospholipids were also shown to decrease serum cholesterol levels in rats (*170*) and to reduce the intestinal absorption of cholesterol (*171*), and they have been found to improve memory retention and increase acetylcholine concentrations, a neurotransmitter that decreases in concentration in cases of Alzheimer's disease (*172*, *173*). One phospholipid in particular, phosphatidylcholine, is a significant source of choline, an important nutrient in brain development, liver function, and cancer prevention (*174*), and a diet including egg phosphatidylcholine was found to enhance "maze-learning" ability and brain functions in old mice (*175*).

In recent years, the role of unsaturated fatty acids, in particular, omega-3 fatty acids, in human health and development has come to light. The health benefits of omega-3 fatty acids are significant and well documented, including prevention and treatment of hypertension, arthritis, and autoimmune disorders, as well as the inhibition of certain cancers, and are essential for fetal brain and visual development (*176*−*178*). Furthermore, they have been implicated in the prevention of some neuropsychiatric disorders, particularly depression, and in dementia, including Alzheimer's disease (*179*). An effort to increase omega-3 fatty acid intake has led to the development of omega-3 fatty acid-enriched eggs, via the manipulation of the laying hens' diet (*180*). It was found that the consumption of four omega-3-enriched eggs per day for four weeks did not significantly increase plasma cholesterol and low-density lipoprotein, but rather decreased plasma triglycerides and blood platelet aggregation (*181*).

4. CONCLUSION

It is now well established that eggs contain numerous substances with potential and demonstrated therapeutic effects, beyond supplying basic nutritional requirements, with several of these already produced on an industrial scale for food or medical applications. It has also been demonstrated that the content of eggs can be manipulated, through various methods including diet or immunization, to target certain functionalities, leading to the concept of the hen as a bioreactor for the production of medically relevant substances. Continued research to identify new and existing biological functions of hen egg components will help to define new methods to further improve the value of eggs, as a source of numerous biologically active compounds with specific benefits for human and animal health, and secure their role in the therapy and prevention of chronic and infectious disease.

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