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Review

Egg white versus *Salmonella* Enteritidis! A harsh medium meets a resilient pathogen

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ABSTRACT

Salmonella enterica serovar Enteritidis is the prevalent egg-product-related food-borne pathogen. The egg-contamination capacity of *S.* Enteritidis includes its exceptional survival capability within the harsh conditions provided by egg white. Egg white proteins, such as lysozyme and ovotransferrin, are well known to play important roles in defence against bacterial invaders. Indeed, several additional minor proteins and peptides have recently been found to play known or potential roles in protection against bacterial contamination. However, although such antibacterial proteins are well studied, little is known about their efficacy under the environmental conditions prevalent in egg white. Thus, the influence of factors such as temperature, alkalinity, nutrient restriction, viscosity and cooperative interactions on the activities of antibacterial proteins in egg white remains unclear. This review critically assesses the available evidence on the antimicrobial components of egg white. In addition, mechanisms employed by *S.* Enteritidis to resist egg white exposure are also considered along with various genetic studies that have shed light upon egg white resistance systems. We also consider how multiple, antibacterial proteins operate in association with specific environmental factors within egg white to generate a lethal protective cocktail that preserves sterility.

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1. Introduction

The avian egg white is an intracellular fluid that functions to protect the developing embryo against invading bacteria. In contrast to the immune system of animals which produces antibodies when needed, the avian egg white can efficiently resist microorganisms over a prolonged period in the absence of any inducible innate or adaptive host defence. Many studies have demonstrated that egg white is a poor medium for bacterial growth due to its harsh physicochemical conditions (alkaline pH, high viscosity, nutritional deficiencies), but also because it contains an arsenal of antimicrobial molecules (Alabdeh et al., 2011; Baron et al., 1997; Clavijo et al., 2006; Garibaldi, 1970; Kang et al., 2006; Lu et al., 2003).

Under healthy breeding conditions, the egg contents, including egg white, is generally sterile just after laying. However, the egg can be infected by external sources due to contamination of the eggshell surface and subsequent penetration of microorganisms into the egg. Eggs are particularly prone to such infection after laying. The eggshell may be exposed to microorganisms from hen faeces, other microorganisms present in the farm environment or downstream, in the environment of the conditioning centres. Cracks in the eggshell increase the risk of internal penetration of contamination. In the case of *Salmonella*, eggs may be also contaminated during their formation in the ovary or in the oviduct of infected hens. However, this type of contamination is sporadic. Indeed, the number of contaminated eggs produced by infected flocks is very limited, as is the number of *Salmonella* cells found within eggs, provided that eggs are fresh (EFSA, 2014).

However, the risk of contamination by *Salmonella* is a real threat for human health and remains a major concern for egg production and processing. In particular, contamination by *Salmonella enterica* serovar Enteritidis is widely studied because it represents the predominant serotype involved in the foodborne diseases (salmonellosis) due to egg or egg product consumption (EFSA, 2014). It is also notable that *S.* Enteritidis is able to survive in egg white in contrast to other similar strains and species (Clavijo et al., 2006; Gantois et al., 2008b; Guan et al., 2006; Lock and Board, 1992). For these reasons, this bacterium is the target in many studies on the antimicrobial activity of egg white.

The aim of this review is to provide a comprehensive overview of the antimicrobial activities of egg white against Gram-negative bacteria, especially *Salmonella* Enteritidis, under the specific physicochemical conditions encountered in egg white. A few studies involving Gram-positive bacteria are also included in cases that provide additional insight concerning the antibacterial mechanisms of egg white against *S.* Enteritidis. The antibacterial proteins of egg white and their mechanism of action are described along with a consideration of their likely roles, and potential synergistic behaviours, under conditions that correspond to those found within the complex medium of the egg white This review also considers how *S.* Enteritidis is able to resist the defences of egg white and evaluates the evidence provided by genetic studies on the various mechanisms of egg white resistance. In this way, we intend to provide insight into the manner in which *S.* Enteritidis attempts to thwart the multifactorial defensive ploys imposed by egg white.

2. Egg white composition: general data

Egg white is a heterogeneous medium consisting of four physically separate layers (Guérin-Dubiard et al., 2010):

(i) the chalaze layer which corresponds to spiral filaments ranging from the egg yolk to the two ends of the egg. They allow maintenance of egg yolk in suspension in a central position;
(ii) the external liquid egg white which is in direct contact with the shell membranes;
(iii) the thick egg white which is fixed to the ends of the egg and has the appearance of a gel;
(iv) the internal liquid egg white which is located between thick egg white and egg yolk.

The proportions of liquid and thick egg whites depend on many factors such as the weight of the egg, the laying rate, the strain, the age and health status of the hen, and especially the duration and method of egg storage (Sauveur, 1988). Egg white is globally an aqueous solution of proteins (10.6%) containing small amounts of carbohydrates (0.9%) and minerals (0.5%). Some of the proteins are present in large quantities, such as ovalbumin which represents more than 50% of total proteins; others are found in trace amounts (see Guérin-Dubiard et al., 2010 for a review). Fifty percent of carbohydrates are represented by free glucose. Other sugars are incorporated into the polysaccharide moieties of the egg white glycoproteins. The monomeric units are galactose, mannose, glucosamine, galactosamine and sialic acids.

Table 1
Egg white minerals (adapted from Nys and Sauveur, 2004).

Mineral	Quantity (mg/100 g)
Chloride	175
Sulphur	163
Sodium	155
Potassium	140
Phosphate	18
Magnesium	10
Calcium	8
Zinc	0.12
Iron	0.1
Copper	0.02
Manganese	0.007
Iodine	0.003

Table 2
Egg white vitamins (adapted from Nys and Sauveur, 2004).

Vitamin	Quantity (μg/100 g)
Thiamine (vitamin B1)	10
Riboflavin (vitamin B2)	430
Niacin (vitamin B3)	90
Pantothenic acid (vitamin B5)	250
Pyridoxine (vitamin B6)	10
Biotin (vitamin B8)	7
Folic acid (vitamin B9)	12
Cobalamin (vitamin B12)	0.1