

BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Achieving sustainable production of eggs

Volume 1: Safety and quality

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Contents

Series list	xi
Introduction	xv
Part 1 Egg composition and chemistry	
1 Composition and properties of eggshell <i>Maureen Bain, University of Glasgow, UK</i>	3
1 Introduction	3
2 Introduction to eggshell formation	4
3 The inorganic components of the eggshell	7
4 The organic components of the eggshell: eggshell membranes	9
5 The organic components of the eggshell: the eggshell matrix	10
6 The organic components of the eggshell: cuticle and pigment	14
7 Case study: OC-116 as a genetic marker for improved eggshell quality	15
8 Summary and future trends	17
9 Where to look for further information	17
10 References	18
2 Composition and properties of egg white <i>Kaustav Majumder, University of Nebraska, Lincoln, USA and Yoshinori Mine, University of Guelph, Canada</i>	25
1 Introduction	25
2 The structure of egg white	28
3 Proteins in egg white: an overview	29
4 Ovalbumin	31
5 Ovotransferrin	32
6 Ovomuroid	34
7 Ovomucin	35
8 Lysozyme	36
9 Minor proteins in egg white	38
10 Carbohydrates, minerals and vitamins in egg white	40
11 Summary	41
12 Where to look for further information	41
13 References	42
3 The nutritional and physiological functions of egg yolk components <i>Yasumi Horimoto, University of Guelph, Canada and Hajime Hatta, Kyoto Women's University, Japan</i>	49
1 Introduction	49
2 Egg yolk proteins	51
3 Egg yolk lipids	56
4 Egg yolk cholesterol	64
5 Egg yolk antibody (IgY)	67

6	Other bioactive components derived from egg yolk	79
7	Conclusion	83
8	Where to look for further information	84
9	Abbreviations	85
10	References	86

Part 2 Safety

4	Pathogens affecting table eggs	99
	<i>Kapil Chousalkar, University of Adelaide, Australia and Kylie Hewson, Australian Chicken Meat Federation, Australia</i>	
1	Introduction	99
2	Pathogen contamination of eggs: an overview	99
3	<i>Salmonella</i> spp.	102
4	<i>Campylobacter</i> spp. and other bacteria	104
5	Yeasts and moulds	105
6	Conclusions and future trends	106
7	References	106
5	Mechanisms for the transmission of pathogens into eggs	111
	<i>Sophie Jan and Florence Baron, Agrocampus Ouest-INRA, France</i>	
1	Introduction	111
2	Microbial hazards associated with egg consumption	112
3	Vertical transmission	115
4	Horizontal transmission	117
5	Secondary contamination: trans-shell penetration	119
6	The effect of antimicrobial molecules on the behaviour of microorganisms in egg white	121
7	The influence of the physicochemical and environmental conditions on the egg white	125
8	Migration and behaviour of microorganisms in egg yolk	127
9	Conclusion	128
10	Where to look for further information	129
11	References	129
6	Sampling and detection of <i>Salmonella</i> in eggs	141
	<i>Richard K. Gast, United States Department of Agriculture, USA</i>	
1	Introduction: bacterial pathogens in eggs	141
2	Contamination of eggs with <i>Salmonella</i>	142
3	Detection of <i>Salmonella</i> on or inside eggshells: sampling, pooling and incubation	146
4	Detection of <i>Salmonella</i> on or inside eggshells: conventional culture methods	147
5	Detection of <i>Salmonella</i> on or inside eggshells: rapid detection methods and egg yolk antibody detection	150
6	Egg testing and <i>Salmonella</i> control: conclusions and challenges for future research	152

7	Where to look for further information	153
8	References	153
7	Understanding the natural antibacterial defences of egg white and their regulation <i>Nicolas Guyot, Sophie Réhault-Godbert, Yves Nys, INRA, France; and Florence Baron, INRA – Agrocampus Ouest, France</i>	161
1	Introduction	161
2	Egg white antimicrobial molecules	162
3	Environmental and physicochemical conditions controlling bacterial growth and dispersion in egg white	171
4	Variability of egg white natural defences induced by hen-associated factors	174
5	Environmental variability of physicochemical and antibacterial properties of egg white during egg storage	177
6	Environmental variability of physicochemical and antibacterial properties of egg white during egg incubation	182
7	Conclusions	184
8	Where to look for further information	185
9	References	185
8	The effects of laying hen housing systems on egg safety and quality <i>Deana R. Jones, US Department of Agriculture, Agricultural Research Service, USA</i>	195
1	Introduction	195
2	Indicator organisms in monitoring egg safety	196
3	<i>Salmonella</i> spp.	199
4	Other pathogens and chemical contaminants	203
5	Egg quality	206
6	Summary	208
7	Future trends in research	208
8	References	209
9	Egg washing to ensure product safety <i>Margaret Sexton, Primary Industries and Regions, South Australia (PIRSA), Australia</i>	215
1	Introduction	215
2	Egg properties relevant to the washing process	216
3	General cleaning principles	217
4	Cleaning principles applied to egg washing: time, temperature, agitation and use of chemicals	219
5	Cleaning principles applied to egg washing: rinsing, drying and decontamination of equipment	223
6	Monitoring, process control, verification and validation	224
7	Summary	226
8	Where to look for further information	226
9	References	227

10	New developments in packaging of eggs to improve safety and quality <i>Pietro Rocculi, University of Bologna, Italy</i>	229
1	Introduction	229
2	Shell egg packaging in low- and high-barrier materials	230
3	Modified atmosphere packaging (MAP) of shell eggs	232
4	Case study: combination of CO ₂ MAP and active packaging with humidity regulator for shell eggs storage	234
5	Future trends in research	238
6	Where to look for further information	239
7	References	239
Part 3 Sensory and nutritional quality		
11	Egg quality: consumer preferences and measurement techniques <i>Bart De Ketelaere, Katholieke Universiteit Leuven, Belgium; Koen De Reu, Institute for Agricultural and Fisheries Research (ILVO), Belgium; and Steven Vermeir, Katholieke Universiteit Leuven, Belgium</i>	243
1	Introduction	243
2	Consumer perceptions of egg quality and consumer preferences for eggs	244
3	Instrumental techniques for measuring eggshell quality	248
4	Instrumental techniques for measuring egg freshness	252
5	Instrumental techniques for measuring other quality attributes	257
6	Conclusions and future perspectives	260
7	Where to look for further information	261
8	References	262
12	Determinants of egg appearance and colour <i>C. Hamelin, CCPA, France and F. Cisneros, DSM, Switzerland</i>	267
1	Introduction	267
2	Consumer purchasing decisions: choosing to eat eggs and choosing which eggs to eat	267
3	The shell	272
4	The albumen	278
5	The yolk	280
6	Conclusion	285
7	Where to look for further information	285
8	References	286
13	Understanding and improving the shelf-life of eggs <i>Juliet R. Roberts, University of New England, Australia</i>	293
1	Introduction	293
2	Factors affecting egg internal quality	294
3	Factors affecting egg microbiological safety	296
4	Technologies for increasing the shelf-life of eggs	298
5	Coatings to increase the shelf-life of eggs	301
6	Summary and future trends	310

7	Where to look for further information	311
8	References	312
14	The nutritional role of eggs	319
	<i>Tia M. Rains and Mitch Kanter, Egg Nutrition Center, USA</i>	
1	Introduction	319
2	The nutrient composition of chicken eggs	319
3	The relationship between egg consumption and human health and disease	324
4	Future trends: emerging health benefits	328
5	Summary	329
6	Where to look for further information	329
7	References	330
15	Nutraceutical benefits of eggs	337
	<i>Hoon H. Sunwoo and Naiyana Gujral, University of Alberta, Canada</i>	
1	Introduction	337
2	The eggshell and its membrane	338
3	Egg white proteins	339
4	Egg yolk proteins	341
5	Egg lipids	344
6	Summary	345
7	Future trends in research	347
8	Where to look for further information	347
9	References	348
16	Enhancing the nutritional profile of eggs	355
	<i>Erin M. Goldberg and Neijat Mohamed, University of Manitoba, Canada and James D. House, University of Manitoba and the Canadian Centre for Agri-Food Research in Health and Medicine, Canada</i>	
1	Introduction	355
2	Egg nutrient composition and role in the human diet	356
3	Omega-3 PUFA: biological factors influencing the n-3 composition of eggs	356
4	Omega-3 PUFA: enhancing the n-3 profile of eggs	359
5	Omega-3 PUFA: sensory attributes of enriched eggs	363
6	The vitamin profile of eggs	367
7	Minerals and trace elements in eggs	369
8	Dietary combinations	371
9	Conclusion	371
10	Where to look for further information	372
11	References	372
17	Molecular breeding techniques to improve egg quality	383
	<i>Anna Wolc, Iowa State University, and Hy-Line International, USA; and Janet E. Fulton, Hy-line International, USA</i>	
1	Introduction	383
2	Whole-genome methods to improve egg quality	384
3	Improving external egg quality	388

4	Improving internal egg quality	390
5	Genomic selection	391
6	Targeting candidate genes for particular traits	392
7	Gene expression and regulation	395
8	Summary and future trends	396
9	Where to look for further information	397
10	References	397

Introduction

The quality of the egg encompasses its chemical composition, nutritional quality as a human food source, sensory qualities including appearance and special nutraceutical benefits for human health. Eggs contain all essential nutrients for human health except for dietary fibre and vitamin C. This volume also discusses handling, packaging and storage of eggs, conditions that affect the quality of the product that reaches the consumer. The microbiological safety of eggs is of paramount importance as eggs have been implicated in outbreaks of food-borne illness, mainly caused by *Salmonella*. Fortunately, by virtue of its role in nature, the egg possesses many anti-microbial properties, and these properties can be maintained and enhanced for the benefit of consumers. This volume, *Achieving sustainable production of eggs Volume 1: Safety and quality*, explores the wealth of research addressing these themes.

Part 1 Egg composition and chemistry

Part 1 consists of three chapters on egg composition and chemistry of the eggshell, egg white and egg yolk, respectively. The integrity of the eggshell is of primary importance because it ensures the safe delivery of the egg's contents from the point of production to the consumer, as well as influencing the microbiological safety of the egg's contents, as outlined in **Chapter 1**. The layers of the eggshell perform different functions. The mammillary layer and its bond with the shell membranes is the foundation of the eggshell. The palisade layer forms the bulk of the thickness of the eggshell. The surface cuticle is a protective layer that impedes the ingress of bacteria into the egg's contents. The eggshell consists of both the inorganic calcite and an organic matrix, with the synthesis of the organic components determining the dynamics of eggshell production. At the time of publication, 699 proteins have been identified in the eggshell. Most characteristics of eggshell quality have moderate to high heritability, so genetic selection by breeder companies plays a vital role in maintenance and improvement of eggshell quality. In recent years, the availability of high-throughput technologies in fields such as proteomics and transcriptomics has facilitated the study of the organic components of the eggshell. The goal of the commercial industry is to extend worldwide the life span of layer flocks to around 100 weeks, at the same time maintaining eggshell quality.

Chapter 2 describes the structure and role of the egg white (albumen). As for the eggshell, modern techniques have facilitated the identification of many major proteins in egg white (78 identified to 2007), including ovalbumin, ovotransferrin, ovomucoid, ovoglobulins and ovomucin. In addition, there are many 'minor' proteins. Various proteins influence the properties of egg white, from textural and functional to anti-microbiological properties. The allergens in egg white are ovalbumin and ovomucoid. Ovomucin is largely responsible for the jelly-like structure of egg white and plays a major role in the egg white thinning during storage. Egg white contains many antimicrobial substances including lysozyme, ovotransferrin, ovomucin, ovocystatin and avidin, as well as carbohydrates, minerals and vitamins. In the food industries, egg white is used for its functional properties of gelation and foaming.

Egg yolk composition and chemistry is outlined in **Chapter 3**. Egg yolk consists of 15.7–16.6% proteins (lipoprotein, livetin, phosvitin, riboflavin-binding protein, biotin-binding protein, thiamine-binding protein, egg yolk transferrin, proteins with cholinesterase activity), 32–35% lipids (neutral lipids, phospholipids, cholesterol), minerals and vitamins, antibodies and bioactive substances such as sialic acid. Egg yolk immunoglobulins (IgY) can be selectively produced in eggs for human therapeutic purposes. Egg yolk can be separated into two main fractions by dilution and centrifugation: plasma (supernatant – 85% low-density lipoproteins, 15% livetins) and granules (precipitate – 70% high-density lipoprotein or lipovitellin, 16% phosvitin). Egg yolk can be fortified with desirable substances such as omega-3 fatty acids, folic acid and vitamins by enriching the diet of the hens. Continuing development in proteomics of egg yolk proteins has great potential for identifying novel egg proteins and their biological functions for use in human health. Egg yolk lipids are used in human infant formula because they closely mimic the composition of human breast milk. In the food industries, egg yolk is used as an emulsifier. Recent studies have shown that earlier concerns about consumption of egg yolk causing elevation of plasma cholesterol in humans are unfounded as eggs contain low levels of saturated fatty acids which are the main cause of elevated plasma cholesterol.

Part 2 Safety

Part 2 comprises Chapters 4–10 on the food safety of eggs and egg products. **Chapter 4** lists the main pathogenic bacteria associated with eggs. Although a large number of microorganisms (bacteria, yeasts, moulds) may be found on the outside of eggs, the organisms of greatest concern are *Salmonella* spp. and, to a lesser extent, *Campylobacter* spp. In many parts of the world, *Salmonella* Enteritidis is the main cause of egg-associated food-borne illness. *S. Enteritidis* is capable of entering the egg during its forming in the hen's oviduct. It is also able to pass across the shell of a fully-formed egg. To date it appears to be the only type of *Salmonella* capable of such vertical transmission. In Australia, where *S. Enteritidis* is not endemic in commercial laying hen flocks, *S. Typhimurium* is the main cause of egg-associated illness. **Chapter 5** explores the mechanisms by which pathogens of concern to human health are transmitted into the contents of eggs, with emphasis on *S. Enteritidis*. Although *S. Enteritidis* is able to enter an egg via the hen's oviduct (primary or vertical transmission), transmission of other pathogens appears to occur exclusively by horizontal transmission from the outside of the eggshell into the egg contents. Other pathogens associated with eggs are discussed including other serovars of *Salmonella* such as *Typhimurium*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Campylobacter jejuni* and *Listeria monocytogenes*. Mechanisms of trans-shell penetration (horizontal transmission) are discussed, including risk factors such as cracks or defects in the shell and/or cuticle, storage conditions and water condensation on the eggs. Chapter 5 also covers the natural antimicrobial properties of all regions of eggs.

Chapter 6 reviews the literature on methods of sampling and detection of *Salmonella* in eggs. This information is critical to effective monitoring of commercial eggs. Emphasis is on *S. Enteritidis*, although other bacteria associated with eggs are mentioned. In commercial production systems, pathogens such as *Salmonella* arise mainly from environmental sources. It is difficult to detect bacteria inside eggs so sampling needs to involve large numbers of eggs. Storage time and temperature influence the ease with which bacteria

can reach the egg contents. Bacteria are detected on the outside of eggshells by immersing the whole eggs in a nutrient broth in special bags. Shells may be crushed to release bacteria contained in the pores of the shells. Detection of bacteria in egg contents requires pooling and incubation of samples to increase the chances of detection. Following pre-enrichment, samples are plated onto selective agar, incubated and colonies selected for further analysis. Rapid detection methods have increased the speed at which the process can be conducted. Egg yolk antibody detection provides additional information.

Chapter 7 provides a more detailed description of the antimicrobial properties of egg white and addresses ways of enhancing these natural defences. Detailed information is provided on the properties and role of lysozyme; ovotransferrin; the proteinase inhibitors ovomucoid, ovomucoid, ovostatin and cystatin; the vitamin-binding proteins avidin and riboflavin-binding protein; the defensins AvBD11 and Gallin (OvoDA1) and others including Tenp (Transiently Expressed in Neural Precursor) (Ovoglobulin G2); Ovalbumin-related protein X (OVAX) and recently identified proteins VMO-1, pleiotrophin and beta-microseminoprotein-like. Factors influencing bacterial growth and spread in egg white include temperature, pH, viscosity, carbon dioxide levels and ionic composition. This chapter also reports on the effects of physiology, genetics, age and immune status of the hen on egg white antimicrobial properties. The effect of changes occurring during egg storage, on the antimicrobial properties, of the egg white are also discussed in detail. The use of modified atmosphere packaging (MAP) and cryogenic cooling of eggs can improve the properties of stored eggs. Also discussed are the changes that occur during incubation of embryonated eggs and how these affect the antimicrobial properties of the egg white.

The egg industry, all around the world, has experienced a trend of moving away from conventional cages, towards cage-free production systems. Conventional cages are banned in the EU and egg production in Australia has been moving progressively towards free-range production. **Chapter 8** reviews the effect of housing system on egg quality and safety. Social pressures resulting from concerns about hen welfare have resulted in this shift towards cage-free production, although this production system does not result in a product of higher quality. The chapter reviews the types of microorganisms that are found on eggs. Some of these are indicators of general cleanliness and others are potentially pathogenic for humans. Research studies comparing housing systems have produced contradictory results, although non-cage systems are more likely to result in higher levels of bacteria on the outside of eggs and this is particularly the case for floor eggs. Strain of hen, flock management, management of the nest boxes and stocking density have all been shown to influence egg cleanliness. Challenge studies using *Salmonella* indicate that non-cage production systems do not necessarily result in worse outcomes for food safety. Similar results were obtained when commercial farms were surveyed for the presence of *Salmonella* and *Campylobacter* with housing system not having a consistent effect on flock microbiological status. Various studies have investigated the effect of production system on eggshell quality and egg internal quality. Although some studies have reported best-quality eggs from conventional cages, others have found no consistent relationship. It appears that some of the earlier egg quality problems that occurred in non-cage production systems have been overcome by improvements in the design of the production systems and in flock management. Ensuring that hens lay in the nest boxes in cage-free systems is essential for the maintenance of egg quality and safety.

Chapter 9 evaluates the effect on food safety of the egg-washing process. Egg washing is not permitted for first quality eggs in Europe, but is commonly practiced in other countries including the United States and Australia. Whereas properly conducted egg

washing can improve the microbiological safety of eggs, it can actually make the situation worse if washing is not carried out correctly. A series of steps need to be carried out and important considerations are temperature; time; type, concentration and application of the cleaning chemical used (including agitation, use of brushes); rinsing; application of sanitizer; and drying of the eggs. This needs to be followed by monitoring, process control and verification. Care needs to be taken to avoid damage to the cuticle of the eggshell and to avoid ingress of the egg washing solutions into the egg contents.

The type of packaging used for transport of eggs from the processing plant to the consumer is important, as reviewed in **Chapter 10**. Most egg packaging used throughout the world is 'low barrier', meaning that it is very permeable to air and water vapour, but has the advantage of minimizing the build-up of water condensation on the eggs. The loss of carbon dioxide and water vapour for eggs housed in low barrier packaging results in changes in egg quality during storage. These losses can be reduced by using high barrier packaging but at the expense of increased condensation on the eggs. MAP enables maintenance of egg quality. Carbon dioxide atmosphere packaging improves egg albumen quality in terms of Haugh unit, coagulation and foaming, whipping and gelling properties. Although studies have shown no adverse effect of the MAP on the microbiological safety of the eggs, it is recommended that an absorbent be included in the packaging to reduce condensation of water vapour on the outside of the eggs.

Part 3 Sensory and nutritional quality

Part 3, Chapters 11–17, covers the sensory and nutritional quality of eggs. Maintenance of egg quality is of fundamental importance to the egg industries as consumers are increasingly discriminating in their choice of food items. **Chapter 11** examines consumer preferences for egg quality and the ways in which quality can be measured. Quality indicators of concern to consumers are cleanliness, egg size, shell colour and shell colour uniformity, shell strength and integrity (absence of cracks and defects), albumen quality, yolk colour, absence of meat and blood spots. Preferences for some of these measures vary among countries. Some consumers are strongly influenced by animal welfare considerations and the type of production system. Others focus on health benefits and are prepared to pay a premium for specialty eggs such as organic, or eggs fortified with nutrients such as omega-3 fatty acids. A range of different equipment types are available for measurement of eggshell quality and egg internal quality in research studies and in quality assurance laboratories associated with commercial facilities. However, in commercial egg-processing operations, equipment for measuring quality needs to be compatible with the in-line processing equipment used. Such methodologies are being extended to detection of microbiological agents, as well as of eggs produced in one production system (e.g. conventional cage), but are being sold as having been produced in a different production system (e.g. free range). Measurement of egg appearance and colour is further explored in **Chapter 12**. Egg consumption per annum varies greatly among countries, with Mexico and Japan having the highest consumption at more than 300 eggs per year per person. The attractiveness of eggs as a food item is influenced by many of the quality measurements described in Chapter 12 and also by packaging and marketing. This chapter discusses the regional differences in consumer preferences and outlines ways in which egg appearance and colour can be achieved.

Shell eggs are regarded as having a limited shelf life and **Chapter 13** investigates ways of improving the shelf life of eggs. Shelf life is important both in terms of the sensory qualities of eggs and also in terms of their microbiological safety. This chapter outlines some of the ways in which shelf life is determined by regulators in different countries and ways in which it may be extended. Of primary importance is the handling and storage of eggs, with refrigeration being one of the most important factors. Rapid cooling has been investigated, although it is important that this process does not induce microcracks in the shells of the eggs. Pulsed visible light, electrolysed water, MAP, ultraviolet radiation, ionizing radiation, whole egg pasteurization and ultrasonic treatment have all been investigated, at least experimentally. A range of egg coating substances have been tested: oils and waxes, carbohydrate coatings such as those based on cellulose or chitosan, emulsions of oils and chitosan, proteins and synthetic polymers. Oils, both mineral and vegetable, have excellent moisture-proofing properties, whereas chitosan coatings have antimicrobial properties, which make the combination of these two substances promising for use in extending the shelf life of eggs. One commercial provider reports use of whole-egg pasteurization followed by coating with food-grade wax as extending the shelf life significantly.

Chapter 14 reviews the nutritional role of eggs for humans. Eggs are one of the most affordable, accessible and nutrient-dense foods available around the world. The proteins, lipids, carbohydrates, vitamins, minerals and other functional nutrients in an egg not only are sufficient to support the transition from fertilized cell to newborn chick, but also provide unique nutrient contributions to the human diet. Eggs contain proteins (12.6%), lipids (9.5%), vitamins and minerals. There have been concerns about the association between egg consumption, coronary heart disease (CHD) and blood lipids. However, recent research and analyses of data from a large number of studies have failed to support any association between egg consumption and CHD. There is also no clear evidence to implicate egg consumption in the development of type II diabetes. Because of their effect on satiety, eggs can play a useful role in weight loss. In addition, eggs are the source of many bioactive compounds which promise to be useful for human health. The uses of eggs are further explored in **Chapter 15**, which reviews the nutraceutical benefits of eggs. Biological functions of eggs, reported in the literature, include anti-inflammatory, anti-hypertensive, anti-microbial, anti-oxidant, blood cholesterol/lipid-lowering, immune modulatory, anti-toxin/allergen and skin/cartilage/bone strengthening. Eggshell powder is a natural source of calcium and microelements (magnesium, fluorine, strontium, copper, manganese and zinc), which have a positive effect on bone density. Components of eggshell membranes can assist in maintenance of healthy joints and as treatment for wounds. The section on egg white complements Chapter 2. Egg white proteins that have nutraceutical benefits are ovalbumin, ovotransferrin, ovomucin, ovomucoid, avidin, lysozyme and cystatin. Egg yolk proteins with nutraceutical benefits are lipoprotein, livetins and phosvitin, and lipids with nutraceutical properties are fatty acids and phospholipids. Efficient methods for large-scale isolation and purification of biologically active constituents of eggs are needed for these products to be commercially viable.

Chapter 16 describes how the nutritional profile of eggs can be enhanced by supplementing the diet of hens with bioactive substances such as omega-3 fatty acids, vitamins, minerals and carotenoids that are then deposited into the eggs. Various sources of omega-3 fatty acids may be used including plant sources such as flax and marine sources such as microalgae and fish. Attention needs to be paid to sensory qualities of the eggs produced. Water-soluble B vitamins are deposited into the egg albumen whereas

the lipid-soluble vitamins (A, D, E, K) are deposited into yolk. Carotenoids are deposited into yolk. Egg enrichment with the minerals selenium, iodine, manganese, chromium, zinc, copper, cobalt and boron has been investigated. Combinations of supplements have also been trialled. Enriched eggs are generally viewed as a specialist food item, but have the potential to increase dietary intake in selected groups of humans.

Chapter 17 addresses molecular breeding techniques for improving egg quality. Since the release of the chicken genome sequence in 2004 (Hillier et al., 2004), the range of technologies available for genetic improvement of the multiple traits that define egg quality has increased greatly. The genetic improvement techniques include whole-genome methods, genomic selection, the targeted gene approach and investigations of gene expression and regulation. Genome-wide association studies have identified regions of the genome associated with variation in the different egg quality traits. These regions are called quantitative trait loci as it is presumed that they contain genes that influence the quantitative traits. These studies are able to identify candidate genes, leading to marker-assisted selection. Egg production, along with the various indicators of eggshell quality and egg internal quality, can be considered individually and in combination. Genomic selection relies on using whole-genome information to predict breeding values of animals rather than trying to identify specific genomic regions. In the targeted gene approach, genes with potential to influence the traits of interest are identified. These candidate genes are then investigated in more detail. The thousands of genes that constitute the chicken genome do not work independently. They work together within genetic regulatory networks of interacting genes that influence gene expression. Proteomics is the study of large numbers of proteins all of which are expressed at one time or in one tissue. By combining information from genetic networks with proteomic studies of specific tissues, it is possible to understand when genes are turned on or off, and in which tissues or regions of individual organs this occurs. This knowledge can help in understanding traits of interest for improving egg production. The recently developed genetic manipulation tool CRISPR (Cong et al., 2013) allows rapid and highly specific editing of genes. The genome is altered to produce a very specific and desired change in the genes to improve a particular trait. All these methods complement each other in achieving better understanding of genetic determination of egg-quality traits.

Summary

The chapters in volume 1 outline the structure and function of the main egg components: eggshell, egg white and yolk. The microbiological challenges to the food safety of eggs are discussed, as are ways of detecting pathogens, enhancing the egg's natural defences against pathogens and ways of minimizing food safety risks in the egg industries. The appeal of eggs as a food product is critical to the profitability and sustainability of the egg industries, so ways of maintaining and enhancing the sensory and nutritional quality of eggs are outlined. Finally, the way in which modern molecular breeding techniques improve egg quality is discussed.

Index

- ACC. *see* Amorphous calcium carbonate (ACC)
phase
- ACE. *see* Angiotensin-converting enzyme (ACE)
- Age and laying cycle 175
- Agitation, and egg washing 217, 221
- Air cell size, and egg quality 254–255
- Albumen. *see also* Egg white
and egg packaging 233–234
and egg washing 217
quality of 278–280, 390
- American Heart Association 65
- Amorphous calcium carbonate (ACC)
phase 8–9
- Angiotensin-converting enzyme (ACE) 56
- Anomalies detection, and egg quality 257–258
- Antibacterial properties, of egg white 177–181
- Antimicrobial molecules
egg white 162–163
lysozyme 121–122
other proteins and peptides 124
ovotransferrin 122–123
protease inhibitors 123–124
vitamin-chelating proteins 123
- AvBD11 169
- Avidin 39, 168
- Bacterial contamination, and egg
quality 258–259
- BEUC. *see* European Consumer Association
(BEUC)
- BHT. *see* Butylated hydroxytoluene (BHT)
- Blood and meat spots 391
- Butylated hydroxytoluene (BHT) 359
- Calcite crystals, and growth mechanisms 8–9
- Campylobacter* 105
and egg safety 203–204
- Carbohydrates
coatings 304–307
in egg white 40
- Cardiovascular disease (CVD) 319, 324–326,
359
- CD. *see* Crohn's disease (CD)
- CF. *see* Cystic fibrosis (CF)
- Chemical application, and egg washing 218
- Chemical concentration, and egg washing 218,
222–223
- Chemical contamination, and egg
safety 205–206
- Cholesterol, egg yolk
international guidelines for daily intake 66
roles of 64–65
United States and Japanese history 65–66
- Cleaning chemicals, and egg washing 218,
221–222
- Cleaning principles, egg
agitation 217, 221
temperature 217, 220–221
time 217, 219
- Clustered Regularly Interspaced Short
Palindromic Repeat (CRISPR) 396
- Coatings, egg shelf-life
carbohydrate 304–307
oil–chitosan emulsions 307–309
oils and waxes 302–304
protein 309–310
synthetic polymers 310
- Coronary heart disease (CHD). *see*
Cardiovascular disease (CVD)
- Cracks, and egg washing 216–217
- CREMPs. *see* Cysteine-rich eggshell membrane
proteins (CREMPs)
- CRISPR. *see* Clustered Regularly Interspaced
Short Palindromic Repeat (CRISPR)
- Crohn's disease (CD) 78
- Cryogenic cooling and egg shelf-life 298
- Cultured fish infections 78–79
- Cuticle 14–15
and egg washing 216
- CVD. *see* Cardiovascular disease (CVD)
- Cystatin/ovocystatin 39
- Cysteine-rich eggshell membrane proteins
(CREMPs) 9
- Cystic fibrosis (CF) 77–78
- Decontamination, of egg washing
equipment 224
- Defensins 169
- Delipidated egg yolk protein 55–56
- Dental caries 74–76
- DIAAS. *see* Digestible Indispensable Amino
Acid Score (DIAAS)
- Diagnosis tool, IgY as 70
- Dietary combinations, and egg nutrient
composition 371
- Digestible Indispensable Amino Acid Score
(DIAAS) 320
- Drying, and egg washing 218–219, 224
- Egg appearance and colour
albumen 278–280
consumer purchasing decisions
factors affecting 269–270
packaging and marketing 270–272
perception 268–269
regional differences 270
and eggshell 272–273
consumer preferences for 273–275
factors influencing 276–278
overview 267

- and yolk
 - consumer preferences for 280–281
 - factors affecting 282–285
- Egg consumption
 - and coronary heart disease (CHD) 324–326
 - and type 2 diabetes (T2D) 326–327
 - weight loss and satiety 327–328
- Egg contamination. *see also* Pathogen
 - contamination of eggs; *Salmonella* Enteritidis
 - frequency and magnitude 143
 - mechanisms of 142–143
 - quantitative aspects of 143
- Egg freshness, and pathogen transmission 125
- Egg incubation, and egg white 182–184
- Egg internal quality, and egg shelf-life 294–296
- Egg lipids 322–323
 - fatty acids 344–345
 - phospholipids 345
- Egg microbiological safety 296–298
- Egg nutrient composition
 - dietary combinations 371
 - and lipids 322–323
 - minerals and trace elements in 369–371
 - Omega-3 PUFA, n-3 composition
 - biological factors influencing 356–359
 - marine sources of 362–363
 - plant sources of 362
 - sensory attributes of
 - enriched eggs 363–367
 - strategies to enhance 359–361
 - overview 319–320, 355–356
 - proteins 320–322
 - role in human diet 356
 - vitamins and minerals 323–324
 - fat-soluble vitamins 367–369
 - water-soluble vitamins 369
- Egg packaging
 - low- and high-barrier materials 230–232
 - modified atmosphere packaging (MAP)
 - case study 234–238
 - functional properties of albumen 233–234
 - pathogen and spoilage bacteria 234
 - physico-chemical properties 232–233
 - overview 229–230
- Egg production 385–388, 393–394
- Egg Products Inspection Act (1970) 141
- Egg quality
 - and air cell size 254–255
 - and albumen quality 252–254, 390
 - and bacterial contamination 258–259
 - blood and meat spots 391
 - consumer perceptions
 - of animal welfare concerns 246–247
 - of eggs and health 247–248
 - and detection of anomalies 257–258
 - and egg shape 388–389
 - and eggshell colour preferences 245–246
- and egg size 388
 - preferences 246
- gene expression and regulation 395–396
- general consumer surveys 244
- and genome sequence
 - egg production 393–394
 - external egg quality 394
 - internal egg quality 395
 - overview 392–393
- genomic selection 391–392
- mechanical shell quality 248–250
- and odour 255–257
- and origin/fraud detection 259–260
 - overview 243, 383–384
- and shell colour 389–390
- and shell quality 206–207, 389
- visual shell quality 250–252
- whole-genome methods
 - egg production 385–388
 - overview 384–385
 - yolk colour preferences 244–245
 - and yolk quality 390–391
- Egg safety. *see also* Egg quality
 - and *Campylobacter* 203–204
 - chemical contamination 205–206
 - consumer demands 195–196
 - hen housing and 196
 - indicator organisms in monitoring 196–199
 - other pathogens 204–205
 - and *Salmonella* 199–203
- Egg shape, and quality 388–389
- Egg shelf-life
 - coatings
 - carbohydrate 304–307
 - oil-chitosan emulsions 307–309
 - oils and waxes 302–304
 - protein 309–310
 - synthetic polymers 310
 - and egg internal quality 294–296
 - and egg microbiological safety 296–298
 - and electrolysed water 299–300
 - and ionizing radiation 300
 - and modified-atmosphere packaging 300
 - overview 293–294
 - and pulsed visible light 299
 - refrigeration and cryogenic cooling 298
 - and ultrasonic treatment 301
 - and ultraviolet radiation 300
 - and whole egg pasteurization 300–301
- Eggshell
 - description 338–339
 - and egg appearance and colour 272–273
 - consumer preferences for 273–275
 - factors influencing 276–278
 - egg quality and colour preferences 245–246
 - formation
 - overview 4–6
 - and uterine fluid 7

- inorganic components of
 - calcite crystals and growth mechanisms 8–9
 - overview 7–8
- matrix 10–14
- membrane 339
- OC-116 as genetic marker for 15–17
- organic components of
 - cuticle 14–15
 - eggshell matrix 10–14
 - mammillary cores/mammillae/mammillary density 10
 - membrane composition 9–10
 - pigment 15
 - sampling 146
- Egg size
 - and quality 388
 - preferences 246
- Egg washing
 - albumen (protein) and yolk (fat) 217
 - chemical application 218
 - chemical concentration 218, 222–223
 - cleaning chemicals 218, 221–222
 - cracks 216–217
 - cuticle 216
 - decontamination of equipment 224
 - drying 218–219, 224
 - general cleaning principles
 - agitation 217, 221
 - temperature 217, 220–221
 - time 217, 219
 - membranes 216
 - monitoring, process control and verification 219, 224–225
 - overview 215–216
 - porous shell 216
 - rinsing 218, 223–224
 - sanitizer 218, 223
 - thermodynamic properties 217
 - validation 219, 225–226
- Egg white. *see also* Albumen
 - age and laying cycle 175
 - antibacterial properties of 177–181
 - antimicrobial molecules 162–163
 - AvBD11 169
 - avidin 39, 168
 - and bacterial growth
 - other parameters 173–174
 - pH value 172
 - temperature 171
 - viscosity 172–173
 - carbohydrates in 40
 - cystatin/ovocystatin 39, 168
 - defensins 169
 - and egg incubation 182–184
 - Gallin (OvoDA1) 169
 - genetics 175–176
 - and hen-associated factors 174–175
 - and immune stimulation 177
 - lysozyme 36–38, 163–165
 - minerals in 40
 - and nutrition 176
 - ovalbumin (OVA) 31–32, 170
 - overview 25–28, 161–162
 - ovoflavin/ovo-flavoprotein/riboflavin-binding protein 40
 - ovoglobulin 38–39
 - ovoinhibitor 167–168
 - ovomucin 35–36
 - ovomucoid (OVM) 34–35, 167
 - ovostatin 168
 - ovotransferrin (OTf) 32–34, 165–167
 - physicochemical and environmental conditions in
 - and egg freshness 125
 - and environmental temperature 125–126
 - and inoculum state 125
 - pH effect 126–127
 - and synergetic effects 127
 - protease inhibitors 167
 - proteins in 29–30
 - avidin 341
 - cystatin 341
 - lysozyme 341
 - ovalbumin 339–340
 - ovomucin 340
 - ovomucoid 340–341
 - ovotransferrin 340
 - stress and disease 176–177
 - structure of 28–29
 - vitamins in 40–41
 - VMO-1 (pleiotrophin and beta-microseminoprotein-like) 170–171
- Egg yolk
 - cholesterol
 - international guidelines for daily intake 66
 - roles of 64–65
 - United States and Japanese history 65–66
 - egg appearance and colour
 - consumer preferences for 280–281
 - factors affecting 282–285
 - and egg quality preferences 244–245
 - and egg washing 217
 - as emulsifier 62–64
 - feed effects
 - overview 60
 - ω -3 fatty acid fortified eggs 60–61
 - folic acid fortified egg 79–81
 - lipids
 - components of 57–58
 - nutritional characteristics of 58–60
 - overview 56–57
 - lutein and zeaxanthin 83
 - migration and behaviour of
 - microorganisms in 127–128
 - overview 49–51

- passive immunization 71
 - neutralizing toxins 71–72
 - Newcastle disease (ND) virus 72
 - rabies virus 72–73
 - xenotransplantation 73
- phospholipids (PLs)
 - application in pharmaceutical, medical and cosmetic areas 62
 - applications in food industry 62–64
- proteins
 - delipidated egg yolk protein 55–56
 - granule proteins 53–55
 - lipovitellin (HDL) 54
 - livetins 53, 342–343
 - low-density lipoprotein (LDL) 51–53, 342
 - new egg yolk proteins 55
 - phosvitin 54–55, 343–344
 - plasma proteins 51–53
 - sialic acids 81–83
- Egg yolk antibody (IgY)
 - detection of 151–152
 - as diagnosis tool 70
 - as immuno-adsorbent ligand 70–71
 - oral administration of
 - Crohn's disease (CD) 78
 - cultured fish infections 78–79
 - cystic fibrosis (CF) 77–78
 - dental caries 74–76
 - Helicobacter pylori* 76–77
 - human rotavirus (HRV) 73–74
 - overview 67–68
 - purification of 69–70
 - rapid detection methods 150–151
 - versus serum antibody 68–69
- Emulsifier, egg yolk as 62–64
- Environmental temperature, and pathogen transmission 125–126
- European Consumer Association (BEUC) 244
- External egg quality 394

- Fat-soluble vitamins 367–369
- Fatty acids
 - egg lipids 344–345
- Feed effects, egg yolk
 - overview 60
 - ω -3 fatty acid fortified eggs 60–61
- Folic acid fortified egg 79–81

- Gallin (OvoDA1) 169
- GBP-B. see Glucan-binding protein B (GBP-B)
- Gene expression and regulation 395–396
- Genetics, and egg white 175–176
- Genome sequence, and egg quality
 - egg production 393–394
 - external egg quality 394
 - internal egg quality 395
 - overview 392–393
- Genome-wide association studies (GWAS) 384
- Genomic selection, and egg quality 391–392

- Glucan-binding protein B (GBP-B) 76
- Glypican-related integral membrane proteoglycan family (GRIPs) 13
- GRIPs. see Glypican-related integral membrane proteoglycan family (GRIPs)
- GWAS. see Genome-wide association studies (GWAS)

- HDL. see Lipovitellin (HDL)
- Helicobacter pylori* 76–77
- Hen-associated factors, and egg white 174–175
- Hen housing, and egg safety 196
- HLB. see Hydrophobic–lipophilic balance (HLB)
- Horizontal pathogen transmission, in eggs 117–119
- HRV. see Human rotavirus (HRV)
- HSV. see Hue–saturation–value (HSV)
- Hue–saturation–value (HSV) 251
- Human rotavirus (HRV) 73–74
- Hydrophobic–lipophilic balance (HLB) 62

- IBD. see Inflammatory bowel disease (IBD)
- Immune stimulation, and egg white 177
- Immuno-adsorbent ligand, IgY as 70–71
- Inflammatory bowel disease (IBD) 78
- Inoculum state, in eggs 125
- Inorganic components, of eggshell
 - calcite crystals and growth mechanisms 8–9
 - overview 7–8
- Internal egg quality 207–208, 260, 294–296, 395
- Ionizing radiation, and egg shelf-life 300

- LDL. see Low-density lipoprotein (LDL)
- Lipids, egg yolk
 - components of 57–58
 - nutritional characteristics of 58–60
 - overview 56–57
- Lipoprotein receptor proteins (LRPs) 395
- Lipovitellin (HDL) 54
- Livetins 53
- Low-density lipoprotein (LDL) 51–53
- LRPs. see Lipoprotein receptor proteins (LRPs)
- Lutein, and egg yolk 83
- Lysozyme 36–38, 121–122, 163–165

- Mammillary cores/mammillae/mammillary density 10
- MAP. see Modified atmosphere packaging (MAP)
- Mechanical eggshell quality 248–250
- Membranes
 - composition 9–10
 - and egg washing 216
- Microbial hazards, in eggs
 - other than *Salmonella* 114–115
 - S. Enteritidis* 112–114
- Minerals. see also Vitamins
 - in egg white 40
 - and trace elements in eggs 369–371

- Modified atmosphere packaging (MAP)
 case study 234–238
 and egg shelf-life 300
 functional properties of albumen 233–234
 pathogen and spoilage bacteria 234
 physico-chemical properties 232–233
- Monitoring, egg washing 219, 224–225
- Mono-unsaturated fatty acids (MUFAs) 58
- Moulds, and yeasts 106
- MUFAs. *see* Mono-unsaturated fatty acids (MUFAs)
- ND. *see* Newcastle disease (ND) virus
- Neural tube defects (NTDs) 79
- Neutralizing toxins 71–72
- Newcastle disease (ND) virus 72
- New egg yolk proteins 55
- NTDs. *see* Neural tube defects (NTDs)
- Nutrition, and egg white 176
- Nutritional characteristics, of egg yolk lipids 58–60
- Odour, and egg quality 255–257
- Oil-chitosan emulsions 307–309
- Oils and waxes coatings 302–304
- Omega-3 PUFA, n-3 egg nutrient composition
 biological factors influencing 356–359
 fatty acid fortified eggs 60–61
 marine sources of 362–363
 plant sources of 362
 sensory attributes of enriched eggs 363–367
 strategies to enhance 359–361
- Oral administration, of IgY
 Crohn's disease (CD) 78
 cultured fish infections 78–79
 cystic fibrosis (CF) 77–78
 dental caries 74–76
Helicobacter pylori 76–77
 human rotavirus (HRV) 73–74
- Organic components, of eggshell
 cuticle 14–15
 eggshell matrix 10–14
 mammary cores/mammary/mammary density 10
 membrane composition 9–10
 pigment 15
- OTf. *see* Ovotransferrin (OTf)
- OVA. *see* Ovalbumin (OVA)
- Ovalbumin (OVA) 31–32, 170
- OVM. *see* Ovomuroid (OVM)
- Ovocleidin-116 (OC-116) genetic marker 15–17
- Ovoflavin/ovo-flavoprotein/riboflavin-binding protein 40
- Ovo-flavoprotein/riboflavin-binding protein 40
- Ovoglobulin 38–39
- Ovomucin 35–36
- Ovomucoid (OVM) 34–35, 167
- Ovostatin 168
- Ovotransferrin (OTf) 32–34, 122–123, 165–167
- Packaging and marketing 270–272
- Passive immunization 71
 neutralizing toxins 71–72
 Newcastle disease (ND) virus 72
 rabies virus 72–73
 xenotransplantation 73
- Pathogen contamination of eggs. *see also*
 Egg contamination
Campylobacter 105
 other bacteria 105–106
 overview 99–102
Salmonella 102–104
 yeasts and moulds 106
- Pathogen transmission, in eggs
 antimicrobial molecules
 lysozyme 121–122
 other proteins and peptides 124
 ovotransferrin 122–123
 protease inhibitors 123–124
 vitamin-chelating proteins 123
 and egg freshness 125
 and environmental temperature 125–126
 horizontal transmission 117–119
 and inoculum state 125
 microbial hazards
 other than *Salmonella* 114–115
S. Enteritidis 112–114
 migration and behaviour of microorganisms
 in egg yolk 127–128
 overview 111–112
 pH effect 126–127
 and synergetic effects 127
 trans-shell penetration 119–121
 vertical transmission 115–117
- PERV. *see* Porcine endogenous retrovirus (PERV)
- PH effect, in eggs 126–127
- Phospholipids (PLs) 345
 application in pharmaceutical, medical and cosmetic areas 62
 applications in food industry 62–64
- Phosvitin 54–55
- Pigment, and eggshell 15
- PLs. *see* Phospholipids (PLs)
- Polyunsaturated fatty acids (PUFAs) 58
- Porcine endogenous retrovirus (PERV) 73
- Porcine Reproductive and Respiratory Syndrome (PRRS) 396
- Porous shell, and egg washing 216
- PRL. *see* Prolactin gene (PRL)
- PRLR. *see* Prolactin receptor gene (PRLR)
- Process control and verification, egg washing 219, 224–225
- Prolactin gene (PRL) 393
- Prolactin receptor gene (PRLR) 393
- Protease inhibitors 123–124, 167
- Proteins
 coatings, egg shelf-life 309–310
 and egg nutrient composition 320–322
 in egg white 29–30

- avidin 341
- cystatin 341
- lysozyme 341
- ovalbumin 339–340
- ovomucin 340
- ovomucoid 340–341
- ovotransferrin 340
- in egg yolk
 - delipidated egg yolk protein 55–56
 - granule proteins 53–55
 - lipovitellin (HDL) 54
 - livetins 53, 342–343
 - low-density lipoprotein (LDL) 51–53, 342
 - new egg yolk proteins 55
 - phosvitin 54–55, 343–344
 - plasma proteins 51–53
 - vitamin-chelating 123
- PRRS. *see* Porcine Reproductive and Respiratory Syndrome (PRRS)
- PUFAs. *see* Polyunsaturated fatty acids (PUFAs)
- Pulsed visible light, and egg shelf-life 299
- Purification, of IgY 69–70

- QTL. *see* Quantitative trait loci (QTL)
- Quantitative trait loci (QTL) 384–385

- Rabies virus 72–73
- Red-green-blue (RGB) images 251
- Refrigeration and egg shelf-life 298
- Restricted ovulator (R/O) 395
- RfBP. *see* Riboflavin-binding protein (RfBP)
- RGB. *see* Red-green-blue (RGB) images
- Riboflavin-binding protein (RfBP) 40, 168
- Rinsing, and egg washing 218, 223–224
- R/O. *see* Restricted ovulator (R/O)

- Salmonella* Enteritidis 102–104, 112–114, 141–142. *see also* Egg contamination
 - conventional culture methods 147–150
 - and egg safety 199–203
 - and egg testing 152–153
 - location, multiplication and migration 143–145
 - rapid detection methods 150–151
 - and sampling eggshells 146
 - serovar and strain differences 145–146
- Sampling eggshells 146
- Sanitizer, and egg washing 218, 223
- Satiety and egg consumption 327–328
- Saturated fatty acids (SFAs) 58
- Serum antibody *versus* IgY 68–69
- SFAs. *see* Saturated fatty acids (SFAs)
- Shell colour, and egg quality 389–390
- Shell quality, and egg quality 206–207, 389
- Sialic acids, and egg yolk 81–83
- Single nucleotide polymorphisms (SNPs) 385, 393
- SNPs. *see* Single nucleotide polymorphisms (SNPs)

- Stress and disease, egg white 176–177
- Synergetic effects, in eggs 127
- Synthetic polymers, and egg shelf-life 310

- TCV. *see* Transmission colour value (TCV)
- T2D. *see* Type 2 diabetes (T2D)
- Temperature, and egg washing 217, 220–221
- Tenp (transiently expressed in neural precursor/ovoglobulin G2) 169–170
- Thermodynamic properties, and egg washing 217
- Time, and egg washing 217, 219
- TMA. *see* Trimethylamine (TMA)
- TNF. *see* Tumour necrosis factor (TNF)
- Transmission colour value (TCV) 252
- Trans-shell penetration, in eggs 119–121
- Trimethylamine (TMA) 395
- Tumour necrosis factor (TNF) 78
- Type 2 diabetes (T2D) 326–327

- Ultrasonic treatment, and egg shelf-life 301
- Ultraviolet radiation, and egg shelf-life 300
- Uterine fluid, and eggshell 7

- Validation, and egg washing 219, 225–226
- Vascular endothelial growth factor (VEGF) 56
- VEGF. *see* Vascular endothelial growth factor (VEGF)
- Vertical pathogen transmission 115–117
- Very low-density lipoproteins (VLDL) 51
- Visual eggshell quality 250–252
- Vitamins 323–324. *see also* Minerals
 - chelating proteins 123
 - in egg white 40–41
 - fat-soluble 367–369
 - water-soluble 369
- VLDL. *see* Very low-density lipoproteins (VLDL)
- VMO-1 (pleiotrophin and beta-microseminoprotein-like) 170–171

- Water-soluble vitamins 369
- Weight loss and egg consumption 327–328
- Whole egg pasteurization, and egg shelf-life 300–301
- Whole-genome methods, for egg quality
 - egg production 385–388
 - overview 384–385

- Xenoreactive natural antibodies (XNAs) 73
- Xenotransplantation 73
- XNAs. *see* Xenoreactive natural antibodies (XNAs)

- Yeasts, and moulds 106
- Yolk granule proteins 53–55
- Yolk plasma proteins 51–53
- Yolk quality, and egg quality 390–391

- Zeaxanthin, and egg yolk 83