STRATEGIES FOR REINFORCING HYGIENIC QUALITY OF TABLE EGGS

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Abstract

This review describes different strategies which have been explored to reduce food safety risks associated with egg consumption. The first one aims to reinforce the chemical (antimicrobial proteins) protection mechanism of eggs by identifying genes coding proteins involved in egg protection. The following step is to explore phenotypic variability in egg protection against Salmonella related to environmental factors during egg production and storage and the feasibility to select hens with superior anti-microbial alleles using marker assisted selection. A complementary approach is to eliminate eggs at risk by improving the technology for egg grading using non-invasive methods to enhance the detection of eggs with the greatest risk to the consumer. Finally, the risk can be controlled by reducing the degree of egg contamination by developing innovative egg decontamination treatments alternative to egg washing. We explored the decontamination efficiency of hot air, gas plasma sterilization, modified atmosphere packaging of eggs. These approaches were developed under the European programmes RESCAPE.

Key words: egg, antimicrobial, genetic, egg grading, decontamination

INTRODUCTION

The highly competitive market of egg production with its low profit margins has resulted in the development of intensive caged hen egg production throughout the world. This rearing system has been considered as unacceptable to some consumers in terms of animal welfare. Consequently, European Directive 1999/74/EC banned the conventional cage system and imposed its replacement by non-cage or enriched cage systems. The conventional cage system has however benefited from optimisation over three decades, avoiding major hygiene problem and resulting in lower costs relative to alternative systems. There are large variation in use of more welfare friendly systems between European countries (20 to 98% of cage production) due to market forces and differences in legislative constraints. A large survey looked at two serotypes of *Salmonella* prevalence in numerous hen flocks of the 25 European Member States and underlined the large variability in the prevalence of *Salmonella* contamination between countries (0 to 80 % of positive flocks depending on the countries, mainly carried out on the dominant cage system). This reflects the different environments, predominantly in conventional cage production systems and in hen management practise. Legislative constraints and national control programmes on Salmonella including slaughter of breeder hens of salmonella positive flocks have almost certainly had a large influence on salmonella prevalence. Keeping hens on floor system and outdoors is generally considered

to increase the risk of exposure to infectious agents and to favour the prevalence of downgraded eggs due to the higher level of bacterial load in the environment of hens and on eggs (EFSA, 2006) however that might be not the case for Salmonella (Dewulf et al., 2009). Recent development and commercial experience of aviaries and barn systems in Europe led to improvements in the design of systems and management of hens and have partly solved some of the problems. Treatments of eggs such as egg washing, currently banned in Europe with the exception of a few countries may help, but its use remains controversial and limited in Europe. In conclusion, technical progress is still required in alternative or enriched caged system to minimise the risk of egg-borne food diseases. We explored complementary strategies to reduce the risk factors in alternative systems (Strep RESCAPE, 2006-2009). Firstly, genetics and genomics were used to identify novel egg genes coding for proteins with antibacterial properties and markers of these genes were evaluated in commercial pedigree populations to assess the relevance of their use in selection of eggs showing higher protection mechanism. Secondly, phenotypic variability of egg chemical defence was studied during egg production and storage. Thirdly, innovative technology that integrates new knowledges on non invasive techniques was developed to measure egg quality in egg sorting plants. Finally, alternative technologies to egg washing for reducing egg contamination were evaluated*:* (1) hot air pasteurisation, and gas plasma sterilization (2) modified atmosphere packing for preservation of egg quality.

CHARACTERISATION OF ANTI-MICROBIAL DEFENCES OF EGGS

The aims were to identify components of the egg responsible of its antimicrobial properties. Transciptomic comparison of various parts of the oviduct revealed in the magnum, relative to uterus or isthmus, more than 800 genes specifically over-expressed in this compartment where egg white proteins are deposited. Genes were classified according to their potential functions using bioinformatics tools: 1) Proteins with potential antimicrobial properties 2) Proteins involved in antioxidant and inflammatory process 3) Genes coding for proteases and protease inhibitors. A specific affinity chromatography was used for the identification of potential antimicrobial peptides and proteins in egg white: 13 proteins were identified. Among them, OVAX, a serpin (serine protease inhibitor) with no inhibitory activity and AvBD 11, an antimicrobial peptide, were purified and gallin was synthesized by solid-phase peptide synthesis. Their antimicrobial activities were tested against different pathogens. We showed anti-salmonellic activity for AvBD11 and anti-listeria activity for OVAX. AvBD11. In spite of mRNA expression of AvBD-1, -2, -3, -4, -5, -8, -9, -10, -11 and -12, in the different segments of the oviduct (Mageed et al., 2008), only four AvBDs were identified, in the hen egg, by proteomic and transcriptomic approaches: AvBD9 in the uterus; AvBD10 in the eggshell; AvBD11 in the eggshell, the egg white and the vitelline membranes; and gallin in the egg white (Jonchere et al., 2010, Mann, 2007, Mann and Mann, 2008). Synthetic AvBD9 (Herve-Grepinet et al., 2009), recombinant AvBD10 peptide and AvBD11 which contains two beta-defensin motifs with 12 cysteins and 6 disulfide bonds (Herve-Grepinet et al., 2010) have been shown to exhibit antimicrobial activities towards Gram-negative and Gram-positive bacteria. Gallin was identified as a cationic peptide of 41 residues with antimicrobial activity against *Escherichia coli* (Gong et al., 2010).

Lipopolysaccharide Binding (LBP) and Bactericidal/Permeability Increasing (BPI) proteins are well known in mammals for their involvement in defence against bacteria and are key components of the innate immune system which act as the first line of host defence (Bingle and Craven, 2004). Ovocalyxin-36 was the first member of this family of antimicrobial proteins to be identified in the egg. Transcriptomic profiling of hen oviduct expression reveals that OCX-36 was upregulated in uterus

during shell mineralization (Jonchere et al., 2010), as previously revealed by qRT-PCR (Gautron et al., 2007).Additional LBP-BPI like proteins (Tenp, BPIL2) have been identified in egg but their functional characterization has not yet been established (Rehault-Godberg et al., 2010b).

Most microorganisms secrete proteases, which play a major role in various processes associated with proliferation and colonization by pathogens. Inhibitors of serine and cysteine proteases are largely represented in all egg compartments including numerous novel proteins (Rehault-Godberg et al., 2010b) but their antimicrobial effect has not been yet characterized.

GENETIC AND PHENOTYPIC VARIABILITY OF ANTIMICROBIAL EGG ACTIVITY

The objective was to understand the genetic and environmental factors which cause variation in the eggs' defence against the growth of bacteria to improve the antimicrobial defence of the hen's egg and select novel candidate genes.

Phenotypic variability*:* In assessing the antimicrobial activity of egg white the most obvious effect was the storage time. Egg white displayed higher anti-*Salmonella* activity after a few days of storage at 20°C and 37°C (Rehault-Godbert et al., 2010a). The rate of increase in activity was more rapid and pronounced at the higher temperature. However, egg white stored at 20°C retained higher antimicrobial activity compared to storage at 4°C or 37°C when considering the entire storage period. In contrast, storage of egg at 37°C for more than 14 days reduced the bacteriostatic potential of egg white. However, the fluctuation in anti-*Salmonella* activity of egg white could not be related to any variation of trypsin-like, chymotrypsin-like or gelatinolytic activities that potentially account for degradation of antimicrobial egg white proteins.

Genetic variability: The heritability of antisalmonellic activity of egg white and the presence pf loci of candidate gene which contribute to the genetic variation in antimicrobial egg defence were evaluated in a large population of commercial pedigree hens. This study was conducted on 1050 hens from 26 full sib families with an average size family of 40.4 hens. For each hen analysed 3 eggs were pooled on 2 occasions and analysed for their antisalmonellic activity. The phenotypic values for antisalmonellic activity were correlated with alleles of 15 genes (119 SNPs). Alleles of lysozyme and ovostatin were identified as genes that may contribute to variation in salmonella growth in egg white. This property of the egg white to control growth of salmonella was confirmed to have moderate heritability (0.23). Progress of this trait can therefore be obtained by conventional selection but the assessment of this trait remained rather laborious.

NON INVASIVE TECHNIQUES FOR MEASURING EGG QUALITY

Based on their characteristics, some eggs might be subject to more bacterial contamination than others. The objective therefore was to improve the technology used for egg grading as a way to enhance the detection of risky eggs and thus reduce the potential risks of egg bacterial contamination. A non-destructive measurement platform combining crack, spectral and dirt detection has been settled by the University of Leuven. The effective design consisted in a fusion of existing techniques (De Ketelaere et al., 2004; Kempf et al., 2006; Mertens et al., 2010; Perianu et al., 2010) and new measurement concepts in one measurement package. The eggs are checked for cracks and the dynamic stiffness of the eggshell is calculated. The entire egg spectrum is stored and the eggshell colour index (TCV value) is determined. The third operation available on the measurement platform is related to the presence of dirt on the eggshell, dark and white dirt stains are detected and both dark stains percentage and white stains percentage are finally displayed. For the eggshell strength and the crack presence evaluation, the Acoustic Egg Tester was included in the 3 in 1 platform after performing some mechanical and electrical adjustments on the old design. The spectral detection was based

on the classical optical set-up, slightly adapted to the new application. Finally, the dirt detection operation required a completely new design based on the hyperspectral imaging concept. The non invasive quality measurement platform is controlled by a specific software. The final output of the program consists of a crack presence alarm, an eggshell colour index and a dirt percentage. Based on these outputs, the final code is able to give a "risky eggs" warning. The final prototype of the 3 in 1 measurement platform built in Leuven is under validation in large scale experiment in farms in order to produce a "risky eggs" algorithm.

Two monitoring studies were initially performed in order to test the AET (Acoustic Egg Tester) prototype, developed by the University of Leuven, which detects the presence of eggshell cracks and measures the dynamic stiffness (Kdyn): the first monitored 10 flocks at sites in Belgium and the second 11 flocks supplying a sorting plant in France. In addition, daily recording of the Transmission Colour value (TCV) of eggs was carried out throughout the laying period on eggs from hens laying brown eggs. The presence of paler eggs at a flock level revealed a disturbance in the hen nutrition, environmental condition or management. In Belgium it was possible using the daily assessment of egg quality (acoustic or colorimetric methods) to provide valuable information on the protective capacity of eggs. These measurements can either directly indicate "risky eggs" such as cracked eggs or give an indirect indication on the likelihood of occurrence of risky eggs from flocks, for examples when eggs were weaker (Kdyn recording). Some of the observed changes in colour were associated with Bronchitis Infection or mistakes in feed formulation. Overall this has demonstrated the power of continuous measurement and monitoring tools to record abnormalities at the flock level and to potentially prevent risky eggs entering the food change by changing management or veterinary intervention. The prototype of the 3 in 1 measurement platform (combination of acoustic, surface image analysis and external colour recording) can evaluate the crack and dirt presence on the eggshell, the colour index of the eggshell and the eggshell strength. Based on the monitoring concept, the prototype is currently upgraded to be able to give "risky eggs" warnings.

INNOVATIVE DECONTAMINATION TECHNIQUES

The last approach proposed to reduce risk of egg bacterial contamination is the development and assessment of innovative decontamination techniques. The objective was to collect information to implement and standardize appropriate conditions for the application of innovative egg surface decontaminating treatments. Firstly, the University of Bologna explored the thermal effect on egg decontamination and internal properties when eggs were submitted to a couple of air jets impinging on opposite faces (**hot air pasteurisation;** Manfreda et al., 2010; Pasquali et al., 2010). The thermal cycle set up tend to reduce by 90% (one log) the initial population of both *E.coli*, *L. monocytogenes* on experimentally inoculated shell eggs as well as the initial population of aerobic mesophilic bacteria on naturally contaminated eggs collected from organic poultry farm but egg can be recontaminated during storage. The treatments have no impact on the quality indexes. This finding suggests the useful industrial application of the hot air treatment on eggs before packaging for a significant reduction in *S.* Enteritidis, *E. coli*, *L. monocytogenes* and in the indigenous microflora populations naturally infecting the surface of the eggs. Under **gas plasma exposure** (Ragni et al., 2010)**,** bacterial cells can be inactivated by reactive species (OH and NO radicals)**.** The effectiveness of the resistive barrier discharge (RBD) prototype settled in the University of Bologna was significantly improved by increasing the relative humidity value during the treatments thus achieving maximum immediate viability losses ranging between 1.5 and 4.5 Log CFU/eggshell. The inoculated pathogens and the indigenous microflora were characterised by different

inactivation curves, thus indicating different sensitivities to this technology. No recovery of the surviving cells was observed during a 50-day storage at 25°C. No significant treatment effects on egg quality in terms of cuticle and inner surface of the inner shell membrane were observed between the treated and the control eggs. The University of Bologna has also investigated the potential of **packaging technologies** (Rocculi et al. 2010a, b), along with the use of conventional (N_2, CO_2, O_2) and innovative (N_2O, Ar) gases to minimize microbial contamination of eggs and maintain egg quality. Physico-chemical and microbiological properties of eggs packed in air, 100 % O_2, and 100 % CO_2 and stored at 4, 20 and 36 °C up to 45 days of storage were evaluated. All packed eggs showed a strong decrease in the weight loss. Eggs packed in CO_2 showed a higher decrease in pH, a better maintenance of Haugh Unit and a better stability of the foam obtained from the egg white, confirming previous findings observed at different storage temperatures. Among all gases tested (Air, 100 % CO_2 and 100 % O_2), 100% O_2 combined with a chilled temperature showed a statistically significant high bacterial load reduction both on the eggshells and the albumen. One exception was observed for *L. monocytogenes* experimentally contaminated eggs for which 36 °C represent the best condition for bacterial load reduction on the eggshell regardless to the gas used for packaging and O_2 showed the lowest bacterial load reduction in the albumen.

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