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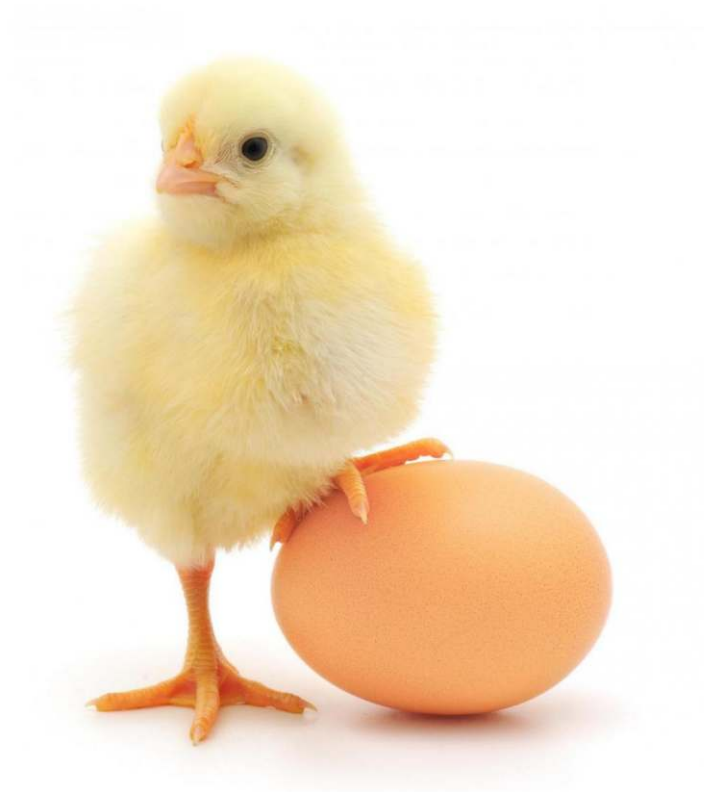

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Insight into Chicken Egg Proteins and Their Role in Chemical Defense Mechanism

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Abstract: Chicken eggs are considered as complete food having a perfect balance of essential amino acids needed for building and repairing of body tissues. Eggs get infected by vertical as well as horizontal transmission of bacteria, thereby posing risk to human health. However, nature has bestowed an egg with inherent protective mechanism to combat these problems. There are 2 main natural defenses in an egg viz. physical and chemical. The physical defense against bacteria invasion is accomplished by eggshell, together with the cuticle and membranes. The chemical defense consists of proteins that exhibit antimicrobial activity and are mainly present in albumen and to a lesser extent in yolk and shell. Eggshell matrix contains many antimicrobial proteins namely ovocleidin-17, ovocleidin-116, ovocalyxin-21, ovocalyxin-25, ovocalyxin-32 and ovocalyxin-36 and they have shown *in vitro* antimicrobial activity against many microbes, mostly because of their strong binding affinity for bacterial polysaccharides. Similar to eggshell, the albumen also contains several antimicrobial proteins with important ones being ovotransferrin, ovomucoid, lysozyme, ovoinhibitor, avidin and cystatin. Ovotransferrin exerts antibacterial effect based on iron deprivation method. Ovomucoid causes inactivation of trypsin enzyme and ovoinhibitor inhibit bacterial and fungal serine proteinase. Lysozyme causes splitting of bacterial cell wall peptidoglycan bond between N-acetylmuramic acid and N-acetyl-glucosamine. Cystatin inhibits sulphydryl proteinases while as avidin shows strong binding affinity for biotin. Yolk proteins include lipovitellin, phosvitin and Livetin. Lipoproteins have been reported to be antibacterial and antiviral. Phosvitin has high metal-chelating ability and γ-livetin (Immunoglobulin Y) binds and immobilize bacteria, thereby exert antimicrobial effect. Following the exploration of mechanism of action and antimicrobial selectivity of all these chicken egg proteins, they could be used as a viable therapeutic alternative in animal and human production.

Key words: Antimicrobial, avian egg, chemical defense, proteins

INTRODUCTION

Chicken eggs form an important constituent of human diet. They are important source of various nutrients viz. protein, fats, vitamins and minerals (Table 1). Chicken eggs are considered as complete food having a perfect balance of essential amino acids needed for building and repairing of body tissues (Watkins, 1995). Eggs get infected by vertical as well as horizontal transmission of bacteria. Vertical transmission of egg occurs by bacterial invasion during egg formation in the reproductive tract of hen (Miyamoto *et al*., 1997), while as horizontal transmission occurs if the shell is exposed to environment contaminants including faecal pathogens of hen (De Reu *et al*., 2006; Gantois *et al*., 2009; Baron *et al*., 2011). However, nature has bestowed an egg with inherent protective mechanism to combat these problems. The chicken egg has different compartments as yolk, albumen (egg white) and eggshell (Hincke *et al*., 2008) which exert defence against invading organisms. There are 2 main natural defenses in an egg viz. physical and chemical. The physical defense against bacteria invasion is accomplished by eggshell, together with the cuticle and membranes. The chemical defense consists of proteins that exhibit antimicrobial activity and are mainly present in albumen and to a lesser extent in yolk and shell (Gautron and Nys, 2006). This review will focus on these antimicrobial proteins present in various parts of an avian egg.

Proteins present in chicken egg: With the advancement in proteomics, a number of proteins have been identified in chicken egg; however, all of them have reported to be not involved in antimicrobial defense mechanism (Rehault-Godbert *et al*., 2011). The antimicrobial egg proteins either cause direct degradation of microbial components or chelation of vitamins, minerals essential for microbial growth and/or inhibition of bacterial proteases involved in the invasion of pathogens (D'Alba and Shawkey, 2015). Gautron and Nys (2006) reported that the antimicrobial proteins in chicken egg are mostly present in albumen and only to a lesser extent in yolk and shell. However, according to Abdou *et al*. (2013) most of these proteins are present in the egg white and egg yolk amounting to 50 and 40%, respectively, with remaining amount in egg shell and egg shell membranes.

Egg shell proteins: The chicken eggshell has been reported to contain six layers viz. outermost cuticle, followed by vertical crystal layer, palisade layer, mammillary layer and finally the outer and inner shell membranes (Nys *et al*., 2004). The avian eggshell constitutes an organic matrix (3.5%) that comprises of eggshell membranes, cuticle and other constituents embedded in the layer of calcium carbonate as reported by Gautron and Nys (2006). They categorized the matrix proteins of eggshell into 3 categories viz. major egg white proteins (ovalbumin, lysozyme and ovotransferrin) localized in basal layer of shell, ubiquitous proteins (osteopontin and clusterin) expressed in other tissues and specific eggshell matrix proteins (ovocleidin-17, ovocleidin-116, ovocalyxin-21, ovocalyxin-25, ovocalyxin-32 and ovocalyxin-36).

The *in vitro* evaluation of specific matrix proteins have shown their antimicrobial activity against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Mine *et al*., 2003; Wellman-Labadie *et al*., 2008). Ovocleidin-17 is a C-type lectin-like protein (Mann and Siedler, 1999) having strong binding affinity for bacterial polysaccharides especially peptidoglycan (Wellman-Labadie *et al*., 2008). Ovocleidin-116 belongs to the secretory calcium-binding phosphoprotein group of proteins (Kawasaki and Weiss, 2006). Ovocalyxin-21 has been reported to show identicalness with gastric secretome component gastrokine-2 and ovocalyxin-25 contains protease inhibitor domain (Gautron and Nys, 2007; Rose and Hincke, 2009). Ovocalyxin-32 is a member of the latex in family of carboxypeptidase inhibitors (Gautron *et al*., 2001). Ovocalyxin-36 has been reported to exert antimicrobial effect by binding the bacterial lipopolysaccharides (Cordeiro *et al*., 2013).

Egg albumen proteins: Egg albumen proteins are antimicrobial, enzymatic and anti-enzymatic, cell growth stimulatory, metal and vitamin binding and immunological (Ibrahim, 1997). Similar to eggshell, the albumen also contains several antimicrobial proteins. As per Kovacs-Nolan *et al*. (2005), ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3.5%) and ovomucin (3.5%) are the major proteins and ovoinhibitor (1.5%), ovoglycoprotein (1.0%), ovoflavoprotein (0.8%), ovomacroglobulin (0.5%), avidin (0.05%) and cystatin (0.05%) minor proteins found in egg albumen. Out of these, ovalbumin localizes in the mammillae of eggshell (Hincke, 1995); ovotransferrin (Gautron *et al*., 2001) and lysozyme (Hincke *et al*., 2000) mainly in the eggshell membranes. Board *et al*. (1994) reported ovotransferrin, ovomucoid, lysozyme, ovoinhibitor, avidin and cystatin as important egg albumen antimicrobial proteins.

Ovotransferrin (also known as conalbumin) is iron-binding protein and exert antibacterial effect based on iron deprivation which is an essential growth factor for most of the microorganisms (Abdou *et al*., 2013). It has broad spectrum of antibacterial activity, especially against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas* spp., *Salmonella enteritidis*, *Streptococcus mutans* and *Staphylococcus aureus* (Valenti *et al*., 1983; Abdallah and Chahine, 1999; Baron *et al*., 2000), besides antiviral activity (Giansanti *et al*., 2005). Rzedzicki and Stepien-Pysniak (2009) reported that the antibacterial property of ovomucoid occurs due to inactivation of trypsin enzyme that is essential during multiplication of bacteria. Likewise, ovoinhibitor has been reported to inhibit bacterial and fungal serine proteinase (Sugino *et al*., 1997). Lysozyme is another antimicrobial protein that exerts its effect by splitting the bond between N-acetylmuramic acid and N-acetyl-glucosamine of bacterial cell wall peptidoglycan of some Gram positive bacteria (Masschalck and Michiels, 2003) but Gram-negative bacteria have been reported to be less susceptible to its activity (Wild *et al*., 1997). However, Li-Chan and Nakai (1989) reported that stimulation of phagocytic activity of macrophages by lysozyme as its possible antimicrobial mechanism. Cystatin inhibits sulphydryl proteinases and has been reported to have antimicrobial and antiviral activities (Ebina and Tsukada, 1991). Avidin exerts its antibacterial activity by having strong binding affinity for biotin which is important for growth of many micro-organisms or by binding with surface receptors of various bacteria (Korpela, 1984; Banks *et al*., 1986).

Egg yolk proteins: Yolk is composed of particles (especially granules) suspended in yellow fluid called plasma, which contains proteins. The granules contain high-density lipoprotein (HDL), phosvitin and some low-density lipoprotein (LDL) (15%); while as major LDL (85%) and livetins are present in plasma (Mine and Kovacs-Nolan, 2006; Mann and Mann, 2008). HDL or lipovitellin exists as a complex phosvitin (Sugino *et al*., 1997). The word phosvitin indicates that it contains high phosphorous content and its origin is egg yolk (Mecham and Olcotte, 1949). Phosvitin is a mixture of two polypeptides: α-phosvitin and β-phosvitin (Itoh *et al*., 1983). Phosvitin and lipovitellin are metal-binding proteins with 90% iron in the yolk bound to phosvitin (Greengard *et al*., 1964) and 90% zinc to lipovitellin (Tupper *et al*., 1954). LDL when treated with ether gives a residual fraction called lipovitellenin containing 40% lipids (Sugino *et al*., 1997). Livetins consist of α-, β- and γ-fractions corresponding to hens' plasma albumin, α2-glycoprotein and mixed immunoglobulins respectively (Williams, 1962). γ-livetin is usually referred to as Immunoglobulin Y (IgY) (Leslie and Clem, 1969).

The *in vitro* study has showed the antibacterial activity of lipoproteins in inhibiting the growth of *Streptococcus mutans* (Mine and Kovacs-Nolan, 2006). Moreover,

Table 1: Nutritional composition of an edible portion of about 100 g chicken egg

Component	Quantity	Component	Quantity
Egg shell (%)	10.5	Calcium (mg)	56.0
Egg yolk (%)	31	Magnesium (mg)	12.0
Egg white (%)	58.5	Iron (mg)	2.1
Water (g)	74.5	Phosphorus (μg)	180.0
Energy (Kcal)	162	Zinc (mg)	1.44
Protein (g)	12.1	Thiamine (mg)	0.09
Carbohydrates (g)	0.68	Riboflavin (mg)	0.3
Lipids (g)	12.1	Niacin (mg)	0.1
Saturated fatty acids (g)	3.3	Folic acid (μg)	65.0
Monounsaturated fatty acids (g)	4.9	Cyanocobalamin (μg)	66.0
Polyunsaturated fatty acids (g)	1.8	Pyridoxine (mg)	0.12
Cholesterol (mg)	410	Retinol equivalents (μg)	227.0
Iodine (μg)	12.7	Potassium (mg)	147
Tocopherols (μg)	1.93	Carotenoids (μg)	10
Selenium (μg)	10	Cholecalciferol (μg)	1.8

Source: Miranda *et al*. (2015)

high-density lipoproteins have been reported to be anti-adhesive, thus having role in inhibiting the colonization of *Salmonella typhimurium*, *Campylobacter jejuni* and *E. coli* O157:H7 in chicken (Kassaify and Mine, 2004; Kassaify *et al*., 2005). The antimicrobial study on phosvitin revealed that it has antibacterial activity against *Escherichia coli* under thermal stress which might be due to the high metal-chelating ability of phosvitin (Sattar Khan *et al*., 2000; Choi *et al*., 2004). Immunoglobulin Y has shown *in vitro* antibacterial activity against microbes by binding and immobilizing them, thereby restricting or inhibiting their growth and multiplication (Lee *et al*., 2002; Sim *et al*., 2000).

Conclusion: In order to restrict the entry of microbes into developing embryo and to safeguard human health, nature has bestowed chicken egg with various protective mechanisms. The antimicrobial proteins in various parts of chicken egg make an excellent chemical barrier against different microbes. With further advancement in proteomics, it is expected that newer chicken egg proteins would be identified and the mechanism of action and antimicrobial selectivity of all known egg proteins be explored in order to use them as a viable therapeutic alternative in animal and human production.

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