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

S. Adil
Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Srinagar-190006, Kashmir

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, ovoclyxin-21, ovoclyxin-25, ovoclyxin-32, and ovoclyxin-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 protease. Lysozyme causes splitting of bacterial cell wall peptidoglycan bond between N-acetylmuramic acid and N-acetyl-glucosamine. Cystatin inhibits sulphydryl proteinases while 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 immobilizes 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, 2008). 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 (2008) 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 Sautron 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 (ovooleadin-17, ovooleadin-116, ovocallyx-in-21, ovocallyx-in-25, ovocallyx-32 and ovocallyx-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). Ovooleadin-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). Ovooleadin-116 belongs to the secretory calcium-binding phosphoprotein group of proteins (Kawasaki and Weiss, 2006). Ovocallyx-21 has been reported to show identicalness with gastric secretome component gastrihype-2 and ovocallyx-25 contains protease inhibitor domain (Gautron and Nys, 2007; Rose and Hincke, 2009). Ovocallyx-32 is a member of the latex in family of carboxypeptidase inhibitors (Gautron et al., 2001). Ovocallyx-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 ovooinhibitor (1.5%), ovoglycoprotein (1.0%), ovoaflavoprotein (0.8%), ovomacroaglobulin (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, ovooinhibitor, 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 enteridis, Streptococcus mutans and Staphylococcus aureus (Valenti et al., 1983; Abdallah and Chahine, 1998; Baron et al., 2000), besides antiviral activity (Giansanti et al., 2005). Rzedzicki and Stepień-Pysniak (2009) reported that the antibacterial property of ovomucoid occurs due to inactivation of trypsin enzyme that is essential during multiplication of bacteria. Likewise, ovooinhibitor 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-acetyl muramic 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 sulphhydril 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). LDL or lipopolitelin 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 lipopolitelin are metal-binding proteins with 90% iron in the yolk bound to phosvitin (Greegaard et al., 1994) and 80% zinc to lipopolitelin (Tupper et al., 1954). LDL when treated with ether gives a residual fraction called lipopolitelin 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 (IγY) (Leslie and Clem, 1969). The in vitro study has shown the antibacterial activity of lipopolitins 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

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Component</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>Egg shell (%)</td>
<td>10.5</td>
<td>Calcium (mg)</td>
<td>56.0</td>
</tr>
<tr>
<td>Egg yolk (%)</td>
<td>31</td>
<td>Magnesium (mg)</td>
<td>12.0</td>
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<tr>
<td>Egg white (%)</td>
<td>58.5</td>
<td>Iron (mg)</td>
<td>2.1</td>
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<tr>
<td>Water (g)</td>
<td>74.5</td>
<td>Phosphorus (μg)</td>
<td>180.0</td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>162</td>
<td>Zinc (mg)</td>
<td>1.44</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>12.1</td>
<td>Thiamine (mg)</td>
<td>0.09</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>0.68</td>
<td>Riboflavin (mg)</td>
<td>0.3</td>
</tr>
<tr>
<td>Lipids (g)</td>
<td>12.1</td>
<td>Niacin (mg)</td>
<td>0.1</td>
</tr>
<tr>
<td>Saturated fatty acids (g)</td>
<td>3.3</td>
<td>Folic acid (μg)</td>
<td>65.0</td>
</tr>
<tr>
<td>Monounsaturated fatty acids (g)</td>
<td>4.9</td>
<td>Cyanocobalamin (μg)</td>
<td>86.0</td>
</tr>
<tr>
<td>Polysaturated fatty acids (g)</td>
<td>1.8</td>
<td>Pyridoxine (mg)</td>
<td>0.12</td>
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<tr>
<td>Cholesterol (mg)</td>
<td>410</td>
<td>Retinol equivalents (μg)</td>
<td>227.0</td>
</tr>
<tr>
<td>Iodine (μg)</td>
<td>12.7</td>
<td>Potassium (mg)</td>
<td>147</td>
</tr>
<tr>
<td>Tocopherols (μg)</td>
<td>1.93</td>
<td>Carotenoids (μg)</td>
<td>10</td>
</tr>
<tr>
<td>Selenium (μg)</td>
<td>10</td>
<td>Cholecalciferol (μg)</td>
<td>1.8</td>
</tr>
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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.

REFERENCES


