Effects of housing systems for laying hens on egg quality and safety

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Introduction

Prior to the promulgation of the EC Directive 1999/74, a scientific report on the effects of existing housing systems on the welfare of laying hens was required. Such report only included five pages of discussion on egg quality issues, and 13 lines and two references related to egg safety. In 2004, a new report (EFSA, 2005) dedicated a full chapter to food safety aspects, but at that time scientific information was still quite scarce. Its main recommendations were: a) to promote research on the effects of housing systems for laying hens on egg quality and safety; b) to classify the official data on *Salmonella* prevalence and on residues and contaminants in eggs, according to their different origins; and c) further changes or improvements in the design of housing systems for layers should be always evaluated taking into account their effects on egg safety. Then several European research projects started, such as LayWel and EggDefence (2004-2006), and Rescape and Safehouse (2006-2009).

In 2012, the implementation of the EU Directive was completed and conventional cages (CCs) were prohibited. In the meanwhile, the new furnished cages (FCs) evolved very fast and continuously, as well as the different non-cage systems (NCS), and still are evolving. The scientific research and the growing experience at commercial level have promoted relevant changes in the design and management of such systems. In the most recent years, many papers have been published on the effects of layers housing systems on the hygienic quality of eggs and, more specifically, on the risks of *Salmonella* contamination of eggs. New knowledge on the consequences on commercial quality and nutritional value is much more scarce.

The aim of this review is to update the scientific information published, after the latest ones presented in European meetings (Kan, 2007; Dewulf *et al.*, 2009; De Reu et al., 2009a; Rossi *et al.*, 2010), and the more recently published one by Holt *et al.* (2011). Thus, mainly papers from such dates will be analyzed.

Nutritional value and sensory quality

The effects of the production system on the nutritional value of eggs remains controversial. Earlier reviews of a small number of studies (Belyavin, 1988; Sauveur, 1991) did not find conclusive differences. More recent papers still show conflicting results (Rakonjac *et al.*, 2014). Holt *et al.*, (2011) remarked that only a few controlled experiments have been published in peer-reviewed journals, and also that, since grasses and forage vary in different regions, the potential changes in yolk composition due to free-range foraging may also differ.

To clarify this subject, Anderson (2011) organized the analysis in 4 different laboratories of eggs from hens from the same origin and consuming the same feed, but differing in their access or not to the outdoors. Interestingly, there were significant differences among laboratories on reported results for the content of all nutrients except for cholesterol. Eggs from the range production environment had a total fat, MUFA and PUFA content greater than that of eggs produced by caged hens. Levels of n3-fatty acids were also higher in free-range (FR) eggs (169 *vs.* 141 mg/100g). Whether this constitute a viable nutrient increase is questionable, since recommended nutritional intake by EFSA amounts to 2g/day and thus an n3-enriched food product should have at least 300 mg/100 g. Vitamin A and E and cholesterol contents were not affected by the layers housing system.

On the contrary, Hidalgo *et al.* (2013) did not find significant variations in lipid content in yolks from birds housed in cages, barn or an organic system in a whole cycle of lay. Eggs from organic hens had lower MUFA and higher PUFA values, reflecting the different fatty acid profile of their feed, and probably the consumption of a PUFA rich grass in the range. The authors concluded that the influence of feeding on the fatty acid composition seemed to be more important than the intrinsic characteristics of the housing system. Van Ruth *et al.* (2011) found a different carotenoid profile in organic eggs when compared to free-range or barn eggs, which was proposed as a reliable method to identify possible frauds. Results may be related to the prohibition of using synthetic carotenoids in organic production.

Differences in the rate of lay and thus in nutrient deposition per egg may be another influencing factor. Karsten *et al.* (2010) performed a small-scale study with three different types of pastures, but limiting the hens' consumption of commercial feed to 70 g/hen/day in all cases. Compared to eggs from the caged hens, pastured hens eggs had twice as much vitamin E and long-chain PUFA, 2.5-fold total n3 fatty acids and an increase of 40% in the vitamin A concentration. Vitamin E content was significantly higher in the eggs from hens reared on a grass-dominant environment range. However, pastured hens had a 15% less egg production and weighed 14% less.

It is quite surprising that studies on the supposed differences in egg sensory attributes are almost inexistent. Rossi (2007) performed a sensory evaluation of hard boiled eggs from different production systems, demonstrating no influence on egg taste.

Egg commercial quality

Many experiments have been performed over the years to check the effects of moving from CCs to FCs on several shell and interior quality parameters, or comparing eggs from caged layers to eggs from the different NCS. Most of the published research was conducted in small research flocks and housing systems, which may not directly correlate with the outcomes in a commercial production setting.

With respect to egg size, when comparing FCs with CCs a common finding is a slight decrease in average egg weight (EFSA, 2005), what is often confirmed by reports from the industry. However, research results show a rather wide degree of variability. A possible explanation is that the studies were rarely conducted with the same strains of laying hens, which adds to the variation between studies because genetic differences exist in egg size (Holt *et al*., 2011). Sometimes no differences are seen (Tactacan *et al*., 2009; Onbasilar *et al*., 2015). A recent study with FCs showed that group size and litter provision had no effect on egg weight (Huneau-Salaün *et al.*, 2011).

Stanley *et al.* (2013) did not found significant differences between cage and barn eggs in average egg weight or egg components weight. However, Jones *et al.* (2014) found that eggs from aviary and FCs were significantly heavier than those produced in CCs.

From the LayWel database (LayWel, 2006 b) a trend towards heaviest eggs appears in conventional cages and small furnished cages, while lighter eggs correspond to the other systems. The differences in egg weight, in particular between free-range and cage production, could also be due to differences in feeding levels, and/or in environmental temperature

Most of the research work to date suggests no differences in egg internal quality among types of cages, and inconsistent results in NCS. Karcher *et al.* (2015) did not find differences in Haugh units between conventional and furnished colony cages. Albumin height and HU were significantly greater in eggs from CC in the study published by Jones *et al.* (2014). On the contrary, Chodová *et al*. (2013) obtained lower Haugh units and higher albumen pH in eggs from a barn system, which also showed greater deterioration of egg quality with time. This aspect is not confirmed by Jones *et al.* (2014) after 12 weeks of cold storage. Concerning yolk colour, Martínez-Alesón and Hamelin (2014) performed a market study evaluating samples from 78 commercial brands of eggs produced in the four housing systems, and sold in the Spanish and Portuguese market. They did not find differences except for NCS eggs sold in Portugal, whose yolk tended to be paler and more variable in colour.

Direct or indirect shell strength measures (such as shell thickness and weight, shell percentage and density, shell deformation, or resistance to breakage) also seem to be more dependent upon other factors, such as genetics, age, feed levels, or even egg size, than on the housing system itself. The contrasting results found in the literature do not support a clear influence of the housing system on shell strength. Karcher *et al.* (2015) noted that dietary changes more directly influenced shell parameters, as opposed to the type of cage. Jones *et al.* (2014) found greater static compression strength in eggshells from FCs than in aviary eggs, but no significant differences for shell dynamic stiffness.Modifying some details of cage design, as type of nest floor (Valkonen *et al.*, 2010), or substrate for pecking and scratching (Kalmendal *et al.*, 2013), did had no effect on shell strength parameters.

Van den Brand *et al.* (2004) observed that FR eggs were more variable than cage eggs for most measured parameters, remarking that it is more difficult to maintain a constant quality in FR eggs throughout the laying cycle. Ferrante *et al.* (2009) and Dalle Zotte *et al.* (2013) studied egg quality in eggs from commercial organic farms compared with barn or cage eggs, with contrasting results for shell thickness. In Australia, Samiullah *et al.* (2014) compared eggs from a cage and an FR farm, observing more ultra-structural shell defects and paler shell colour, and also inferior shell strength measures. Variability and a progressive loss of shell colour seem to be a common commercial problem in FR eggs. Roberts *et al.* (2013) studied the loss of shell colour, which occurred only in the FR system, by relocating some birds in cages. This change resulted in a rapid improvement of shell colour.

As noted by Bain (2011), any stress or disease that compromises the health of the birds will result in an increased incidence of egg abnormalities. The FR environment is less controlled than that of cage or barn facilities, and there are more risks for laying hens to suffer from some bacterial or parasitic diseases (Lay *et al.*, 2011). Some recent examples of the effects of stress on shell quality have been provided by Nasr *et al.* (2012) and Alm *et al.* (2015). They found respectively a negative correlation between keel bone fractures and several shell strength parameters, and a positive influence of restricting access of newly-housed pullets to the litter area for the first two weeks.

Cracked and dirty eggs

In 2005, Tauson reviewed the state of the art of furnished cages and concluded that the by then most developed models provided similar production results to those of CCs. However, at that time differences in dirty and cracked eggs still existed between models. Design and location of nests, perches and litter, as well as group size, were all considered important factors.

The potential for improving egg quality in furnished cages by rather simple methods was demonstrated by Wall and Tauson (2002), and introduced in practice in several FC models: e.g., using egg saver wires or curtains, receiving the egg into the egg cradle at a slower speed; the running of the egg belt in short distances at intervals, to compensate for high concentration of eggs into a smaller space in the egg cradle than in a CC; and location of the litter area well away from the nest. For example, Tactacan *et al.* (2009) did not observe any increase in egg breakage in cages provided with an egg-saver wire; Callejo *et al.* (2013) quantified the reduction of cracked eggs around 40%, using every day three automatic advances of egg belts, adapted to the width and location of nests.

Many of the results published in the last few years about egg production and eggshell quality of eggs from furnished cages, were obtained under experimental conditions or in new-built EC farms. However, in some countries as Spain, many conventional cages were transformed into furnished cages; farms with several houses connected by an egg-conveyor are also very common. A wide study under commercial conditions was conducted over 25 months by Hernándiz (2013) in two farms. In the first one there were 4 laying houses with adapted furnished cages (AFC) and 2 with new ones (NFC), and in the second one there were 4 houses equipped with the same NFC model. Dirty and cracked eggs were detected using Moba Omnia® grading machines, which included the electronic devices Egg Inspector® and Crack Detector®, as well as by conventional candling. Data were compared with those obtained in the former 25 months in the first farm, then equipped with CCs.

The percentage of dirty eggs in CCs (5.8 ± 1.7%) increased after the cages were adapted (9.0 ± 1.9%). This increase was probably due to the absence of an egg saver and an automatic egg belt advance system. Moreover, the cages and their nests were narrower, and equipped with Astroturf®. Eggs from new-built FCs showed the lowest percentage of dirty eggs (5.1 ± 1.8%). The percentage of broken-cracked eggs in CCs (3.7 ± 1.3%) increased when they were adapted (4.3 ± 1.9%), being nearly the same in FCs (4.2 ± 1.3%). Dirty eggs from AFC were mainly dusty and re-calcified eggs, especially at the beginning of the laying cycle. Broken eggs and star cracks accounted for the majority of eggshell quality problems, but hairline cracks were very prevalent in FCs and frequently not detected by candling. However, in the second and newly built farm, with a packing plant where the collecting and grading systems were especially adapted, egg quality obtained in NFCs was significantly better than when using CCs. Visual inspection removed only 28% of the broken-cracked eggs, whereas the Crack Detector® removed 62%. This experiment illustrates the variety of situations that can be found in commercial practice, not always corresponding to research protocols, and also the importance of acute measurement methods so as to not draw wrong conclusions.

Current trends in FCs design are to increase group size (up to 100 birds) and replace the old litter box with a mat provided with bedding, automatically distributed. Very often the substrate consists of layer feed. However, in many cases, no litter is supplied under commercial conditions (Huneau-Salaün *et al.*, 2011). Furthermore, nest floor and even litter mats are frequently made of plastic and perforated with the same flush as wire floor, to prevent accumulation of faeces on them, and thus to reduce soiled eggs. Some manufacturers try to provide brighter light intensity in the pecking and scratching area (PSA) and a darker environment in the nest area, by installing LEDs into the cages. However, the design and placement of cage amenities must fulfill both the zootechnical requirements prevailing in commercial egg production and behavioural needs (Guinnebretière *et al.,* 2013). Knowledge on hens' behaviour and physiology is also useful to modify FC designs or to adapt the management to improve egg quality.

Use of nests. A good use of nest boxes is of paramount importance to avoid an excess of dirty and cracked eggs. However, a high use of nests also involves the concentration of eggs in a limited space of the egg cradle, thus increasing the risk of breakage. Conversely, a low nest box use may decrease the external quality of eggs. As first described by Mallet *et al.* (2006), eggs laid outside the nest are more often broken and dirty than those laid in the nest (Huneau-Salaün *et al.*, 2011; Guinnebretière *et al.*, 2012; Tuyttens *et al.*, 2013; Onbasilar *et al.*, 2015). This can be mainly attributed to eggs laid in the scratch pad area of the cage, especially if it shows attractive features for laying, e.g. a floor of turf or abundance of remaining litter particles.

Other factors affecting nest use can be an insufficient adaptation to the nests, and differences among genotypes in nest use. Tactacan *et al.* (2009) obtained in FCs a number of dirty eggs three times greater than in CCs, due to a late transfer from rearing house because of a delay in the assembly of FCs, and Tuyttens *et al.* (2013) observed a nest use of only 70.8% after pullets reared on litter were transferred at 18 wk. These data illustrate the importance of establishing a suitable period of adaptation before entering in lay. Also, nest use increases with age, especially over the first weeks of lay (Wall, 2011; Guinnebretière *et al.*, 2012; Tuyttens *et al.*, 2013).

From the LayWel database (LayWel, 2006a), it seems that flocks of White Leghorns used the nests in small and medium FCs better than brown birds, situation also observed in large FCs by Huneau-Salaün et al. (2014). However, in other recent studies such difference has not been found, although some interactions were detected, depending on group size or nest lining (Wall, 2011; Wall and Tauson, 2013); also the opposite situation has been observed (Onbasilar *et al.*, 2015).

A crucial factor could be the oviposition timing, related to the lightning program used. Investigations using individual electronic identification and single nest boxes have shown differences between hybrids in their oviposition time, and this fact should be taken in account to avoid misplaced eggs due to unavailability of nests when needed (Icken *et al.*, 2012). Most brown eggs were laid about two hours after the lights turned on, and had already reached the maximum rate of daily production at just three hours after the lights came on, whereas a high percentage of the white egg layers started looking for a nest three hours after daylight begun and laid most of their eggs six hours after the beginning of daylight. The nest visits of the White Leghorn line were mainly within a period of two hours, whereas the brown-egg line spread its nest visits to more than four hours. Tumová *et al.* (2013) also found that each genotype had a particular laying pattern, and white-egg strains tend to lay eggs later during the day. Housing on litter delayed time of oviposition in comparison to cage housing.

Callejo *et al.* (2014), using a brown hybrid, observed that almost 50% of daily eggs were laid 3 h after lights turned on, reaching more than 80% in the following three hours.

Nest lining. In the past, cage manufacturers mainly proposed an artificial turf mat for nest lining. Hens do prefer this type of nest floor (Struelens et al., 2008b; Valkonen *et al.*, 2010; Huneau-Salaün *et al.*, 2011; Guinnebretière *et al.*, 2012). However, even perforated artificial turf mats are soiled by faeces, leading to more dirty eggs. Thus, plastic mesh was later proposed as a more hygienic alternative.

Guinnebretière *et al.* (2012) observed similar frequencies of soiling in eggs laid in nests with both types of lining, although those with artificial turf scored as dirtier than plastic mesh mats. Valkonen *et al.* (2010), using smooth perforated plastic nest flooring, report a 40% increase in the incidence of cracked eggs, according to the packing plant data, but also a reduction in dirty eggs. However, Tuyttens *et al.* (2013) and Wall and Tauson (2013) did not find any difference between turf and plastic netting in % of dirty and broken eggs. The bare cage floor as a nest bottom resulted in a significantly lower use of the nest, and tendencies for inferior egg quality. Struelens *et al.* (2008a) also observed the shorter duration of nest visits and less sitting in nests with coated wire mesh flooring.

Group/cage sizes. Compared to smaller designs, large furnished cages provide more space overall that may better support nesting. Crowding and competition for the nest may occur in larger group sizes since more hens must share a restricted number of resources, and often only a single nest is provided for a large group of hens. However, there are contrasting results, probably because of differences in cage designs. Bignon *et al.* (2010), using experimental cage models, found a higher level of broken eggs in cages housing 46 laying hens than in those housing 30 birds. Wall (2011) compared small FCs (8-10 hens by cage) with larger cages (20, 40 hens), observing in the latter less use of nests and 30% increase of dirty eggs, even higher in white layers.

However, Hunneau-Salaün et al. (2011), working with cages for 20, 40 or 60 birds found an overall percentage of eggs laid in the nest greater than 95%. This one was slightly lower in the smaller cages, where the proportion of dirty eggs was also higher (1.6 vs. 1.2%). As each hen in all three group sizes had 67 cm^2 of nest, the overall area of the nest increased with the number of hens per cage. In the study performed by Guinnebretière *et al.* (2012) with cages for 60 hens, 87.3% of eggs were laid in the nest over the entire laying period, whereas 7.8% of eggs were collected in the PSA, and only 4.9% of eggs elsewhere in the cage. In total, 3.8% of dirty eggs were recorded, with high variability between treatments. The percentage of broken eggs remained low in all treatments (0.8%).

A recent study (Hunniford *et al.*, 2014) tried to clarify the effect of floor/nest space allowance and overall cage/nest size on the nesting behaviour. Cages housed 28, 40, 55 or 80 birds, and nest space varied between 70 and 100 cm2 for each hen. More eggs were laid in the nest area in small cages (91.7%) than in large (77.2%), with a non-significant effect of space allowance. Most of the remaining eggs appeared in the scratch area. There was a distinct peak in time of lay by hens in large cages, with most eggs laid between 08:00 h and 09:00 h (32.6%), which was significantly higher than the percentage of eggs laid in the small cages at this time (23.2%). The birds in the smaller cages appeared to be more willing to aggressively compete to lay their eggs in the nest area, while more birds in larger cages chose to lay their eggs in the PSA area, probably because the crowding in the nests at that time. The overall use of nest in large FCs was low, and the authors related it to the less attractive nest floor (plastic mesh), and the semi-enclosed design of the scratch area, also provided with a more desirable surface for egg laying (smooth plastic mat).

Litter provision and mat design. Litter provision could have an effect on nesting rate and egg cleanliness by enhancing the attractiveness of the pecking and scratching area for laying. However, in the experiments performed by Huneau-Salaün *et al.* (2011, 2014) nesting rate and frequency of dirty or cracked eggs were unaffected by litter provision (none, feed or wheat bran). The distribution of litter begun several hours after putting lighting on, when most of the hens had already laid. Also, the litter was quickly dispersed or pecked by the birds after distribution and did not remain on the mat. Moreover, the pecking and scratching area was more brightly lit than the nest and was thus less attractive for nesting. Kalmendal *et al.* (2013) did not observe differences in soiled eggs when using sawdust or pelleted straw as litter substrate.

Guinnebretière *et al.* (2012) tested different types of materials (artificial turf or rubber) for the pecking and scratching area (PSA). Dirtiness and breakage were more frequently observed in eggs laid in the PSA and elsewhere in the cage than in those laid in the nest, whatever the linings in the PSA.

However, if the litter mat was made of rubber, total dirty eggs significantly increased. Both PSA linings were more heavily soiled than wired areas due to droppings stuck in the mats. Hygiene was poor on artificial turf mats, but eggs laid on PSA covered with a rubber mat were much dirtier (32%), and also the frequency of broken eggs increased. Moreover, rubber mats in PSA were rapidly destroyed and proved to be unsuitable. Hens laid a 7.8% of eggs in the PSA; in appearance, the use of wheat bran as litter made the PSA more attractive for nesting.

Perches. Hester (2014) has recently reviewed the effect of perches in egg quality. Previous research has shown the influence of some features of perches on the risk to increase broken or soiled eggs. However, some studies have reported no effect of perches in laying cages on the percentage of cracked eggs (Hester *et al.*, 2013). The diverse results could be due to the use of different perch heights and designs, as well as shell integrity differences among the genetic strains.

Perch placement should be of sufficient height from the cage floor (minimum of 6 cm) to allow eggs to roll down the sloped floor to the collection area (Struelens and Tuyttens, 2009). It seems that hens preferences on perch height (high perches), as observed by Struelens *et al.* (2008a) do not favour optimum egg quality. Based on observations at the commercial level indicating that using low perches halved the proportion of cracked eggs, Tuyttens *et al.* (2013) compared a height of only 5 cm with 23 cm high perches. Low perches were more frequently used during the light period and less at night, and sitting time was shorter than in elevated perches; however, the percentage of broken eggs was significantly lower (2.0 vs. 4.6%).

Perch access may result in a greater percentage of dirty eggs as compared to eggs from caged hens without perches (Hester *et al.,* 2013). Eggs can become trapped in cages with perches perhaps due to less movement of hens on the floor of the cage as compared with hens in cages without perches. Since eggs may arrive later at the collecting area as compared to cages without perches, greater fecal contamination could occur leading to more dirty eggs. Less hen traffic under the perches may not allow manure to fall through the wire cage floor to the manure collecting area as effectively as compared to cages without perches. Guinnebretière *et al.* (2012) tested cages with perches located in the middle part of the cage. The wire under perches scored inferior hygiene than the rest of the wired area. Indeed, droppings accumulated on the wire floor under the perches because hens stayed on them during the night, and droppings under the perches did not cross the wire floor by bird walking.

Microbial load

Furnished cages. The first and scant reports on the hygienic quality of eggs of eggs produced in the first FC models showed a significantly higher bacterial load, around +1 log unit, on the shell of eggs from FCs (Cepero et al., 2000, 2001; Mallet *et al.*, 2003). Counts were around 5-6 or even 3-4 log units, in the range of an acceptable quality. Further studies also found a greater microbial contamination (total, *Enterococcus* and *Enterobacteriaceae*) in the eggshells from FCs when comparing to CCs (Wall *et al.*, 2008). However, results obtained in different models of FCs varied considerably.

According to De Reu *et al.* (2008), in experimental studies systematic but not great differences have been found. However, at the commercial level, the variability between farms using FCs is relatively high, from 4.2 to 5.2 log units (De Reu *et al.*, 2009c). Thus, to draw valid conclusions it may be necessary to evaluate the results of numerous farms. Huneau-Saläun *et al.* (2010b) took samples in 28 commercial farms and obtained higher counts of mesophilic bacteria in FCs than in CCs. Such differences, however, were only of 0.2-0.6 log units, also showing a great variability within both types of cages.

Mallet *et al.* (2006) demonstrated higher shell microbial counts of eggs laid on the wire floor, and especially in the litter area, than those found in eggs laid in the nest. This finding, which outlines the importance of a proper nest use, has been later confirmed by other researchers (Huneau-Salaün *et al.*, 2011; Guinnebretière *et al.*, 2012) in modern large cages (20, 40, 60 birds). They also reported a lack of effect of group size.

These studies were also addressed to verify the effect of litter mat lining and substrate. Huneau-Salaün *et al.* (2010b) compared results of 21 commercial farms with CCs and 7 with FCs, using the feed as substrate in the pecking and scratching area. Counts of mesophilic bacteria were significantly higher in FCs (5.1 *vs*. 4.4 log units), but not higher than those found when using other substrates. Guinnebretière *et al.* (2012) found higher eggshell counts of mesophilic bacteria in eggs laid if PSA mat was made of rubber instead of artificial turf. However, nest lining (turf or plastic mesh) had no effect on shell microbial load.

Non Cage Systems. In studies performed 30-40 years ago, a high level of microbial contamination of eggs from backyard and free-range hens was shown (Sauveur, 1991). In recent times, eggshells produced on commercial farms using NCS (not including floor-laid eggs) still had higher microbial load (De Reu et al., 2006a, 2009c; Huneau-Salaün *et al.,* 2010b; Hannah *et al.,* 2011a), and/or *E.coli* and coliform counts (Singh *et al,* 2009; Englmaierová *et al*., 2014), but this increase usually amounts to only around one log unit. However, there is a wide variation depending on the place of egg laying; in eggs laid on the floor, bacterial counts are much higher (≥ 7 log units). Although data on egg contents contamination are scarce, it seems to be very low (De Reu *et al*., 2006b; Rossi *et al*., 2010).

De Reu *et al.* (2008) pointed out in their review that the differences found in experimental studies with respect to cage eggs are not high (whenever the eggs laid on the floor are not included). But, at the commercial level are often smaller, usually of 0.5-1 log units, with a tendency to be greater in aviaries. Sometimes counts for coliforms are even slightly smaller than in CC. De Reu *et al.* (2009c) studied 13 laying houses from 3 EU countries (6 with FC, and 7 with access to range). Counts for total aerobic bacteria in shells of NC eggs arose to only 0.2 log units more than in FC eggs, difference with a small relevance from the hygienic point of view. In most cases, the counts for *Enterobacteriaceae* were near to detection limit (< 10 CFU), and not significantly different. In all housing systems, counts in egg contents were very low (≈ 2%). However, a wide variability among farms using the same system has been observed (De Reu *et al*., 2009a; Huneau-Salaün *et al*., 2010b).

At the moment of egg grading and packaging, the differences between NCS and cage eggs are diluted (De Reu *et al*., 2006a). In addition, eggshell bacterial load diminishes over the storage period. De Reu *et al.* (2008) concluded that the effects of the housing system are variable, and related to layer facilities design and husbandry factors that may exert their influence. Rossi *et al.* (2010) stated that it was not foreseeable that the increase of NCS in the EU could have significant consequences for the hygienic quality of eggs.

The latest published research, coming from outside the EU, seems to support the trends cited above. In the USA, Jones and Anderson (2013) compared eggs from hens housed in CC, barn and FR, detecting higher levels of *Enterobacteriaceae* in eggshells from FR eggs, but only in some strains of birds, because of a different tendency to lay eggs on the floor. Parisi *et al.* (2014) found that numbers of *Enterobacteriaceae* in FR eggs averaged 1.0 log CFU higher than in eggs from CC hens. Jones *et al.* (2012) reported that the greatest number of coliform isolates (62%) was collected primarily from FR production, being *E.coli* the primary isolate (55%), especially in FR nest boxes (44%). Only 15% of these isolates were from CC production. FR egg shells also showed a larger diversity of organisms. More recently, however, Jones *et al*. (2015) published results for four production periods comparing eggs from CC, FC, and a FR aviary. Aviary eggshell pools had the greatest levels of aerobic contamination (4.9 vs. 2.7 log CFU), and coliform levels were low for all housing systems.

Stanley *et al.* (2010) found significantly higher bacterial shell counts in fresh eggs from a floor system than in cage eggs (5.9 log CFU vs. 4.0 log CFU). This difference disappeared in eggs collected eight hours after being laid. Hannah *et al.* (2011a) observed that shell bacterial levels after washing were similar for eggs from hens housed in caged and cage-free environments. In Australia, Sammiullah *et al.* (2014) compared eggs from one FR and one cage commercial farms. They found a significantly lower total microbial load in cage eggs, but the overall bacterial load was low in both systems.

The observed trend to a higher sell contamination in eggs from NCS can be controlled using good husbandry and hygiene practices, as a more frequent collection of eggs, and especially the prevention of floor lay. Other critical points in NCS can be the accumulation of eggs in egg belts, putting next boxes directly on the litter, and the contamination of nests (Rodenburg *et al*., 2005). Nevertheless, the differences observed with respect to facilities with CC or FC can be very variable, from 5 to 15 or even 100 times greater (DeReu *et al*., 2009c).

In some trials, more *Enterobacteriaceae* and total microorganisms were detected in the dust present in the ambient of aviaries and other NCS (Manfreda *et al*., 2007; Huneau-Salaün *et al*., 2010b). The relationships between air microbial load (by m^3), dust concentration, and the contamination of the eggshell are well known. This fact could be more evident in aviaries (Le Bouquin *et al.*, 2013; Zhao *et al.,* 2015), where population density and ambient dust use to be higher than in other NCS. These findings suggest that a better ventilation may improve the egg microbial quality in NCS.

Salmonella and other zoonotic bacteria

Eggs produced in NCS could have, in theory, a higher risk of contamination by *Salmonella*. This hypothesis is related to the greater exposition of the hens to environmental factors, and to their closer contact with feces and vectors (EFSA, 2005). At the moment, more and better information on this important subject is available (Dewulf *et al.,* 2009; Holt *et al.*, 2011). There are two primary sources of information, epidemiological studies performed on commercial farms, and trials carried out in experimental conditions.

Epidemiological studies. To date, the study of prevalence in the UE in 2004-05 is the research conducted at the greatest scale: 5,310 flocks in 25 European countries were investigated; 30.8% of fecal samples were positive for *Salmonella spp*, and 18.3% to S. *Enteritidis*. In a later report (EFSA, 2007) the influence of the results of the housing system and other variables was evaluated. The flocks housed in cages were more frequently positive to S. *Enteritidis* than layers housed in NCS. Within NCS, the risk was higher in hens housed in barn systems. That situation was also seen for S. *Typhimurium* and the other Salmonella serovars detected. The report concluded “the facilities with cages are probably more contaminated with Salmonella." Nevertheless, the data displayed a considerable variability, and thus should be carefully considered (Dewulf *et al.*, 2009).

Epidemiological studies, performed in different EU countries over hundreds of commercial farms, show variable results, but mostly indicate a higher risk of S. *Enteritidis* in laying houses with cages (Methner *et al.*, 2006; Much *et al.*, 2007; Wales *et al.*, 2007; Snow *et al.*, 2007; Namata *et al.*, 2008). This finding does not necessarily imply a cause-effect relationship between cage housing and the infection or excretion of Salmonella. Firstly, differences in the methodology of sampling and analysis often exist (Dewulf *et al.*, 2009). Even if only taking fecal samples, methods may have a different sensitivity since in manure belts the bacteria are concentrated, but in litter they are much more scattered (Holt *et al.*, 2011).

Wales *et al.* (2007) emphasized the high variability of the prevalence within each system, between flocks of a same farm, and also over time. Epidemiological studies have identified several risk factors for Salmonella infection and shedding (Carrique-Mas *et al.*, 2009b; Huneau-Salaün *et al.*, 2009; Snow *et al.*, 2010; Van Hoorebeke *et al.*, 2010b). According to Dewulf *et al.* (2010), the observed links in the epidemiological studies are likely related to factors associated with the housing system, such as age of infrastructure, previous infections, or incomplete cleaning, and not with the system itself.

The more compromised situations with respect to such risk factors are usually more frequent in laying hen farms with cages. The flocks housed in cages are commonly much more populated, and there are more flocks in every farm. The risk of cross-contamination can increase in the multiage complexes (Carrique-Mas *et al.*, 2009b), because of the proximity and communication between facilities. Dewaele *et al.* (2012 a, b) controlled in Belgium some S. *Enteritidis* persistently positive layer farms during successive laying cycles. Cross-contamination was demonstrated between houses, and between laying houses and the egg-collecting area, which in most farms it was identified as a critical point. Snow *et al.* (2010), studied 380 laying houses of several systems, and calculated an OR of only 0.06 if the system all in-all out was used (a value similar to the estimated effect of vaccination against *Salmonella*).

Other studies have confirmed deficiencies in cleaning and disinfection of many houses of cages, and the greater effectiveness reached in the small and much more detachable facilities for FR hens (Carrique-Mas *et al.*, 2009a; Van Hoorebeke *et al.*, 2010a; Huneau-Salaün *et al.*, 2010a). The old age of the buildings, which leads to more frequent breaches of biosecurity, is another important factor (Van Hoorebeke *et al.*, 2010b); in many countries, NCS facilities are of more recent construction than those with cages. Wallner-Pendleton *et al.* (2014) studied 40 U.S. cage-free layer farms of different sizes, 55% of them with access to pasture area; S. *Enteritidis* appeared in 7 of them. The authors suggested that the most significant risks for *Salmonella* contamination were the presence of infected rodents, and the absence of a S. *Enteritidis* vaccination program.

Studies with fewer farms involved show contrasting results, such as a more frequent presence of *Salmonella* in organic environment and eggs, compared to CCs (Manfreda *et al.*, 2007), or no significant differences among CCs, FCs and aviaries (Pieskus *et al.*, 2008). Van Hoorebeke *et al.* (2010b) studied 29 flocks without finding significant differences, which was attributed to a low level of infection. The greatest proportion of positive samples (1/3) was obtained in organic flocks, and the lowest (1/10) in hens housed in the barn systems, being CCs and FR hens at the same level (2/8).

It has been suggested that in FR farms there is a greater risk of S. *Typhimurium* or "exotic" serovars (Carrique-Mas and Davies, 2008), but this concern has not been confirmed to date (EFSA, 2007; Van Hoorebeke *et al.*, 2010b). Snow *et al.* (2007) investigated data from 454 laying houses, without finding significant differences among systems (ST prevalence, 2-3%), except in organic farms (not detected). In a further study, Snow *et al.* (2010) studied 380 laying houses, where the prevalence of S. *spp* within cage farms arose to 26%, but it was between 5.4 and 7.7% in NCS. The relative risk (OR, odds ratio) of the hens in NCS, with respect to caged flocks, was estimated between 0.16 and 0.26.

Studies in France in more than 500 farms showed that in cage farms the prevalence of ST and S.*spp* was of 14% and 34%, respectively, but in NCS decreased to 5 and 9%, respectively. The best results were obtained in the FR farms (Mahé *et al.*, 2008; Huneau-Salaün *et al.*, 2009). Van Hoorebeke *et al.* (2010b), within the framework of the EU project Safehouse, analyzed 292 flocks from 5 European countries. 10% were positive for Salmonella; the greatest proportion was found in farms with CCs (28.8%), the lowest in organic farms (2.8%), and between 6 and 8% for the rest part of systems. They identified as the most important risks factors the old age of the building, the greater number of hens in each operation, and the higher number of hens in a flock. All of these factors were more frequently linked to cage farms. The prevalence intra-flock was found between 0 and 7%, but in some FR flock it reached 27.5%. Results of the EU research project Safehouse rather show the lack of effect of the housing system, and also the absence of an increased transmission in NCS (Dewulf *et al.*, 2010).

Experimental studies. Recently, several pilot studies have been performed in the USA. Jones *et al.* (2012) took samples over a full laying cycle in a FR facility from the floor, grass, and nest boxes, and also in a CC laying house. *Salmonella* was detected in all treatments, but there were no significant differences in prevalence for any of the sample types. The greatest number of *Salmonella*-positive samples (shell emulsions and environmental swabs) was found in CC production, where more dust was present on the CC equipment surfaces as wells as on eggs. Another interesting finding was the detection of *Campylobacter*-positive shell emulsion pools in CC and FR nest boxes, but not in the egg contents. A low frequency of Listeria detection occurred during this study. In contrast, Parisi *et al.* (2014) did not detect *Salmonella* in any of the eggs collected from CC hens, but its prevalence in FR eggs was 2.4%. Prevalence of *Campylobacte*r recovered from FR eggshells was also significantly higher (26.1 vs. 7.4%).

Some experiments have tried to deepen current knowledge on the dynamics of S. *Enteritidis* transmission and shedding, using experimental infection. De Vylder *et al.* (2009) verified that in CCs the colonization of bird organs was significantly smaller on the 1st and 7th days after the infection; spleen and liver were the organs most frequently colonized. The layers housed in CCs had a significantly smaller *Salmonella* excretion. The percentage of positive cloacal swabs decreased with time in all systems, but this diminution was more accentuated in the birds housed in FCs and aviary. At the end of the test (21 days post-infection), the caeca were found as *Salmonella*-positive in the 46.5, 45, and 42% of the birds housed in CC, FC, and aviary, respectively. There were no significant differences between systems in the number of positive ovaries and oviducts. Thus, the authors concluded that increases in the colonization by *Salmonella* of hens housed in NCS are not expectable.

In further research, De Vylder *et al.* (2011) studied the transmission of S. *Enteritidis*, after introducing birds infected within groups of hens housed in different systems, observing a trend to increase in birds housed in a floor system and an aviary. The frequency of contaminated eggs (5.8%) in the aviary was significantly greater than in eggs from the barn system (1.8%), CC (1.3%) and FC (1%). The colonization of the internal organs by SE was not correlated with its faecal excretion.

Studies conducted in the USA showed quite similar results. Hannah *et al.* (2011b) studied in five sequential trials the horizontal transmission of *Salmonella* among experimentally infected laying hens, housed in cages or barn. It was not significantly different, except for S. *Typhimurium*, where hens housed on wood shavings showed the greatest transmission, as well as for *Campylobacter*. S.

Enteritidis was detected in all organs at significantly higher frequencies than in layers housed in CC (Gast *et al.*, 2013), but no significant differences (3.6% vs. 4.0%) were observed for the frequency of egg contamination (Gast *et al.,* 2014a). In both types of cages, the horizontal spread of S. *Enteritidis* (22.7% vs. 27.1%, respectively) was statistically similar (Gast *et al.*, 2014b).

Residues and contaminants in eggs

Nearly all veterinary drugs and feed additives available on the market may result in residues in eggs. As MRL values are not set for non-target animals (thus a "zero" tolerance), if detected in eggs, any drug not licensed for use in layers, will constitute a violation of legal limits. The levels reported in eggs are low, and the carry-over percentage from feed to egg is around or below 1%. (Kan and Meijer, 2007). A higher prevalence of pathologies in NCS is often reported, in particular those caused by parasites (Lay *et al.,* 2011). Thus, the risk of undesirable residues of antimicrobials, anthelmintics and anticoccidials in eggs could be higher in theory in those produced in NCS.

EU data on residues in eggs do not clarify this subject, since for non-compliant samples the housing system of hens is not reported. Also, priorities for detecting the different substances are not the same in all EU countries and vary from year to year. According to the last EFSA report (2014), in the year 2012 the number of non-compliant targeted egg samples was only a 0.2% (23 over 12,596), a lower level than in previous years. No prohibited substances were found, and only four samples showed residues of antimicrobials over their respective MRLs. The largest part of these samples (56%) was due to residues of anticoccidials, amounting to 0.35 % of the total samples analysed for this substance group.

Carry-over of drugs or additives from one medicated feed batch to the next non-medicated one during either manufacturing, transport or even at the farm can occur. Thus, inadequate control of contamination of feed may result in residues in eggs exceeding legal limits. For this reason, in the EU Reg. 124/2009, considering that "unavoidable transfers" may exist, MRLs in eggs were established for all anticoccidials (Kan and Meijer, 2007). In Poland, residues of coccidiostats were found in a 4.5% of egg samples. A high percentage (31%) of non-compliant feed samples collected during follow-up investigations was observed, which confirmed that feed cross-contamination may be the reason for the occurrence of coccidiostat residues in food (Olejnik *et al.*, 2011).The reported incidence of coccidiostat residues in eggs in the EU does indicate that good control has not yet been achieved. De Reu *et al.* (2009b) investigated the presence of these substances in eggs sold in the Belgian market, analyzing samples of 47 references. They found coccidiostat residues in almost all the categories, as cage (1 positive/16), floor systems (0/5), free-range (1/12), organic (2/7), and backyard eggs (1/7). In all the cases, their MRLs were below the UE limits.

The chemicals relevant to food safety include environmental exposures to persistent organic pollutants such as dioxin and PCBs, pesticides, and heavy metals. The most widely chemical contaminations of eggs associated with free-range or organic flocks are increased levels of dioxin-like compounds (Holt *et al.*, 2011). Research among layer farms in the Netherlands and other EU countries has shown that organic eggs contain more dioxin than conventional ones, and that a significant number of organic farms produce eggs with a dioxin content that exceeds the EU standard (Schoeters and Hoogenboom, 2006; De Vries *et al.*, 2006, Kijlstra *et al.*, 2007). In the latest EFSA report on residues in food, six non-compliant samples were reported for dioxins and PCBs by three Member States.

The hens' intake of dioxins from various sources leads to an increase in the dioxin content of eggs. These sources include plants, feed, soil, worms, and insects. Plants and commercial organic feed appear to be relatively unimportant as sources of dioxins, but not much is known about the non-commercial feed offered to laying hens in small organic farms or backyard flocks. Consumption of worms and insects and particularly ingestion of soil are important causes of high dioxin levels in eggs (De Vries *et al*., 2006). The availability of dioxins from contaminated soil fed to hens was estimated to be between 40 and 60%, as opposed to 80 and 100% availability of dioxins from a solution in oil (van Eijkeren *et al.*, 2006). Environmental dioxin pollution is due to historical waste burning and various industrial processes, and then risks can be higher in densely populated areas, or in facilities near pollutant industries (Schoeters and Hoogenboom, 2006). Contamination levels in soil should be kept low, and should be controlled in areas with free foraging chicken.

When the levels of PCBs and persistent organochlorinated pesticides were measured in eggs from backyard flocks, the median and median values of the sums of DDT's were 457.2 and 63.4 ng/g fat, respectively. PCDD/Fs and dioxin-like PCBs concentrations ranged from 1.50 to 64.8 pg TEQ/g fat, which supposes a 5-79% of the tolerable weekly intake by exposure to home-produced eggs only (Van Overmeire *et al.*, 2009). The time hens spend scavenging outside may enhance soil ingestion (Kijlstra et al., 2007). As hens on small organic farms spend more time outside than hens on free-range farms, organic eggs are bound to have higher dioxin contents. In contrast, almost none of the larger egg laying farms (> 1,500 hens) had problems maintaining their egg dioxin level below the EU standard of 3 pg TEQ (De Vries *et al.*, 2006).

In FR eggs from backyard flocks in Belgium, the heavy metals lead, mercury, cobalt and thallium had median concentrations 2 to 6 times higher than those of commercial eggs. The Pb concentrations correlated well with those found in soils (Van Overmeire *et al.*, 2006). In Latvia, eleven trace elements were quantitatively determined in egg samples collected from large-scale poultry farms, organic farms, and domestic farms. The most variable range of concentrations and the highest content of elements were determined for egg samples derived from organic farms while egg samples from backyard farms and poultry farms mostly contained lower content of elements. An environmental contaminant as Pb was not detected in egg samples from large-scale cage poultry farms (Vincevica-Gale *et al.*, 2013.)

However, raising hens in alternative housing does not necessarily lead to higher chemical residues in eggs. In the Canary Islands, Luzardo *et al.* (2013) did not find differences in the content of PCBs or OCPs of eggs in relation to its production type. Two samples, one free-run and one organic, significantly exceeded the current European Commission (EC) limit of 2.5 pg TEQPCDD/F g lipid, but the rest were well below this limit. However, the concentrations of polycyclic aromatic hydrocarbons in conventionally produced eggs were almost four times higher than in free-run or organic eggs. Anecdotal incidents with dioxins and PCBs have been also documented in cage or barn facilities, due to certain binders used in feed, the presence of acaricide residues after a treatment poorly performed, the use as litter of pentachlorophenol-treated wood shavings, or even steam given off by insulating materials (Kan, 2007; Holt *et al.*, 2011).

In Catalonia (Spain), from 2000 to 2008 three surveys were carried out to determine the daily intake of various chemical contaminants by the population. Exposure to these environmental contaminants through egg consumption, given in percentages of the total dietary exposure to each pollutant, was lower than 2.5% for most contaminants, including Pb. The only exceptions were PCDD/Fs, with a 6.9% vs. the previous 1.7%, and the pesticide Hexachlorobenzene, with a 3.2%, but decreasing from the last 7.6% (Domingo, 2014). It was concluded that nutritional benefits of the regular consumption of hen eggs clearly outweigh the potential health risks derived from potential exposure to a number of chemical pollutants through that consumption.

Conclusions

Many production factors can affect the different aspects of commercial egg quality (i.e. age of hens, genetics, feeding, temperature, egg freshness), and thus care is needed when the effects of housing systems are compared. If the management and feeding conditions are adequate, no relevant consequences are found in the majority of scientific studies, although in non-cage systems a greater variability in several commercial egg quality parameters is often observed. Nutritional value or egg flavour does not show significant differences in practice.

In recent years, there have been many improvements in design and management of both furnished cages and non cage systems, and many new features have contributed to solve some former problems related with egg quality, in particular the increased incidence of dirty and cracked eggs. A good egg quality can be obtained in the newest large furnished cages as well as in non-cage systems. However, design and management of the pecking and scratching area and litter substrates remain a weak point in furnished cages that need to be improved.

At present, there is much more and better scientific information on the impact of housing systems on the hygienic quality and safety of the egg. The eggshell microbial contamination obtained in furnished ages has been found to be only slightly higher than in eggs produced in the old conventional cages. In non-cage systems, the bacterial load of the eggshell may be 10 times greater, but still within acceptable levels, and it is influenced by the dust level in the atmosphere, which is generally greater in aviaries. However, there may be big differences between farms within the same housing system.

Salmonella prevalence is at present lower in these systems than in cage facilities, due, among other factors, to the different dimension and structure of the alternative sector, and the earlier and usually greater use of vaccination against Salmonella. The presence of dioxin/PCB's and other chemical residues may constitute a real risk for free-range and organic farms, if they are located on contaminated lands, or near pollutant industries.

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