FACTORS CONTRIBUTING TO IMPROVEMENT IN EGG QUALITY

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Abstract
The criteria for assessing external and internal quality of eggs include such diverse and important aspects as safety, nutritional, organoleptic and technological properties of eggs and is influenced by a broad range of factors such as the physiology, genetics, nutrition and management of the hens. Genetics are an efficient tool to improve quality but historically egg productivity was the first priority producing spectacular improvement. Selection for egg quality was used mainly to avoid any negative shift in eggshell quality or internal defect. However, internal quality has taken on greater importance for about 10 years and development of molecular genetics is promising for selecting hens with superior egg quality. Nutrition is important for controlling eggshell quality and can successfully enrich the egg in some minor components of interest for human nutrition. The system of production influences the hygienic quality of eggs. This is particularly true in the alternative systems to cage production. Egg safety can have a very large impact on egg consumption and is highly dependant on government regulation. A promising area for improvement is the development of non invasive and rapid physical techniques to measure eggshell and internal egg quality at the egg packing plant. The sensitivity of this technique can enlarge the number of downgraded egg but eliminate egg at risk for the consumers and is also providing new tools for genetic selection. Egg quality has therefore been continuously improved as has the demand for higher quality eggs from the consumers!

Keywords: egg quality genetic nutrition rearing

INTRODUCTION
The external and internal quality of eggs is influenced by a broad range of factors. This is because egg quality criteria includes such diverse and important aspects as safety, nutritional and organoleptic properties or technological properties for cooking, all of which must be controlled from farm to fork. For the poultry breeder, farmers, food, egg sorting and marketing companies, the main priorities are to deliver a safe product which is accepted by the consumers. Therefore, breeders want hens to deliver a consistent product with well established characteristics concerning the appearance of the product, the integrity of the shell to avoid contamination of the egg, a particular weight, a specific colour of the eggshell and yolk and good processing properties reflecting the “freshness of the egg”. Along the whole chain the objective is to avoid any defect in external or internal appearance of the product or any deviation from the expected taste of the product. For some niche markets, the nutritional value of the egg will be important when extra value is added because of the enrichment of egg in specific nutrients such as fatty acid, vitamins or trace elements. The first prerequisite for the consumers will be that no harm should come to them from the egg. Safety of the product is therefore the first priority and its importance is only appreciated when it fails. Outbreaks of disease or contamination can cause large drops in consumption with severe economic consequences. Consequently, numerous rules and legislation have been settled in most countries to ensure microbiological egg safety and the absence of chemical residues in eggs. In addition, producers face constraints concerning animal welfare. This is resulting in changes in egg production system with a ban in Europe of conventional cage systems in favour of alternative systems such as furnished cages, barn of free range. Finally, throughout the world, the consumption of table eggs is shifting to liquid egg products. This tendency towards
egg separation and processing underlines the importance of the technological quality of eggs and also offers new opportunities and challenges for controlling the hygienic quality of eggs. This review aims to analyse the factors, genetic, hen nutrition, methods for measuring egg quality, which have contributed or will in the near future be of importance to pursue the improvement in the egg quality.

**IMPACT OF GENETIC SELECTION ON EGG QUALITY**

For at least 30 years tremendous efforts have been made to improve egg production and egg quality but priorities in breeding companies have evolved throughout this period. Initially feed efficiency and increasing egg number was the prime goal and only latterly has egg quality traits received more attention. The changes in priorities in selection will be illustrated with an example describing the change in performance and quality traits of a European brown commercial line. During the period 1981-1991, the improvement was mainly in egg production (+30 eggs, +2kg egg mass) and feed conversion (minus 35g/ kg egg/year). During the following 10 years (1991-2001), feed efficiency was further improved by slightly lowering the hen weight but mainly by increasing the egg mass. Egg number was increased because of an earlier sexual maturity and increased egg production peak. Hens produced 26 more eggs because of this and of an improvement in persistency (25 weeks of >90% production in 2001). The improvement in egg mass (180g/hen/year) and reduction in feed intake (24g/kg of eggs) was obtained without changing mean egg weight. Selection has focused on limiting increases in egg weight with age, by trying to get a heavier egg earlier and then limiting egg weight increases with age. Eggshell quality remained crucial and in addition, in brown egg laying hens, the intensity and homogeneity of the eggshell colour were controlled. Finally, since 1995, because of the increase in the demand for liquid egg products but also as a consequence of the demands from certified production system, including higher quality products (e.g. “label” appellation) selection was implemented on the technological properties of egg white (mainly Haugh unit).

Since 2000, the demand of consumers in Europe for welfare and environmental standards was reinforced by new regulations. This has favoured segmentation of the egg market with more eggs coming from non-cage systems, (France 26 %, UK 37%, Germany 57%, Sweden 63 % and Netherlands 84 % (Magdelaine, 2007)). Consequently, selection for viability in these systems was carried out and resulted in lower mortality (4-5 % in 2007 in France compared to 8% in 1990). It is well established that egg quality (egg shell, Haugh unit) decreases when hens age. Consequently, to further improve egg mass, it was necessary to reduce the number of downgraded eggs and, therefore, to select hens for higher egg quality at the end of the laying period. Egg shell quality, egg shell colour for brown eggs and eggshell appearance such as roughness of the eggshell, and dark brown spots (which might be confused by consumers in some countries with fly faeces) have been considered toward the end of the laying period (>50 weeks) since 2000. Amongst the internal egg quality parameters the technological properties of egg white (Haugh unit) were considered a priority particularly for certified alternative productions. The heritability of albumen height is estimated to be 0.23 and, in egg quality random tests carried out in Germany (1997/1999), Haugh unit values increased in white and brown eggs (Flock et al., 2001). Currently, selection has produced improvement of 0.8 Haugh units since 2001. Finally, an important internal quality parameter is the percentage of egg yolk. Selection to increase the number of eggs and improve feed efficiency might have negatively affected the egg yolk/white ratio and modified dry matter content of the egg as increasing water content is likely to be metabolically ‘cheaper’. However, this does not seem to have occurred (Flock et al., 2001).

By traditional quantitative genetics, the progress in laying hen performance and in some egg quality traits has been clear to see. In such breeding techniques, a trait was considered as a black box but in reality it is under the control of numerous genes. Knowledge of the chicken genome has developed extremely quickly, the full genome sequence of the chicken became available in 2004 (International chicken genome
sequencing, 2004) and large scale EST resources in 2002 (Boardman et al., 2002). Tremendous efforts have been made to develop genetic markers to localise the sources of statistically significant genetic variability to specific regions of a chromosome (Abasht et al., 2006; Albers et al., 2007).

For egg quality, genome scans to identify loci were based on medium density microsatellite maps (Vilkki, 2007). The QTL database (Abasht et al, 2006; Vilkki, 2007) revealed 113 QTL for eggshell quality. They were observed on chromosomes 1, 2, 4, 5, 7 and Z, chromosomes 1 and 2 for egg white traits and chromosome 8 for fishy taint. The majority of these quantitative trait loci are for egg weight and eggshell quality and those concerning internal quality (Haugh units or albumen height) remained limited. A notable example for organoleptic qualities was described by Vilkki et al. (who demonstrate that the fishy odour in brown eggs is associated with a QTL in chromosome (2007) and more precisely to the mutation of a single gene, the chicken FMO3 gene located in the QTL region associated with this trait. This observation has been used by breeding company to eliminate fishy taint from their brown egg lines which can occur when hens are fed rape seed (canola) meal.

The occurrence of SNP has been recently explored for candidate genes involved in the fabric of the physical barrier, the eggshell and those coding for antimicrobial activity of the egg white. Significant associations have been found between SNP in ovalbumin, ovocleidin 116 and ovocalyxin 32 with eggshell characteristic such as quasi static compression and mammillary layer thickness (Dunn et al., 2006). In candidate gene involved in the natural defences of the egg against microbial penetration or growth, surprising numbers of SNPs which alter the amino acid sequence have been observed (Domahidi et al., 2007). It remains to be seen if the genes coding for antimicrobial proteins of the eggwhite (protease, antiprotease, defensins) are associated with salmonella growth. This is being assessed by an European consortium on a commercial laying hen line in which we previously estimated a heritability of 0.16 for the growth of salmonella in egg white (Sellier et al., 2007).

**EFFECT OF NUTRITION AND HEN HOUSING SYSTEM**

Housing systems for laying hens has evolved in the last 10 years in Europe because of the prospect of banning the conventional cage system in favour of alternative systems (EU council directive 1999/74/EC) but also because the consumer demand for eggs from alternative system of production. The introduction of a new farming system can potentially introduce risk in comparison to the well established conventional cage system especially for the hygienic quality of the egg. However, the effect of the hen production system on sensory, nutritional, or hygienic quality of eggs remains controversial (Fiks-van Niekerk, 2005; De Reu et al., 2008). Nutritional management on the other hand has been relatively stable for the last 15 years, the main modifications being the ban of animal proteins and that of additives.

**Egg composition, nutritional value and internal properties:** In terms of its composition in major elements, the egg displays very consistent composition with regard to its content in total proteins, essential amino acids, total lipids, phospholipids, phosphorus, iron, etc., and only hen age and genetic origin can slightly modify the level of these components (Nys, 2001; Seuss-Baum, 2007). As the bird ages, total egg weight increases and with that increase comes a simultaneous increase in the weight of egg yolk. Likewise the genetic origin of the bird as mentioned previously can on a long term basis slightly modify the yolk/albumen ratio, although when comparing current commercial lines, the variation is small.

In contrast, it has been established for 30 years that the composition of hen diets can have a large influence on the fatty acid profile and the concentration of some vitamins and trace elements. It is possible to increase by ten fold the polyunsaturated fatty acid content (PUFA) belonging to the n-3 (ω3) series, by using flax oil or grains and marine products, and the n-6 (ω6) series by using soybean, sunflower or carthame oils (Jiang and Sim, 1992). With such manipulation, the ω3/ω6 ratio can range from
>30 to <2. It is also possible to incorporate conjugated linoleic acid (CLA) into the egg yolk but it will have a detrimental effect both on rate of laying and yolk texture. Increase in dietary iodine, selenium, E, D and A vitamin is acknowledged to promote a ten fold increase in yolk contents. The high anti oxidant effect of vitamin E is particularly useful when the yolk has a high concentration of PUFAs. Hen diets can induce a similar increase in the egg content of the water soluble vitamins B2, B12, B1, biotin, folic acid and pantothenic acid. So the innovation is not really in the method to modify the egg composition by hen diets even if recent literature helps in reaching a precise composition, but rather in the demonstration of the potential of such modifications for human nutrition and of added value for the enriched eggs (Sparks, 2005; Seuss-Baum, 2007).

It has been concluded that the level of cholesterol in the yolk (210 mg/egg) is not strongly genetically determined (Elkin, 2006), although modern hybrid lines tend to produce eggs with a lower cholesterol content than observed in traditional breeds. In terms of dietary manipulation, PUFA rich oils, cellulose rich fibres, corn kernels, yucca or garlic meals, etc. have all been tried in order to decrease the cholesterol content, with inconsistent effects limited to minus 5-10% (Nys, 2001; Seuss-Baum, 2007). The biggest change has been the fact that more recent epidemiological studies provide evidence for a revision of the effect of cholesterol in human health which should result in better acceptance of eggs in the diet (see review of Seuss-Baum, 2007). In addition, human nutritionists might have come to realise the fact that, in humans the mean consumption of lipids/day in Europe is 25g, 21g and 2-3g of milk, meat and egg lipids respectively. The contribution of eggs is therefore very limited.

Bird housing (cage or free-range) does not directly modify the egg composition, although an indirect response is observed when PUFA and tocopherol rich grass is available. The main effects of rearing hens on litter compared with caging is a decrease in egg weight (-0.5-1g), in yolk proportion (-0 to 5 %) and inconsistent variation (less than ±3%) in total lipid, protein and dry mater (Nys 2001). When comparing various systems of hen production in experimentally control condition with birds of the same age, Rossi (2007) also showed no relevant differences for egg composition. The system of hen production had no effect on blood and meat spots. Haugh unit or pH were also not affected neither was the sensory evaluation of hard boiled eggs demonstrating no influence on egg taste. However, the internal appearance (albumen) of eggs from caged hens was less favoured in this study. Ultimately, the management by the producer had more influence than the rearing system.

**Effect of mineral nutrition on eggshell quality:** Shell breakage is of major concern because it continues to explain the origin of 80-90% of current downgrading and has significant economic consequences for table egg production. New egg sorting systems recording the vibration of the eggshell with electronic sensors are currently used at the egg packing station. This method is more sensitive in detecting hairline cracks, and consequently results in an increase in the incidence of down graded eggs, which is minimal in the early laying period but increases from 12 to more than 20 % at the end of the laying period, depending on programmes of management, nutrition and the environmental conditions of the hen during the rearing and laying period. Eggshell breakage depends on eggshell solidity and on the insult to eggs after oviposition, the latter being influenced by the housing of the hens and egg transportation. Eggshell mechanical strength is influenced not only by the amount of material but also by the fabric of the eggshell ceramic. Numerous examples in the literature demonstrate that defects in hen nutrition can affect the deposition of calcium carbonate in the uterus but evidence for any influence on eggshell structure or crystallographic properties is scarce and to date has only been demonstrated in the case of a deficiency of key nutrients in enzymatic systems such as Cu or Mn (Mabe et al., 2003). Nutritional factors have, therefore to be optimised to prevent any additional decreases in eggshell quality to those induced by hen age or unfavourable environmental conditions.
Calcium nutrition is a key element for eggshell quality. One of the crucial periods is the transition from immature pullet to laying hen. The levels of calcium have to be increased 2 weeks before the onset of egg production to facilitate the development of the medullary bone and more importantly to avoid hens producing the first eggs without the additional supply of dietary calcium, which would result in a negative calcium balance and lower eggshell quality, from which the hens would never recover. Hens mature earlier nowadays and therefore, pullets may produce their first eggs before transfer to the layer house which usually coincides with the introduction of the layer diet. It is therefore recommended to introduce a higher calcium diet (2.5 % or 3.5) before the onset of egg production (14-16 weeks of age). Any risk of lowered feed consumption due to excess of calcium can be alleviated by improving the presentation of the diets (size of particles, use of crumble).

The hen exports daily 2.2 g calcium and, therefore, requires at least 4 g calcium. A low level of dietary calcium (<3%) increases hen mortality and depresses egg production (Keshavarz, 1998a, b). The selection on egg number is nowadays associated with production of eggs laid very early in the morning or even before the light is on. Consequently, the morning feed intake cannot contribute to the supply of calcium for the eggshell the formation of which is completed 1.5 h before oviposition. The introduction of midnight feeding can improve synchronisation of calcium intake and eggshell formation and therefore eggshell quality. The daily kinetics of intestinal calcium absorption is indeed of great importance because of the lack of coincidence between the deposition of calcium for shell formation in the uterus during the night and the calcium intake during the day. The specific appetite for calcium in hens favours the storage of calcium and food in the crop and compensates partly for this gap. Presence of coarse calcium particles allows the expression of this physiological capacity and, in addition reinforces the secretion of acid which is stimulated by crop dilatation. This aspect has been intensively reported in the literature and numerous data have demonstrated the value of using large particles of calcium. The positive effect of particulate calcium, compared to ground calcium has been demonstrated in more than 50 % of the studies (>350 assays since 1927) and the inconsistency of the results can be explained when looking carefully into the factors which modify the eggshell response to particulate calcium (Nys, 2001). Coarse particles of calcium improve especially eggshell quality when given to hens towards the end of the laying period (62 vs. 30% of the trials), in a hot climate (78 vs. 43 %) or when hens are fed low to medium levels of dietary calcium.

Numerous studies have shown that eggshell quality is lowered by high dietary levels of available phosphorus the negative effect being significant when the dietary non phytate phosphorus is higher than 0.35-0.4 % (Nys, 2001). The European mineral group of the WPSA recommended twenty years ago that available phosphorus should be incorporated in the diet at a constant level of 0.28 % throughout the laying period. The relevant question in the current situation of tremendous increase in cost of inorganic phosphorus remains how far is it possible to lower the supplementation of inorganic phosphorus using phytase (300UP) which is generally considered to spare 0.8-1g non phytate phosphorus. In most studies, it seems necessary to supply 1g non phytate phosphorus in presence of phytase to avoid any negative effect on egg production.

Hygienic quality: We have already said that the housing system has no consistent effect on egg composition. This is in contrast with the observation that the hygienic status of eggs from free range or open housing systems is generally considered as lower where the eggs can be in contact with litter. De Reu et al. (2008) concluded that the contamination of the eggshell with aerobic bacteria is higher for nest eggs from non cage system compared to those of conventional or furnished cages. In addition, in floor systems, the hygienic quality of the egg is lowered as a consequence of the higher percentage of floor eggs (Ficks van Niekerk, 2005). De Reu et al. (2008) also concluded in their review that the differences for eggshell
Contamination are very limited between the furnished or conventional cage system for eggs laid in the nest but prevalence of contamination increased if eggs are laid in other parts of the cages (Mallet et al., 2006). These authors however underlined that in commercial conditions, the hygienic difference tend to be lower compared with experimental situations. It is noteworthy however that conventional cage systems have been optimised because of their long use in contrast to the new furnished cages which are still under development. That explains the heterogeneity of the data for furnished cages on egg contamination in the literature. As producers become used to the new systems it is expected that this variation will reduce. The differences between floor and cage system results from the tremendous increase in dust and bacteria in the air in aviary system compared to cages (up to 100 fold) as aerobic bacteria in air and on eggshell are correlated (De Reu et al, 2008). Another important point is the observation that the contamination on the eggshell results predominantly from gram-positive bacteria and is therefore different from the internal egg microflora, which is mainly gram-negative bacteria and less under influence of the environment of the egg (dust, soil or faeces, Board and Tranter, 1995). The relationship between the egg surface contamination and the main bacterial pathogen for humans (Salmonella enteritidis) is not clearly established. In the European countries were the difference in Salmonella prevalence is very large (EFSA, 2006) the importance of the vertical and horizontal transmission between flocks and hens is not established neither is it known the influence of the housing system. In conclusion, the development of new egg production systems might, at least in the short term, threaten the external hygienic quality of eggs but more information is needed to be able to draw clear conclusions on the risk for human health.

**HANDLING AND PROCESSING OF EGGS**

Uniformity and high quality of eggs is an important issue for the companies in charge of egg packing and marketing. Candling and visual inspection combined with some quantitative measures of egg quality (Haugh units, Dry Matter, presence of blood spots) on a very limited number of eggs was traditionally carried out in egg sorting plants with the risk of false evaluation due to operator fatigue. However, the total number of eggs handled and the rate of grading (more than 120 000 eggs per hours) has seen the end of visual inspection by humans. Therefore, the use of automated non invasive techniques using sensors technology (acoustic or optic in the near infrared and fluorescent spectroscopy) has great potential for detecting egg quality very rapidly in egg grading machines (Karoui et al., 2005). The development of such technologies will allow egg to be sorted at high speed not only on eggshell integrity and egg weight, as is possible now, but also on some internal parameters, such as pH and freshness (Haugh unit). It will allow sorting of eggs for specific purposes or eliminating eggs unsafe for human consumption. Currently, crack detection of the eggshell is carried out in commercial conditions using vibration analysis of the eggshell: The detection of difference in oscillation responses of the eggshell after being impacted in four points allows the detection of 90% of cracked eggs with only 1 % of false positives (De Ketelaere et al, 2000). This technique is sensitive and able to reveal some very small micro-cracks. The improvement in the ability to detect cracks might explain why a relatively high percentage of downgraded eggs are observed in commercial conditions despite progress in selection and hen nutrition for over the last 15 years. Optical techniques (Karoui et al., 2005; Berardinelli et al., 2007) are promising to evaluate albumen quality (pH, haugh units and viscosity) or to detect blood spots but are not yet adapted to commercial conditions. Alternatively, computerised video imaging can efficiently detect and differentiate different dirt stains on eggs (Mertens et al., 2005) but it remains too slow to be integrated in a grading machine. Optimizations of these techniques (acoustic, spectral and visual measurements) are currently being developed for integration in a platform to detect risky eggs for human consumption by a European consortium (see [http://www.rescape-](http://www.rescape-))
project.eu). Overall grading and sorting eggs can be a very effective way to improve egg quality for the consumer. An alternative approach to improve egg safety is the decontamination of the whole egg. Egg washing is carried out in numerous countries but remain a controversial issue (EFSA, 2005) and is still banned in Europe. Alternative methods of egg surface decontamination (microwave, gas plasma sterilization, packaging technologies, use of chitosans) are therefore also being evaluated in the EU project Rescape.

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