

Emerging Issues: Social Sustainability of Egg Production Symposium

The impact of different housing systems on egg safety and quality¹

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ABSTRACT A move from conventional cages to either an enriched cage or a noncage system may affect the safety or quality, or both, of the eggs laid by hens raised in this new environment. The safety of the eggs may be altered either microbiologically through contamination of internal contents with *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis) or other pathogens, or both, or chemically due to contamination of internal contents with dioxins, pesticides, or heavy metals. Qual-

ity may be affected through changes in the integrity of the shell, yolk, or albumen along with changes in function, composition, or nutrition. Season, hen breed, flock age, and flock disease-vaccination status also interact to affect egg safety and quality and must be taken into account. An understanding of these different effects is prudent before any large-scale move to an alternative housing system is undertaken.

Key words: enriched cage, noncage system, egg safety, egg quality, hen

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INTRODUCTION

Despite the announcement by the European Union (EU) in 1999 that conventional cages for laying hens would be banned, funding for research into various housing programs and their effects on egg safety and quality only recently has been made available in the EU (Safehouse Project, 2010) and that research is ongoing. In the United States, as in the EU, a paucity of information is available with regard to alternative hen housing systems and the safety and quality of eggs produced on farms utilizing such systems. This white paper provides a summary of the current knowledge regarding how different hen housing systems influence the safety and quality of eggs produced and how changing different elements of an egg production system might affect the safety of egg products flowing into the human food chain.

Food Safety

Two primary food safety concerns confront the consuming public with respect to eggs: microbiological safety and chemical contamination. The microbiological integrity of eggs and egg products as it relates to *Salmonella* contamination, primarily with *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis) remains the overriding issue, although the importance of chemical contamination of egg contents should not be underestimated.

Microbiology

The Egg Products Inspection Act of the early 1970s (USDA, 1971) essentially brought an end to egg-associated *Salmonella* infections in humans, at least for a short time. Provisions of the act stipulated that cracked and dirty shell eggs were no longer acceptable for direct sale but rather had to be sent to the processing plant for pasteurization. This eliminated the primary source of *Salmonella* egg contamination, with a subsequent decrease in the incidence of eggborne human salmonellosis. Intact shell eggs were considered safe, essentially sterile, food products until a study published by St Louis et al. (1988) linked the increasing number of human foodborne *Salmonella* Enteritidis outbreaks

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in the northeast United States to eggs contaminated with that organism. The implication of those outbreaks was that *Salmonella* Enteritidis was inside the egg, not just on the external surface. Subsequent studies indicated that the organism can gain entrance to the internal contents of the egg both through deposition into the egg during its formation before the addition of the shell in utero (Okamura et al., 2001) and via invasion through pores of the shell during egg laying (Messens et al., 2005). As such, factors that affect the infection within the hen as well as environmental situations such as those described below, which increase shell contamination after the egg is laid, play a role in the *Salmonella* Enteritidis problem.

The production of *Salmonella* Enteritidis-contaminated eggs in an infected flock is sporadic and the reported incidence is low: 0.3% of eggs (2.28/10,000 eggs for *Salmonella* Enteritidis PT4; Kinde et al., 1996a,b), 1.1% of eggs (from 2 naturally infected flocks in Great Britain; Humphrey et al., 1989), and 0.56% of eggs (from 15 naturally infected flocks; Humphrey et al., 1991). Risk assessments performed in the United States estimated that only 1 in 20,000 (0.005%) eggs are contaminated with *Salmonella* Enteritidis (Ebel and Schlosser, 2000), but with over 70 billion eggs produced annually in the United States (USDA/NASS, 2009), an estimated 3.5 million eggs contaminated with *Salmonella* Enteritidis could potentially enter into the marketplace each year. Reports from the Centers for Disease Control show that *Salmonella* Enteritidis is one of the primary serovars involved in human salmonellosis (Anonymous, 2008) and in those *Salmonella* Enteritidis outbreaks where a food vehicle was identified, 75% of the foods were predominantly egg-based or had eggs ingredients (Braden, 2006). *Salmonella* Enteritidis remains the principle *Salmonella* serovar responsible for egg contamination, but it is not the sole agent in this problem. The *Salmonella* serovars *Salmonella* Typhimurium (Carramiñana et al., 1997) and *Salmonella* Heidelberg (Hennessy et al., 2004) have also been implicated in eggborne human *Salmonella* outbreaks and must be considered in the overall problem.

Detection. It is crucial that samples used in testing flocks for the presence of *Salmonella* Enteritidis and the other important *Salmonella* serovars give a relatively clear indication of the infection situation within a house. However, the monitoring of layer flocks for *Salmonella* based on detection of infection in the eggs or hens remains problematic. The low number and sporadic production of *Salmonella* Enteritidis-positive eggs rules out egg sampling as the routine monitoring method for flock infection. Furthermore, because *Salmonella* Enteritidis infection does not typically cause mortality in layer flocks, the routine culturing of sick, dying, or cull hens to detect infected flocks is not a sensitive method. The routine necropsy and culturing of healthy hens is time-consuming, wasteful of birds, and does not produce accurate results. In contrast, samples collected from the environment (dust, feces, and surface wipes)

tend to more readily indicate the presence of *Salmonella* Enteritidis in the flock. For instance, based on samples collected from houses with known infected hens, Poppe et al. (1992) compared the results of environmental cultures (feces and dust) against organ cultures obtained directly from killed hens (ceca, liver, spleen, and reproductive tissues). Although 22/48 (45.8%) of environmental cultures positively detected *Salmonella* Enteritidis, only 26/300 (8.7%) of the hens were culture-positive. Phage typing and plasmid profiles of the environmental isolates were identical to those recovered from the hens, indicating that the environmental *Salmonella* Enteritidis originated from the birds. Similar results were reported by Mutalib et al. (1992) and demonstrate that although only a proportion of birds may be infected at any one time, the birds' continual excretion of *Salmonella* Enteritidis will progressively increase *Salmonella* Enteritidis levels in the surrounding environment and enhance the chance for organism detection. Davies and Wray (1995a) made the following statement based on the findings of van de Giessen et al. (1991), Mutalib et al. (1992), and Poppe et al. (1992): "...although some routine monitoring for *Salmonella* is carried out in breeding flocks, bacteriological techniques based on samples taken from individual birds and pooling of samples are likely to show low sensitivity compared with indirect environmental monitoring." It should be noted that a positive environmental sample does not definitively say that a flock is infected, only that the organism is present. However, due to the low sensitivity of direct flock testing, environmental monitoring should be employed as a more sensitive method for examining differences in the prevalence of *Salmonella* between egg production systems. Recent studies from the European Food Standards Agency (EFSA, 2004) in the EU used environmental sampling, 5 fecal and 2 dust samples/flock, to determine *Salmonella* prevalence in EU layer flocks. Dust samples were found to more readily detect the presence of *Salmonella* than fecal samples (EFSA, 2006). Because of these findings, it is anticipated that similar environmental testing regimens will be implemented for our future US studies.

It is unclear whether or how different production systems impact on-farm *Salmonella* infection rates. The environmental testing of layer flocks in the EU showed a higher prevalence of *Salmonella* in flocks housed in conventional cages compared with those housed on the floor. This was observed in studies from multiple countries (Germany: Methner et al., 2006; United Kingdom: Wales et al., 2007; Snow et al., 2009; France: Mahé et al., 2008; Belgium: Namata et al., 2008). Furthermore, in a retrospective epidemiological study in Denmark (Mølbak and Neimann, 2002) found that eggs from conventional cages were associated with human *Salmonella* Enteritidis disease, whereas no association was found with eggs from free-range or organic operations. Conversely, other researchers have detected a lower incidence of *Salmonella* in conventional cage systems than cage-free systems (United States: Kinde et al., 1996b;

Germany: Schaar et al., 1997; Netherlands: Mollenhorst et al., 2005), and a survey by USDA/Animal and Plant Health Inspection Service National Animal Health Monitoring System Layers '99 found that pullets raised in conventional cages have lower *Salmonella* Enteritidis incidences than floor-raised pullets (USDA/APHIS, 2000a). Reasons for the disparity in results are unknown. Three of 4 of the studies showing higher incidence in conventional caged layers versus noncage systems were conducted on flocks within 9 wk of end of lay (Methner et al., 2006; Mahé et al., 2008; Snow et al., 2009) compared with only 1 study in which lower incidence in conventional cage layers was observed (Mollenhorst et al., 2005). *Salmonella* incidence tends to increase with flock age (Wales et al., 2007) and the higher incidence in conventional cage facilities may be a reflection of sampling logistics: feces, and their resident salmonellae, are localized in manure pits beneath the cages rather than being disseminated over a wide area in floor-raised facilities. The study by Mollenhorst et al. (2005) used serology rather than culture methodology to detect the flock infection status; therefore, *Salmonella* location within the facility was less of a variable.

Confounding Factors. One of the factors that can affect the prevalence of *Salmonella* on premises is flock size. Based solely on flock *Salmonella* Enteritidis infection rates, Snow et al. (2009) reported that large flocks (>30,000) in the United Kingdom exhibited an increased incidence of infection compared with smaller flock size holdings (1,000 to 2,999; 3,000 to 9,999). Because the average United Kingdom laying facility capacity was 30,000, 12,500, and 3,900 for conventional cage, barn, and free-range houses, respectively (Carrique-Mas et al., 2009), the large flock designation ostensibly reflects conventional cage production, whereas the smaller flock designation would be free-range and barn systems. In 2000, the average flock size in the United States was reported to be 63,000 layers (USDA/APHIS, 2000b), indicating that large flocks are the norm in this country and may be necessary to ensure that sufficient eggs are available for public consumption and for economic viability. This will be discussed further in the white paper "Economic and market issues on the sustainability of egg production in the United States: Analysis of alternative production systems." In a large-scale study of US layer operations, houses containing more than 100,000 layers were 4 times more likely to be environmentally positive for *Salmonella* Enteritidis than similar houses containing fewer than 100,000 hens (USDA/APHIS, 2000a). Possible explanations for this increase in incidence may be the higher densities of birds in these facilities with the concomitant increased volume of contaminated feces and dust (Davies and Breslin, 2004), the restricted hen movement cage houses are potentially a more attractive location for *Salmonella*-carrying rodents (Carrique-Mas et al., 2009), and the difficulty in effectively accessing and cleaning the cages, drinkers, and accessories in the building (Carrique-Mas et al., 2009). Reaching an understanding of why

larger flock sizes increase the likelihood of *Salmonella enteritidis* infection, especially as it relates to the role of conventional cage versus noncage systems, before establishing guidelines to eliminate the problem is of critical importance. Flock sizes tend to be smaller in noncage systems (Mahé et al., 2008; Carrique-Mas et al., 2009) and this can be problematic when attempting to dissect the relative roles of hen densities and housing systems in exacerbating *Salmonella* problems. Finding and sampling noncage US facilities that incorporate larger flock sizes should therefore be a priority to help answer these questions.

As alluded to earlier, *Salmonella* can potentially penetrate through the eggshell (Messens et al., 2005). Thus, shells contaminated by fecal and environmental *Salmonellae* can be an important potential source of this organism. There has been little systematic investigation of *Salmonella* contamination of eggshells from different production systems or on the effects of production system on internal bacterial contamination of eggs. However, in a recent review, DeReu et al. (2008) observed that, in general, aerobic bacterial counts on eggshells are lower from caged (conventional and furnished) than from noncaged (aviary and floor) flocks, and this difference is very marked when eggs laid outside of the nest boxes in the noncage flocks are included. However, this difference was not seen when gram-negative bacteria or *Enterobacteriaceae* were counted (De Reu et al., 2005, 2008), an especially significant observation considering that *Salmonella* is both gram-negative and a member of the *Enterobacteriaceae*. A United Kingdom Food Standards Agency survey found a *Salmonella* prevalence of 0.34% among 4,753 retail boxes, containing 6 eggs each, with all isolates coming from the subset (50%) of conventional caged flocks (Food Standards Agency, 2004). The difference in prevalence between cage and noncage sources was not considered significant. Such evidence as there is to date, which is summarized by De Reu et al. (2008), does not indicate a markedly differing risk of external or internal contamination between systems, provided that floor-laid eggs are removed from the retail chain and sent most likely to a breaking facility.

Stress is yet another confounding factor because exposure to stressful situations can affect the health of a flock. Stressors such as rehousing (Hughes et al., 1989), thermal extremes (Thaxton et al., 1974), transport (Rigby and Pettit, 1980), initiation of egg lay (Jones and Ambali, 1987), and molting (Holt, 2003) have all been shown to exacerbate infection susceptibility in poultry and represent both welfare and potential food safety problems. Different housing conditions may elicit stress responses in a flock, depending on the particular breed used. Campo et al. (2008) demonstrated that certain hen breeds exhibit significantly higher stress responses when raised in deep litter versus free-range housing conditions, compared with other breeds. This stress may subsequently manifest itself in increased *Salmonella* in the flock. The topics of stress and hen health are discussed in more detail the white paper "A com-

parison of hen welfare in relation to multiple housing systems.” Certain concurrent diseases can also affect the incidence of *Salmonella* in a flock. Illness due to *Eimeria* (Arakawa et al., 1992; Qin et al., 1995), infectious bursal disease virus (Wyeth, 1975; Phillips and Opitz, 1995), and reticuloendotheliosis virus (Motha and Egerton, 1983) has been shown to increase the severity and persistence of *Salmonella* infections. Hens residing in a floor setting may be exposed to disease situations less frequently observed in cage-raised hens, in particular *Eimeria* and some of the immunosuppressive viruses (Fossum et al., 2009). This may in turn alter the health dynamic of the flock and increase the opportunity for *Salmonella* entry.

Salmonella Prevention. Biosecurity, and specifically the control of people and equipment on the farm, is critical to prevent the introduction of *Salmonella* and other disease organisms to the farm. Along with this, limiting hen exposure to potential *Salmonella* vectors such as rodents (rats and mice), insects, and wild birds and mammals will reduce the potential for the introduction of this organism into the flock and transmitting it among existing flocks or between an old flock to a new flock after cleaning and disinfection (Greenberg et al., 1970; Harein et al., 1970; Henzler and Opitz, 1992; Davies and Wray, 1995b,c; Gray et al., 1999; Olsen and Hammack, 2000; Mian et al., 2002; Wales et al., 2009). Different housing systems influence the relative effectiveness of biosecurity measures and the on-farm levels of potential *Salmonella* vectors and thus affect the success of remediation and prevention measures. This may be especially germane for free-range housing, in which the hens spend a portion of their time outdoors, which increases their interactions with wildlife. In addition, the soil environment contaminated by infected free-range flocks will be difficult to disinfect and could serve as a persistent source of *Salmonella* for future birds raised in that facility. The potential land-water contamination coming from these outdoor establishments is also a significant concern and need to be addressed. On the other hand, many of the conventional cage systems are older structures, >20 yr old (USDA/APHIS, 2000b), with the concomitant buildup of manure and dust. These facilities are difficult to clean and disinfect because of the deep manure pits and the complexity of the cage system (stacked cages, drinkers, and manure belts) and this potentially results in carryover of salmonellae from flock to flock (Carrique-Mas et al., 2009). Both cage and noncage systems have their own biosecurity issues that need to be addressed before the superiority of one system over another can be determined.

Another important *Salmonella* prevention tool is vaccination. Long an important management tool to prevent or minimize the severity of poultry infectious diseases, vaccination has also been used in the fight against *Salmonella* Enteritidis and the other important *Salmonella* serovars that infect layer flocks. Used in conjunction with good hygiene and farming practices, vaccination is considered an important measure for

reducing on-farm *Salmonella* problems (EFSA, 2007). There are currently 2 primary types of *Salmonella* vaccines used in poultry flocks, live and killed, and both types have their positive and negative aspects. The live vaccine can be mass-administered via water or aerosol and therefore provides an easy mechanism for immunizing flocks. However, it is a living organism; therefore, storage and viability become issues that can be problematic as is the potential for the attenuated vaccine strain to revert back to a more virulent organism. Further, because live *Salmonella* Enteritidis vaccines are not allowed in the United States, only live *Salmonella* Typhimurium vaccines are currently licensed for poultry use in this country. Producers must therefore rely on the protection provided by cross-reactive immunity (Hassan and Curtiss, 1997) if they vaccinate with a live preparation. The killed vaccine is administered via injection to individual birds and provides strong immunity and good protection for the recipients (Gast et al., 1992). However, because it is administered via injection, it is a more labor-intensive method that requires handling of each bird as well as an increased risk of accidental injection of the vaccinating crew. The efficacy of a flock vaccination program was dramatically demonstrated in the United Kingdom. In the late 1990s, the United Kingdom, under the British Egg Industry Council Lion Code of Practice, mandated layer flock *Salmonella* vaccination and this was considered largely responsible for the dramatic decrease in poultry salmonellosis and, ultimately, human salmonellosis in that country (Cogan and Humphrey, 2003). The original vaccination regimen used a killed *Salmonella* Enteritidis preparation, but the newer, easier to administer live attenuated *Salmonella* Enteritidis vaccines have become more prevalent (Cogan and Humphrey, 2003). The EU adopted regulations in 2006 that instituted mandatory vaccination in laying hen flocks with a *Salmonella* prevalence of 10% or more (Anonymous, 2006). Because no such mandatory flock vaccination program exists in the United States, only 14.6% of layers were vaccinated against *Salmonella* Enteritidis in 2000 (USDA/APHIS, 2000a). More recent official information on flock vaccination against *Salmonella* Enteritidis in this country is not available, but discussions with layer industry and vaccine company representatives (P. Holt, personal communication) indicate that current *Salmonella* vaccine use in US flocks is substantially higher. A move to alternative housing systems is not anticipated to have any impact on the effectiveness of live versus killed vaccines. However, the logistics involved in vaccinating floor-raised chickens make the use of preparations that can be mass-administered in the drinking water or via spray (i.e., live vaccines), more appealing candidates than capturing and injecting individual birds. This has been observed in the United Kingdom and Europe, where live vaccines have become the predominant method for immunizing laying hens against *Salmonella* Enteritidis (Davies, personal communication) and it is anticipated that similar events

will occur in the United States as more producers move to noncage systems.

Many factors affect the prevalence of *Salmonella* Enteritidis within a flock, the severity of the infection, and the subsequent deposition of salmonellae into or on the egg. How the birds are housed will certainly exert an impact, although the superiority of one housing method over another remains to be determined. The recovery of *Salmonella* from egg production premises often shows seasonal trends, usually increasing in the summer months and early fall (Wales et al. 2007). Why this occurs is speculative but may be a result of heat stress, which has been shown to suppress immunity in chickens (Regnier and Kelley, 1981) and may therefore potentially exacerbate a flock *Salmonella* problem. Further, flies, known carriers of *Salmonella* and other intestinal pathogens (Greenberg et al., 1970; Olsen and Hammack, 2000), grow to high levels during this time (Olsen and Hammack, 2000) and increase the likelihood of transmitting *Salmonella* around a house. Housing systems may exhibit different strengths and weaknesses under various seasonal and climactic situations with regard to *Salmonella* contamination. As a consequence, sampling regimens need to be developed to ensure equal representation of all housing types under different seasonal situations.

Chemical Contamination. Although the white paper “Environmental impacts and sustainability of egg production systems” documents the influence housing systems can have on hen welfare and the environment due to chemical emissions of ammonia, greenhouse gases, and nutrients such as nitrogen and phosphorus, this paper investigates the role housing systems can have on the level of certain chemical contaminants in the eggs. The chemicals relevant to food safety in this white paper include environmental exposures to 1) persistent organic pollutants such as dioxins or polychlorinated biphenyls, 2) pesticides, and 3) heavy metals. The persistent organic pollutants, certain historic pesticides, and heavy metals present a food safety concern because these chemicals bioaccumulate through the food chain and are not readily cleared from the body. Therefore chronic exposures can lead to body burdens that may result in adverse health effects, and these exposures need to be minimized. Although registered pesticides should not pose a food safety risk when proper withdrawal times are applied, any increased use of pesticides due to different housing systems could increase the chance of violative residues in eggs.

Independent of the housing systems, the exposure to these classes of chemicals from water or commercial feeds is expected to be uniform across all large production settings. However, exposure to several of these chemicals could potentially be greater in free-range systems than in other systems because free-range hens come in direct contact with the outdoor environment and ingest soil or organisms in the soil. Contamination is also possible when hens in indoor cage-free housing come in contact with litter or barn posts and walls. In

addition, free-range or litter-raised hens may be given additional veterinary drugs or chemicals to control diseases or parasitic infestations because of their exposure to wild birds or parasites, substances that could contaminate the eggs. The white paper “A comparison of hen welfare in relation to multiple housing systems” reviews several studies that have shown increased bacterial infections and ectoparasite infestations in litter-based and free range systems compared with conventional cages.

The most widely reported chemical contaminations of eggs associated with free-range flocks are increased levels of dioxin-like compounds (Chang et al., 1989; Harnly et al., 2000). These include several classes of chemicals, namely, polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and polychlorinated biphenyls. These compounds are ubiquitous environmental contaminants and are extremely persistent in both the environment and biota.

In a California study, eggs from hens raised on soil contaminated from a nearby pentachlorophenol wood treatment plant had elevated levels of dioxins compared with eggs from conventional cage-reared hens. Eggs from the contaminated site had PCDD/PCDF (PCDD/F) levels up to 100 times higher than those from hens housed in cages and showed a chemical fingerprint similar to that of the soil (Chang et al., 1989). This study demonstrated that accumulation of dioxins readily occurred from soil into eggs and led to the issuance of a health advisory against egg or chicken consumption from this area. In a follow-up study, PCDD/F concentrations in eggs were shown to correlate with the extent of range use by hens, and even relatively low soil levels [0.38 pg of dioxin toxic equivalents (TEQ)/g of soil] could result in significant egg contamination (1 pg of dioxin TEQ/g if egg) when the hens foraged over a large area (Harnly et al., 2000). In Europe, food survey data show that free-range eggs have higher PCDD/F and polychlorinated biphenyl levels than conventional cage eggs (Schoeters and Hoogenboom, 2006). Almost 10% of free-range eggs exceeded the EU maximum residue limit (MRL) for PCDD/F in eggs (3 pg of dioxin TEQ/g of lipid); however, eggs from caged hens were well below this limit (95% percentile = 0.83 pg of dioxin TEQ/g of lipid). There have been reports of elevated dioxin-like compounds in eggs from free-range hens in numerous European countries, including eggs not just from hens in municipal-industrial areas but also those in rural areas where soil dioxin levels were considered low (Schoeters and Hoogenboom, 2006).

Dioxins are not the only potential chemical contaminant of eggs. Pesticides and heavy metals can also contaminate the environment, leading to elevated levels of these compounds in eggs from hens allowed to range on contaminated soil. In Brazil, free-range hens in an area historically treated with dichlorodiphenyltrichloroethane (DDT) for vector control had DDT levels twice the MRL recommended by the Food and Agriculture Organization of the United Nations even though DDT

had not reportedly been used in the preceding 9 yr (Vieira et al., 2001). The residue level was 1,000 times the level of DDT found in commercial eggs purchased at a local market. In free-range 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, presumably due to soil contamination (Van Overmeire et al., 2006). Given the bioaccumulative nature of DDT and heavy metals, these elevated chronic exposures cause a concern that increasing body burdens of these chemicals may result in adverse health effects.

As mentioned above, the use of contaminated litter and building materials can also introduce chemical residues to eggs produced in indoor noncage systems. In one instance, pentachlorophenol-treated wood shavings were used as litter, and eggs from hens raised on the litter had PCDD/F levels almost 30 times the EU MRL (Diletti et al., 2005). Presumably, hens ingested contaminated wood through pecking, which led to the transfer of PCDD/F into the eggs and resulted in elevated concentrations.

Although there are other potential chemical sources of egg contamination, there are few data comparing the use of chemicals to treat diseases, endoparasites, ectoparasites, or nuisance insects under various housing conditions and the resulting egg residue levels. In one study, egg residues of propoxur (2-isopropoxyphenyl-*N*-methyl carbamate), a chemical used to control poultry red mites, were measured after identical applications of the insecticide in different housing facilities (Hamscher et al., 2003). Eggs from aviaries had the lowest mean concentration of propoxur, whereas eggs from conventional cage units had significantly higher levels. The EU MRL were exceeded in 6% of the conventional cage eggs. Eggs from furnished cages were intermediate between the 2 other housing types. The mobility of the hens in the aviaries and furnished cages appeared to decrease exposure to the insecticide as it was being applied. Nuisance insects, especially houseflies, increase dramatically during the summer months (Olsen and Hammack, 2000) and they are particularly evident in conventional cage houses utilizing a deep pit manure management system. To counter the fly problem, the houses must be sprayed or fogged with higher amounts of insecticide, increasing the risk for egg contamination by these agents (Davies, personal communication).

Raising hens in alternative housing does not inherently lead to higher chemical residues in eggs. In a Canadian study, free-range eggs had lower or similar concentrations of dioxin-like compounds than conventional eggs (Rawn et al., 2008). Flock size appears to influence the level of environmental contaminants ingested by hens in free-range systems. In a study of organic farms, Kijlstra et al. (2007) found that flocks of more than 1,500 birds had significantly lower dioxin levels in their eggs compared with smaller flocks. The most likely reason was the limited time spent in the outdoors by the larger flocks. Likewise, although pesticides may be

needed more in litter-based or free-range systems due to increased parasite infestation (see "A comparison of hen welfare in relation to multiple housing systems" white paper), an increase in violative pesticide residues in the eggs is not a given, and the mobility of hens in these systems may even decrease exposure to the pesticide. Many factors related to the hens' mobility and access to extraneous materials or the outdoors, therefore, interact to increase or reduce the problem of chemical residues in eggs, and an understanding of what is germane to the situation will allow for the identification of housing situations that do not compromise food safety by resulting in increased chemical residue levels in eggs. Besides the safety of the food, quality is also an important parameter, which should be addressed when examining the impact of different housing systems.

Exterior Quality. Exterior quality assessments are based on the following characteristics: egg size and shell integrity-shell strength, dynamic stiffness, elasticity, and color.

Egg Size. In the United States, eggs are marketed based on consumer weight classes (USDA, 2000). The use of consumer weight classes ensures a continuity of egg size within an egg carton and guarantees that consumers are receiving a homogenous size distribution. The US market for shell eggs consists of 3 primary weight classes (weight/dozen): medium [21 oz (595 g)], large [24 oz (680 g)], and extra large [27 oz (765 g)]. It is general knowledge that genetic selection is practiced in anticipation of consumer purchasing preferences, including egg size. Furthermore, it is generally known that production practices and physiological stress can directly impact egg size (Cunningham et al., 1960; Gardner and Young, 1972; Summers and Leeson, 1983; Morris, 1985; Keshavarz and Nakajima, 1995); therefore, it is often difficult to determine if housing changes or other factors are affecting egg size or quality.

There is a large degree of variability in the research findings on the effects of various housing systems on egg size. The studies were rarely conducted with the same strains of laying hens, which adds to the variation between studies because there are genetic differences in egg size. Also, most of the published research has been conducted in small research flocks and housing systems, which may not directly correlate with the outcomes in a commercial production setting. When furnished cages were compared with conventional cages within a single flock, no differences were seen for egg weight (Guesdon and Faure, 2004). Several studies have compared conventional cages and various aviary systems. Tanaka and Hurnik (1992) compared conventional cages and aviary production between 27 and 63 wk of hen age and found no differences in egg size between the systems. The initial research of Abrahamsson and Tauson (1995) included 2 experiments with no differences in egg weight between conventional cages and aviary production. A subsequent study conducted by Abrahamsson et al. (1996) found eggs from the conventional cages to have significantly greater egg weight compared with

the 2 aviary systems used. Eggs from free-range production systems have been shown to weigh more on average than those from battery and conventional cages, respectively (Hughes et al., 1985; Hidalgo et al., 2008). Hughes et al. (1985) suspected that the differences in egg weight could be due to differences in environmental temperature between the free-range and caged egg production systems. Free-range eggs have also been found to be broader at the equator compared with conventional cage counterparts (Van Den Brand et al., 2004).

Shell Integrity. As mentioned earlier, USDA regulations allow only intact shell eggs to be marketed to consumers, whereas eggs that are cracked but not voiding their contents (checks) may be further processed to pasteurized or dried egg products (USDA, 2005a). When the shell membranes have been disrupted and the egg contents are being voided (leaker), the egg is considered inedible under US law (USDA, 2005a) and has to be destroyed. The previously mentioned regulations governing the use of downgraded eggs are based on the increased risk of microbial contamination of the egg contents. Downgrades in shell integrity also reduce profits.

Research has produced varied results concerning the incidence of egg cracking in the various production systems. Hens in conventional cages produced significantly fewer cracked eggs than those in get-away cages (as described by the authors) in one study (Abrahamsson et al., 1995). Guesdon and Faure (2004) found a greater percentage of cracked eggs in furnished versus conventional cages. However, if only eggs laid in nests were considered from the furnished cages, there were no differences in cracked eggs between the 2 systems. The use of egg saver wires and long nest curtains reduced the incidence of egg cracks in furnished cages (Wall and Tauson, 2002). Guesdon and Faure (2004) also suggested that there would be fewer cracked eggs if furnished cage nest boxes were better designed.

Findings from a series of studies comparing the percentage of cracked eggs produced in conventional cages versus 2 aviary systems yielded different results. In the first stage of a 2-stage experiment, Abrahamsson and Tauson (1995) found the percentage of cracked eggs to be greatest in the 2-tier aviary, followed by the 3-tier, and finally caged system. Results from the second stage, however, indicated that the 2-tier aviary system was associated with the lowest percentage of cracked eggs. In a follow-up study, Abrahamsson et al. (1996) once again found the lowest percentage of cracked eggs in the 2-tier aviary system, whereas the 3-tier aviary and conventional cages yielded similar results. Two strains of laying hens were used for this study, but no strain effects were observed (Abrahamsson et al., 1996). When a more comprehensive study of laying characteristics was conducted over 5 separate laying cycles in a single 3-tier aviary system, the percentage of cracked eggs was highly variable (Abrahamsson and Tauson, 1998).

Other studies have also compared the incidence of egg cracking in different production systems. A greater

percentage of cracked eggs was seen in conventional cages versus free-range (Hughes et al., 1985). Another study comparing aviaries, conventional cages, and floor pens found greater percentages of cracked eggs in the aviaries and conventional cages than in the floor pens (Tauson et al., 1999). Mertens et al. (2006) detected the highest percentage of cracked eggs at the point of lay in conventional and furnished cages, with lower levels in aviary and free-range production. However, Abrahamsson and Tauson (1998) and Tauson (2002) suggested that production and quality data from aviary systems may not be accurate because cracked or broken eggs could be consumed by the hens, thus preventing the inferior eggs from being counted. This supposition could also be carried over to free-range systems.

Shell Cleanliness. There are various laws in the United States that require retail shell eggs to be washed. Guidelines also exist that describe allowable tolerances for shell staining and coloration after washing (USDA, 2005b). Excessively dirty eggs entering the shell egg processing facility are more difficult, and at times practically impossible, to clean. The primary debris associated with shell eggs consists of dust, dirt, feces, feed, and egg contents. This debris can alter wash water effectiveness.

As with other external quality attributes, the research findings about egg dirtiness are often contradictory. Hens in conventional cages have been found to produce a greater percentage of dirty eggs than those in furnished cages (Abrahamsson et al., 1995). Conversely, Guesdon and Faure (2004) reported that there are more dirty eggs in furnished cages than in conventional cages. However, nest box design has an effect on the percentage of dirty eggs recovered from the nest box (Abrahamsson and Tauson, 1998). Guesdon and Faure (2004) stated that there would be fewer dirty eggs in furnished cages if nest boxes were better designed. For instance, Wall and Tauson (2002) determined that in furnished cages, decreased nest box covering (30% total covering) resulted in decreased nest box usage and increased the percentage of dirty eggs. Thus, producers using a furnished cage system could improve the external quality of their eggs by simply providing additional cover on their nest boxes to encourage nest box use.

When comparing conventional cages and aviary production, hens in aviaries produced significantly more dirty eggs (Abrahamsson and Tauson, 1995; Abrahamsson et al., 1996). When 5 production cycles were evaluated in an aviary system, most of the eggs laid outside of the nest were dirty (Abrahamsson and Tauson, 1998). In a comparison of several production systems, aviaries were found to produce the most dirty eggs, whereas traditional floor pens (as described by the authors) produced the lowest percentage, with conventional and furnished cages falling in the middle (Tauson et al., 1999).

Shell Quality. Shell strength, dynamic stiffness, deformation, and thickness are all recognized as parameters for assessing shell quality. The USDA guidelines

describe acceptable and unacceptable shell quality attributes and the corresponding allowable grade (USDA, 2005b). As a shell egg is downgraded in quality, the profitability of the egg is reduced. The research findings on the effects of housing systems on shell quality are mixed and do not provide a clear indication of which production system maintains eggs with the best shell quality.

When compared with eggs from conventional cages, aviary eggs have been classified as having greater shell thickness, lower shell deformation, greater shell weight, and a greater percentage of shell (Abrahamsson et al., 1996). Consequently, Hidalgo et al. (2008) reported the greatest percentage of shell and shell strength in conventional cage eggs versus all other housing systems monitored (free-range, barn, and organic). When Tanaka and Hurnik (1992) compared conventional cages and aviary production, they detected no differences in shell deformation between 27- to 63-wk-old hens. When 5 aviary production cycles were compared, there were no clear trends in shell quality (Abrahamsson and Tauson, 1998). Guesdon and Faure (2004) saw no differences in shell breaking strength between furnished and conventional cages. Free-range eggs had greater shell thickness and stronger shells compared with conventional cages (Hughes et al., 1985). The authors note that although a significant difference in shell strength was detected, numerically, the difference in shell strength was very small. In a comparison study of conventional cage and free-range production, shell quality decreased in the caged hens with hen age but remained constant or increased in the free-range eggs (Van Den Brand et al., 2004). Mertens et al. (2006) examined the effects of multiple housing systems (conventional cages, furnished cages, aviary, and free-range) on egg quality and reported shell strength to be greatest in aviary eggs and weakest in free-range eggs.

Interior Quality

There are federal quality standards in place for all eggs marketed with the USDA grade shield (USDA, 2005b). The guidelines list acceptable levels for air cell size, blood spots, meat spots, and other quality defects within certain grade standards. Furthermore, interior quality can be assessed based on the following: yolk integrity: vitelline membrane strength, yolk index, color, and viscosity; albumen integrity: Haugh unit [the Haugh unit is a measure of internal egg quality, which is considered the gold standard for egg quality assessment (Haugh, 1937)], albumen height, and viscosity; and functional quality: whipping, emulsification, and foam stability. The US egg quality standards exist to ensure that consistently high-quality eggs reach the consumer. It is generally accepted that egg production practices (such as lighting, nutrition, and environmental temperature) can affect egg quality. The greatest portion of this work was conducted with conventionally caged laying hens, however, and research exploring the

effect of alternative laying hen housing systems on egg quality is limited.

When conventional cage and aviary production were compared, no differences were detected in interior egg quality (Abrahamsson and Tauson, 1995), nor did clear trends emerge for internal egg quality across 5 aviary production cycles (Abrahamsson and Tauson, 1998). More than one strain of hen was used in the multiple cycle aviary study, but there were no consistent strain differences, although replication of strains was limited. A greater incidence of meat spots has been found in aviary versus conventional cage eggs (Abrahamsson et al., 1996). Further research (which did not include aviary production) found the lowest incidence of meat spots in free-range eggs when compared with conventional cages, cage-free, and organic production (Hidalgo et al., 2008). Additional research comparing flocks across all production methods is needed before a complete understanding of production method and interior egg quality can be attained.

Van Den Brand et al. (2004) compared egg quality in hens housed in individual cages and hens housed on range with males. Lay began 3 wk earlier in the caged versus free-range hens. Yolk color was darker in the free-range eggs. In general, the authors found inconsistencies in external and internal quality (as monitored by egg physical and compositional quality) in the free-range eggs. Furthermore, increased variation in free-range egg quality was observed as the hen age increased. Based on these results, the authors determined that more work was needed to gain an understanding of what parameters play important roles in affecting the variability of egg quality from free-range eggs.

Very little research has compared the effects of alternative housing systems on egg quality and functionality. Hidalgo et al. (2008) have produced the most comprehensive report thus far on egg functionality and hen housing systems. In their work, caged, cage-free, organic, and free-range systems were compared. Organic eggs had the greatest whipping capacity and foam consistency along with the lowest Haugh unit scores (indicating poorer egg quality). Cage-produced eggs had the lowest whipping capacity, indicating that they were fresher than other eggs. Hidalgo et al. (2008) attempted to develop a multivariate technique discriminate partial least squares regression to classify the eggs from the different production systems. Successful, consistent discrimination could only distinguish cage from noncage-produced eggs. The most powerful discriminates were found to be shell breaking strength, whipping overrun, protein content, and shell thickness. Although the method could not discriminate each of the production methods, it is encouraging that it was able to continually distinguish cage- from cage-free-produced eggs.

Nutritional Quality

Nutritional quality can be assessed based on compositional quality, which includes total solids, ash, crude

fat, and protein. Commercial eggs are a significant source of fatty acids, cholesterol, and other lipid nutrients in the human diet. As a result of studies extolling the health benefit claims of consuming products high in n-3 fatty acids (Sindelar et al., 2004; Gillingham et al., 2005), a significant amount of effort has been put into enriching eggs with n-3 fatty acids using feed supplementation. Sources rich in n-3 such as fish oil, flaxseed (linseed), canola, and soybean oil have been used to augment layer feeds (Botsoglou et al., 1998; Galobart et al., 2002; Milinsk et al., 2003; Kivini et al., 2004; Millet et al., 2006; Parpinello et al., 2006). A combination of microalgae and rapeseed oil supplementation results in eggs enriched in n-3 and carotenoids (Fredriksson et al., 2006).

An animal's diet has a direct impact on the lipid compounds found in that animal's products. Compared with grain-fed animals, free-range individuals have distinctly different fatty acid profiles and different levels of carotenoids and vitamin E in their meat (Yang et al., 2002; Karadas et al., 2005; Braden et al., 2006; Daza et al., 2007; Fredriksson and Pickova, 2007). The meat from free-range animals is leaner and contains a higher proportion of polyunsaturated fatty acids. Most importantly, the meat has a greater n-3:n-6 fatty acid ratio and one that is in accordance with current nutritional guidelines (Realini et al., 2004, Braden et al., 2006). Although there have been numerous investigations on supplementation of layers' diets to enrich eggs with n-3, vitamins A and E, and other lipid-soluble nutrients, while lowering saturated fats and cholesterol (Galobart et al., 2002; Mendonca et al., 2002; Milinsk et al., 2003; Fredriksson et al., 2006; Bölükbaşı et al., 2007), there has been little work investigating the effects on these nutrients of free-range production systems in which the hens are allowed to forage on pasture.

A recent non-peer-reviewed report based on the findings from 14 independent farms stated that eggs from free-range hens had approximately 4 times the amount of vitamin E, twice as much vitamin A, 8 times as much β -carotene, 3 times as much n-3, and 2/3 the amount of cholesterol compared with conventional cage eggs. (<http://www.motherearthnews.com/eggs.aspx>). Based on a limited number of peer-reviewed publications, it appears that eggs from free-range hens have higher α -tocopherol and α -linolenic acid content when compared with eggs from hens fed a commercial mixed diet (Lopez-Bote et al., 1998). Furthermore, Karadas et al. (2005) showed that free-range hens have higher carotenoid levels in their eggs compared with intensively housed hens. Hidalgo et al. (2008), however, saw no differences in fatty acid composition among conventional cage, free-range, floor, or organic labeled eggs, whereas Cherian et al. (2002) observed similar levels of n-6 and n-3 in white eggs produced in conventional cages compared with certified organic free-range brown eggs. In both of these studies, there was no information on the feeds for the hens and thus no way to determine whether the cage eggs were from hens receiving feeds

supplemented with these fatty acids. Therefore, the scientific literature in some instances agrees with the results of the independent claims cited previously but not in all cases. It is clear that more research needs to be conducted. Further, grasses and forage will vary in different regions of the country and thus the potential changes in yolk n-3 levels due to free-range foraging may also differ.

The European Food Safety Authority has asserted that for foods to be labeled as a source of n-3 fatty acids "the food must contain more than 15% of the Recommended Nutritional Intake (with RNI 2g/day for an adult male) of the omega-3 fatty acids concerned per 100 g or 100 mL or 100 kcal" (EFSA, 2005). This corresponds to 300 mg/100 g of egg weight. It has been shown that to establish this level of n-3 in eggs, hen diets must consist of at least 6 g of total n-3 per kilogram of feed (Garcia-Rebollar et al., 2008). Further, commercial n-3-enriched eggs typically have a minimum eicosapentaenoic acid + docosahexaenoic acid content of around 200 mg/100 g of egg weight (Garcia-Rebollar et al., 2008). Whether these levels can be achieved using free-range production systems remains to be determined.

Furthermore, information dealing with the impacts of different housing systems on the nutritional quality of eggs is minimal worldwide and virtually nonexistent in the United States. Although claims made in the popular press extol the virtue of one housing system over another to enhance egg vitamin and n-3 fatty acid content, few controlled studies have been undertaken to justify such assertions. This underscores the need for a concerted research effort to compare the nutritional composition of eggs from hens raised under different housing conditions and to evaluate the interaction of such parameters as hen breed, flock age, and season. This will enable producers to determine more effectively the housing type that enhances both the productivity and the egg quality of the flocks under their care.

CONCLUSIONS

The current white paper attempted to provide insight into how changing the US egg industry from one that houses its hens in conventional laying cages to furnished cages, aviaries, or a cage-free system affects the safety and quality of eggs produced in these different environments. There is no general consensus demonstrating the superiority of one housing situation over another regarding food safety and egg quality. Further, many variables interact to make decisions regarding the housing situation that much more difficult to attain. Factors such as climate, hen breed, disease status, rodent and insect load, and age of the facility, to name a few, all enter into the equation to enhance the complexity of the situation. Much of the most recent information on this topic result from studies conducted in the EU and this information must then be applied to conditions found in the US industry. Although many similarities do ex-

ist, the EU and US egg industries differ sufficiently to make such extrapolation difficult and new studies geared more to egg production in the United States are warranted. Ultimate US housing decisions need to be based on sound scientific data and this information currently does not exist. It is incumbent upon the US egg industry, allied industries, and government regulatory agencies to provide the means necessary to ensure the expeditious performance of the needed studies.

REFERENCES

- Abrahamsson, P., and R. Tauson. 1995. Aviary systems and conventional cages for laying hens. *Acta Agric. Scand. Anim. Sci.* 45:191–203.
- Abrahamsson, P., and R. Tauson. 1998. Performance and egg quality of laying hens in an aviary system. *J. Appl. Poult. Res.* 7:225–232.
- Abrahamsson, P., R. Tauson, and M. C. Appleby. 1995. Performance of four hybrids of laying hens in modified and conventional cages. *Acta Agric. Scand. Anim. Sci.* 45:286–296.
- Abrahamsson, P., R. Tauson, and K. Elwinger. 1996. Effects on production, health and egg quality of varying proportions of wheat and barley in diets for two hybrids of laying hens kept in different housing systems. *Acta Agric. Scand. Anim. Sci.* 46:173–182.
- Anonymous. 2006. Reducing *Salmonella*: Commission sets EU targets for laying hens and adopts new control rules. <http://europa.eu/rapid/pressReleasesAction.do?reference=IP/06/1082&format=HTML&aged=1&language=EN&guiLanguage=en> Accessed February 20, 2009.
- Anonymous. 2008. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2007. *Morb. Mortal. Wkly. Rep.* 57:366–370.
- Arakawa, A., T. Fukaton, E. Baba, L. R. McDougald, J. S. Bailey, and L. C. Blankenship. 1992. Influence of coccidiosis on colonization in broiler chickens under floor pen conditions. *Poult. Sci.* 71:59–63.
- Bölikbasi, S. C., M. K. Erhan, M. S. Keles, and R. Koçyigit. 2007. Effect of dietary vitamin E on the performance, plasma and egg yolk vitamin E levels and lipid oxidation of egg in heat stressed layers. *J. Appl. Biol. Sci.* 3:19–23.
- Botsoglou, N. A., A. L. Yannakopoulos, D. J. Fletouris, A. S. Tserveni-Goussi, and I. E. Psomas. 1998. Yolk fatty acid composition and cholesterol content in response to level and form of dietary flaxseed. *J. Agric. Food Chem.* 46:4652–4656.
- Braden, C. R. 2006. *Salmonella enterica* serotype Enteritidis and eggs: A national epidemic in the United States. *Clin. Infect. Dis.* 43:512–517.
- Braden, K., K. S. Willian, S. Roberts, J. Gentry, and C. Kerth. 2006. Finishing steers on winter annual ryegrass (*Lolium multiflorum* Lam.) with varied levels of supplementation: Effects on fatty acid profile, shelf life and lipid stability. *J. Anim. Sci.* 84(Suppl. 2):6–7.
- Campo, J. L., M. T. Prieto, and S. G. Davila. 2008. Effects of housing system and cold stress on heterophil-to-lymphocyte ratio, fluctuating asymmetry, and tonic immobility duration of chickens. *Poult. Sci.* 87:621–626.
- Carramiñana, J. J., F. Humbert, G. Ermel, and P. Colin. 1997. Molecular epidemiological investigation of *Salmonella* Typhimurium strains related to an egg-borne outbreak. *Res. Microbiol.* 148:633–636.
- Carrique-Mas, J. J., M. Breslin, L. Snow, I. McLaren, A. R. Sayers, and R. H. Davies. 2009. Persistence and clearance of different *Salmonella* serovars in building housing laying hens. *Epidemiol. Infect.* 137:837–846.
- Chang, R., D. Hayward, L. Goldman, M. Harnly, J. Flattery, and R. Stephens. 1989. Foraging animals as biomonitors for dioxin contamination. *Chemosphere* 19:481–486.
- Cherian, G., T. B. Holsonbake, and M. P. Goeger. 2002. Fatty acid content and egg components of specialty eggs. *Poult. Sci.* 81:30–33.
- Cogan, T. A., and T. J. Humphrey. 2003. The rise and fall of *Salmonella* Enteritidis in the UK. *J. Appl. Microbiol.* 94(Suppl.):114S–119S.
- Cunningham, F. E., O. J. Cotterill, and E. M. Funk. 1960. The effect of season and age of bird 1. On egg size, quality and yield. *Poult. Sci.* 39:289–299.
- Davies, R. H., and M. Breslin. 2004. Observations on *Salmonella* contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella enterica* serovar Enteritidis had been used. *Avian Pathol.* 33:133–144.
- Davies, R. H., and C. Wray. 1995a. Observations on disinfection regimens used on *Salmonella enteritidis* infected poultry units. *Poult. Sci.* 74:638–647.
- Davies, R. H., and C. Wray. 1995b. Mice as carriers of *Salmonella enteritidis* on persistently infected poultry units. *Vet. Rec.* 137:337–341.
- Davies, R. H., and C. Wray. 1995c. Contribution of the lesser mealworm (*Alphitobius diaperinus*) to carriage of *Salmonella enteritidis* in poultry. *Vet. Rec.* 137:407–408.
- Daza, A., A. Mateos, A. I. Rey, I. Ovejero, and C. J. Lopez-Bote. 2007. Effect of duration of feeding under free-range conditions on production results and carcass and fat quality in Iberian pigs. *Meat Sci.* 76:411–416.
- De Reu, K., K. Grijspeerdt, M. Heyndrickx, J. Zoons, K. De Baere, M. Uyttendaele, J. Debevere, and L. Herman. 2005. Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. *Br. Poult. Sci.* 46:149–155.
- De Reu, K., W. Messens, M. Heyndrickx, T. B. Rodenburg, M. Uyttendaele, and L. Herman. 2008. Bacterial contamination of table eggs and the influence of housing systems. *World's Poult. Sci. J.* 64:5–19.
- Diletti, G., R. Ceci, M. R. De Massis, G. Scortichini, and G. Migliorati. 2005. A case of eggs contamination by PCDD/Fs in Italy: Analytical levels and contamination source identification. *Organohologen Compd.* 67:1460–1461.
- Ebel, E., and W. Schlosser. 2000. Estimating the annual fraction of eggs contaminated with *Salmonella enteritidis* in the United States. *Int. J. Food Microbiol.* 61:51–62.
- EFSA. 2004. Opinion of the panel on biological hazards on the requests from the commission related to the use of vaccines for the control of *Salmonella* in poultry. (Request No. EFSA-Q-2003-080). *EFSA J.* 114:1–74.
- EFSA. 2005. Opinion of the scientific panel on dietetic products, nutrition and allergies on a request from the commission related to nutrition claims concerning omega-3 fatty acids, monounsaturated fat, polyunsaturated fat and unsaturated fat (Request No. EFSA-Q-2004-107). *EFSA J.* 253:1–29.
- EFSA. 2006. Preliminary report. Analysis of the baseline study on the prevalence of *Salmonella* in laying hen flocks of *Gallus gallus*. *EFSA J.* 81:1–71.
- EFSA. 2007. Report of the task force on zoonoses data collection on the analysis of the baseline study on the prevalence of *Salmonella* in holdings of laying flocks of *Gallus gallus*. *EFSA J.* 97:1–84.
- Food Standards Agency. 2004. *Salmonella* contamination of UK produced shell eggs on retail sale. <http://www.food.gov.uk/science/surveillance/fsis2004branch/fsis5004eggs> Accessed January 22, 2009.
- Fossum, O., D. S. Jansson, P. E. Etterlin, and I. Vagsholm. 2009. Causes of mortality in laying hens in different housing systems in 2001–2004. *Acta Vet. Scand.* 51:1–9.
- Fredriksson, S., K. Elwinger, and J. Pickova. 2006. Fatty acid and carotenoid composition of egg yolk as an effect of microalgae addition to feed formula for laying hens. *Food Chem.* 99:530–537.
- Fredriksson, S., and J. Pickova. 2007. Fatty acids and tocopherol levels in M. Longissimus dorsi of beef cattle in Sweden—A comparison between seasonal diets. *Meat Sci.* 76:746–754.
- Galobart, J., A. Barroeta, L. Cortinas, M. Baucells, and R. Codony. 2002. Accumulation of α -tocopherol in eggs enriched with omega-3 and omega-6 polyunsaturated fatty acids. *Poult. Sci.* 81:1873–1876.
- Garcia-Rebollar, P., P. Cachaldora, C. Alvarez, C. De Blas, and J. Mendez. 2008. Effect of the combined supplementation of diets with increasing levels of fish and linseed oils on yolk fat composi-

- tion and sensorial quality of eggs in laying hens. *Anim. Feed Sci. Technol.* 140:337–348.
- Gardner, F. A., and L. L. Young. 1972. The influence of dietary protein and energy levels on the protein and lipid content of the hen's egg. *Poult. Sci.* 51:994–997.
- Gast, R. K., H. D. Stone, P. S. Holt, and C. W. Beard. 1992. Evaluation of the efficacy of an oil-emulsion bacterin for protecting chickens against *Salmonella enteritidis*. *Avian Dis.* 36:992–999.
- Gillingham, L. G., L. Caston, S. Leeson, K. Hourtovenko, and B. J. Holub. 2005. The effects of consuming docosahexaenoic acid (DHA)-enriched eggs on serum lipids and fatty acid compositions in stamione-ga-treated hypercholesterolemic male patients. *Food Res. Int.* 38:1117–1123.
- Gray, J. P., C. W. Maddox, P. C. Tobin, J. D. Gummo, and C. W. Pitts. 1999. Reservoir competence of *Carcinops pumilio* for *Salmonella enteritidis* (Eubacteriales: Enterobacteriaceae). *J. Med. Entomol.* 36:888–891.
- Greenberg, B., J. A. Kowalski, and M. J. Klowden. 1970. Factors affecting the transmission of salmonellae by flies: Natural resistance to colonization and bacterial interference. *Infect. Immun.* 2:800–809.
- Guesdon, V., and J. M. Faure. 2004. Laying performance and egg quality in hens kept in standard or furnished cages. *Anim. Res.* 53:45–57.
- Hamscher, G., B. Priess, J. Hartung, M. I. Nogossek, G. Glunder, and H. Nau. 2003. Determination of propoxur residues in eggs by liquid chromatography-diode array detection after treatment of stocked housing facilities for the poultry red mite (*Dermanyssus gallinae*). *Anal. Chim. Acta* 483:19–26.
- Harein, P. K., E. Delas Casas, B. S. Pomeroy, and M. D. York. 1970. *Salmonella* spp. and serotypes of *Escherichia coli* isolated from the lesser mealworm collected in poultry houses. *J. Econ. Entomol.* 63:80–82.
- Harnly, M. E., M. X. Petreas, J. Flattery, and L. R. Goldman. 2000. Polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzofuran contamination in soil and home-produced chicken eggs near pentachlorophenol sources. *Environ. Sci. Technol.* 34:1143–1149.
- Hassan, J. O., and R. Curtiss III. 1997. Efficacy of a live avirulent *Salmonella typhimurium* vaccine in preventing colonization and invasion of laying hens by *Salmonella typhimurium* and *Salmonella enteritidis*. *Avian Dis.* 41:783–791.
- Haugh, R. R. 1937. The Haugh unit for measuring egg quality. *US Egg Poult. Mag.* 43:552–555, 572–573.
- Hennessy, T. W., L. H. Cheng, H. Kassenborg, S. D. Ahuja, J. Mohle-Boetani, R. Marcus, B. Shiferaw, and F. J. Angulo. 2004. Egg consumption is the principal risk factor for sporadic *Salmonella* serotype Heidelberg infections: A case-control study in FoodNet sites. *Clin. Infect. Dis.* 38(Suppl. 3):S237–S243.
- Henzler, D. J., and H. M. Opitz. 1992. The role of mice in the epizootiology of *Salmonella enteritidis* infection on chicken layer farms. *Avian Dis.* 36:625–631.
- Hidalgo, A., M. Rossi, F. Clerici, and S. Ratti. 2008. A market study on the quality characteristics of eggs from different housing systems. *Food Chem.* 106:1031–1038.
- Holt, P. S. 2003. Molting and *Salmonella enterica* serovar Enteritidis infection: The problem and some solutions. *Poult. Sci.* 82:1008–1010.
- Hughes, B. O., P. Dun, and C. C. McCorquodale. 1985. Shell strength of eggs from medium-bodied hybrid hens housed in cages or on range in outside pens. *Br. Poult. Sci.* 26:129–136.
- Hughes, C. S., R. M. Gaskell, R. C. Jones, J. M. Bradbury, and F. T. W. Jordan. 1989. Effects of certain stress factors on the re-excretion of infectious laryngotracheitis virus from latently infected carrier birds. *Res. Vet. Sci.* 46:274–276.
- Humphrey, T. J., A. Baskerville, S. Mawer, B. Rowe, and S. Hopper. 1989. *Salmonella enteritidis* phage type 4 from the contents of intact eggs: A study involving naturally infected hens. *Epidemiol. Infect.* 103:415–423.
- Humphrey, T. J., A. Whitehead, A. H. L. Gawler, A. Henley, and B. Rowe. 1991. Numbers of *Salmonella enteritidis* in the contents of naturally contaminated hens' eggs. *Epidemiol. Infect.* 106:489–496.
- Jones, R. C., and A. G. Ambali. 1987. Re-excretion of an enterotropic infectious bronchitis virus by hens at point of lay after experimental infection at day old. *Vet. Rec.* 120:617–618.
- Karadas, F., N. A. R. Wood, P. F. Surai, and N. H. C. Sparks. 2005. Tissue-specific distribution of carotenoids and vitamin E in tissues of newly hatched chicks from various avian species. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 140:506–511.
- Keshavarz, K., and S. Nakajima. 1995. The effect of dietary manipulations of energy, protein, and fat during the growing and laying periods on early egg weight and egg components. *Poult. Sci.* 74:50–61.
- Kijlstra, A., W. A. Traag, and L. A. P. Hoogenboom. 2007. Effect of flock size on dioxin levels in eggs from chickens kept outside. *Poult. Sci.* 86:2042–2048.
- Kinde, H., D. H. Read, A. Ardans, R. E. Breitmeyer, D. Willoughby, H. E. Little, D. Kerr, R. Gireesh, and K. V. Nagaraja. 1996a. Sewage effluent: Likely source of *Salmonella enteritidis*, phage type 4 infection in a commercial chicken layer flock in Southern California. *Avian Dis.* 40:672–676.
- Kinde, H., D. H. Read, R. P. Chin, A. A. Bickford, R. L. Walker, A. Ardans, R. E. Breitmeyer, D. Willoughby, H. E. Little, D. Kerr, and I. A. Gardner. 1996b. *Salmonella enteritidis*, phage type 4 infection in a commercial layer flock in Southern California: Bacteriological and epidemiologic findings. *Avian Dis.* 40:665–671.
- Kivini, H., E. P. Jarvenpaa, H. Aro, R. Huopalahti, and E. L. Ryhanen. 2004. Qualitative and quantitative liquid chromatographic analysis methods for the determination of the effects of feed supplements on hen egg yolk phospholipids. *J. Agric. Food Chem.* 52:4289–4295.
- Lopez-Bote, C. J., R. Sanz Arias, A. I. Rey, A. Castano, B. Isabel, and J. Thos. 1998. Effect of free-range feeding on omega-3 fatty acid and α -tocopherol content and oxidative stability of eggs. *Anim. Feed Sci. Technol.* 72:33–40.
- Mahé, A., S. Bougeard, A. Huneau-Salaün, S. Le Bouquin, I. Pétetin, S. Rouxel, F. Lalonde, P. A. Beloeil, and N. Rose. 2008. Bayesian estimation of flock-level sensitivity of detection of *Salmonella* spp., Enteritidis and Typhimurium according to the sampling procedure in French laying-hen houses. *Prev. Vet. Med.* 84:11–26.
- Mendonca, C. X., C. R. M. Almeida, A. V. Mori, and V. Watanabe. 2002. Effect of dietary vitamin A on egg yolk retinol and tocopherol levels. *J. Appl. Poult. Res.* 11:373–378.
- Mertens, K., F. Bamelis, B. Kemps, B. Kamers, E. Verhoelst, B. De Ketelaere, M. Bain, E. Decuyper, and J. De Baerdemaeker. 2006. Monitoring of eggshell breakage and eggshell strength in different production chains of consumption eggs. *Poult. Sci.* 85:1670–1677.
- Messens, W., K. Grijspeerdt, and L. Herman. 2005. Eggshell penetration by *Salmonella*: A review. *World's Poult. Sci. J.* 61:71–85.
- Methner, U., R. Diller, R. Reiche, and K. Bohland. 2006. Occurrence of salmonellae in laying hens in different housing systems and conclusion for the control. *Berl. Muench. Tieraerztl. Wochenschr.* 119:467–473.
- Mian, L. S., H. Maag, and J. V. Tacal. 2002. Isolation of *Salmonella* from muscoid flies at commercial animal establishments in San Bernardino County, California. *J. Vector Ecol.* 27:82–85.
- Milinsk, M. C., A. E. Murakami, S. T. M. Gomes, M. Matsushita, and N. E. de Souza. 2003. Fatty acid profile of egg yolk lipids from hens fed diets rich in omega-3 fatty acids. *Food Chem.* 83:287–292.
- Millet, S., K. De Ceulaer, M. Van Paemel, K. Raes, S. De Smet, and G. P. Janssens. 2006. Lipid profile in eggs of Araucana hens compared with Lohmann Selected Leghorn and ISA Brown hens given diets with different fat sources. *Br. Poult. Sci.* 47:294–300.
- Mølbak, K., and J. Neimann. 2002. Risk factors for sporadic infection with *Salmonella* Enteritidis, Denmark, 1997–1999. *Am. J. Epidemiol.* 156:654–661.
- Mollenhorst, H., C. J. van Woudenberg, E. G. M. Bokkers, and I. J. M. de Boer. 2005. Risk factors for *Salmonella enteritidis* infections in laying hens. *Poult. Sci.* 84:1308–1313.
- Morris, T. R. 1985. The manipulation of egg size and egg quality. *Afr. J. Anim. Sci.* 15:120–122.

- Motha, M. X. J., and J. R. Egerton. 1983. Effect of reticuloendotheliosis virus on the response of chickens to *Salmonella typhimurium* infection. *Res. Vet. Sci.* 34:188–192.
- Mutalib, A., P. McDonough, S. Shin, V. Patten, and D. Lein. 1992. *Salmonella enteritidis* in commercial layer farms in New York State; environmental survey results and significance of available monitoring tests. *J. Vet. Diagn. Invest.* 4:416–418.
- Namata, H., E. Méroc, M. Aerts, C. Faes, J. Cortiñas, H. Imberechts, and K. Mintiens. 2008. *Salmonella* in Belgian laying hens: An identification of risk factors. *Prev. Vet. Med.* 83:323–336.
- Okamura, M., Y. Kamijima, T. Miyamoto, H. Tani, K. Sasai, and E. Baba. 2001. Differences among six *Salmonella* serovars in abilities to colonize reproductive organs and to contaminate eggs in laying hens. *Avian Dis.* 45:61–69.
- Olsen, A. R., and T. S. Hammack. 2000. Isolation of *Salmonella* spp. from the housefly, *Musca domestica* L., and the dump fly, *Hydrotaea aenescens* (Wiedemann) (Diptera:Muscidae), at caged-layer houses. *J. Food Prot.* 63:958–960.
- Parpinello, G. P., A. Meluzzi, F. Sirri, N. Tallarico, and A. Versari. 2006. Sensory evaluation of egg products and eggs laid from hens fed diets with different fatty acid composition and supplemented with antioxidants. *Food Res. Int.* 39:47–52.
- Phillips, R. A., and H. M. Opitz. 1995. Pathogenicity and persistence of *Salmonella enteritidis* and egg contamination in normal and infectious bursal disease virus-infected Leghorn chicks. *Avian Dis.* 39:778–787.
- Poppe, C., R. P. Johnson, C. M. Forsber, and R. J. Irwin. 1992. *Salmonella enteritidis* and other *Salmonella* in laying hens and eggs from flocks with *Salmonella* in their environment. *Can. J. Vet. Res.* 56:226–232.
- Qin, Z. R., T. Fukata, and E. Baba. 1995. Effect of *Eimeria tenella* infection on *Salmonella enteritidis* infection in chickens. *Poult. Sci.* 74:1–7.
- Rawn, D. F. K., A. Sadler, S. Quade, W.-F. Sun, I. Kosarac, and J. J. Ryan. 2008. Persistent organic pollutants in Canadian egg yolk from four different hatchery types. *Organohalogen Compd.* 70:586–589.
- Realini, C. E., S. K. Duckett, G. W. Brito, M. Dalla Rizza, and D. De Mattos. 2004. Effect of pasture versus concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Sci.* 66:567–577.
- Regnier, J. A., and K. W. Kelley. 1981. Heat- and cold-stress suppresses in vivo and in vitro cellular immune responses of chickens. *Am. J. Vet. Res.* 42:294–299.
- Rigby, C. E., and J. R. Pettit. 1980. Changes in the *Salmonella* status of broiler chickens subjected to simulated shipping conditions. *Can. J. Comp. Med.* 44:374–381.
- Safehouse Project. 2010. Project short presentation: Summary. <http://www.safehouse-project.eu/index.php?rub=Summary> Accessed January 6, 2010.
- Schaar, U., E. F. Kaleta, and B. Baumbach. 1997. Comparative studies on the prevalence of *Salmonella enteritidis* and *Salmonella typhimurium* in laying chickens maintained in batteries or on floor using bacteriological isolation techniques and two commercially available ELISA kits for serological monitoring. *Tierarztl. Prax.* 25:451–459.
- Schoeters, G., and R. Hoogenboom. 2006. Contamination of free-range chicken eggs with dioxins and dioxin-like polychlorinated biphenyls. *Mol. Nutr. Food Res.* 50:908–914.
- Sindelar, C. A., S. B. Scheerger, S. L. Plugge, K. M. Eskridge, R. C. Wander, and N. M. Lewis. 2004. Serum lipids of physically active adults consuming omega-3 fatty acid-enriched eggs or conventional eggs. *Nutr. Res.* 24:731–739.
- Snow, L. C., R. H. Davies, K. H. Christiansen, J. J. Carrique-Mas, A. J. C. Cook, and S. J. Evans. 2009. Investigation of risk factors for *Salmonella* on commercial egg-laying farms in Great Britain 2004–2005. *Vet. Rec.* 163:649–654.
- St Louis, M. E., D. L. Morse, M. E. Potter, T. M. DeMelfi, J. J. Guzewich, R. V. Tauxe, and P. A. Blake. 1988. The emergence of grade A eggs as a major source of *Salmonella enteritidis* infections. *JAMA* 259:2103–2107.
- Summers, J. D., and S. Leeson. 1983. Factors influencing early egg size. *Poult. Sci.* 62:1155–1159.
- Tanaka, T., and J. F. Hurnik. 1992. Comparison of behavior and performance of laying hens housed in battery cages and an aviary. *Poult. Sci.* 71:235–243.
- Tauson, R. 2002. Furnished cages and aviaries: Production and health. *World's Poult. Sci. J.* 58:49–58.
- Tauson, R., A. Wahlström, and P. Abrahamsson. 1999. Effect of two floor housing systems and cages on health, production, and fear response in layers. *J. Appl. Poult. Res.* 8:152–159.
- Thaxton, P., R. D. Wyatt, and P. B. Hamilton. 1974. The effect of environmental temperature on paratyphoid infection the neonatal chicken. *Poult. Sci.* 53:88–94.
- USDA. 1971. Inspection of Eggs and Egg Products (Egg Products Inspection Act). 9 CFR Part 590. http://www.access.gpo.gov/nara/cfr/waisidx_08/9cfr590_08.html Accessed January 21, 2009.
- USDA. 2000. Standards, Grades and Weight Classes for Shell Eggs. USDA AMS 56. <http://www.ams.usda.gov/AMSV1.0/getfile?dDocName=STELDEV3004376> Accessed January 2, 2008.
- USDA. 2005a. Inspection of Eggs (Egg Products Inspection Act). 7 CFR Part 57. http://www.access.gpo.gov/nara/cfr/waisidx_08/7cfr57_08.html Accessed January 2, 2008.
- USDA. 2005b. Voluntary Grading of Shell Eggs. 7 CFR Part 56. http://www.access.gpo.gov/nara/cfr/waisidx_08/7cfr56_08.html Accessed January 2, 2008.
- USDA/APHIS. 2000a. NAHMS Layers '99 *Salmonella enterica* serotype Enteritidis in table egg layers in the U.S. http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/poultry/layers99/Layers99_dr_Salmonella.pdf Accessed June 7, 2010.
- USDA/APHIS. 2000b. NAHMS Layers '99 Part II: Reference of 1999 table egg layer management in the U.S., January 2000. http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/poultry/layers99/Layers99_dr_PartII.pdf Accessed June 7, 2010.
- USDA/NASS. 2009. Chickens and eggs 2008 summary. February 2009. <http://usda.mannlib.cornell.edu/usda/nass/ChicEggs/2000s/2009/ChicEggs-12-22-2009.pdf> Accessed June 7, 2010.
- van de Giessen, A. W., R. Peters, P. A. T. A. Berkers, W. H. Jansen, and S. H. W. Notermans. 1991. *Salmonella* contamination of poultry flocks in the Netherlands. *Vet. Q.* 13:41–46.
- Van Den Brand, H., H. K. Parmentier, and B. Kemp. 2004. Effects of housing system (outdoor vs cages) and age of laying hens on egg characteristics. *Br. Poult. Sci.* 45:745–752.
- Van Overmeire, I., L. Pussemier, V. Hanot, L. De Temmerman, M. Hoenig, and L. Goeyens. 2006. Chemical contamination of free-range eggs in Belgium. *Food Addit. Contam.* 23:1109–1122.
- Vieira, E. D. R., J. P. M. Torres, and O. Malm. 2001. DDT environmental persistence from its use in a vector control program: A case study. *Environ. Res.* 86:174–182.
- Wales, A., M. Breslin, B. Carter, R. Sayers, and R. Davies. 2007. A longitudinal study of environmental *Salmonella* contamination in caged and free-range layer flocks. *Avian Pathol.* 36:187–197.
- Wales, A. D., J. J. Carrique-Mas, M. Rankin, B. Bell, B. B. Thind, and R. H. Davies. 2009. Review of the carriage of zoonotic bacteria by arthropods, with special reference to *Salmonella* in mites, flies and litter beetles. <http://onlinelibrary.wiley.com/doi/10.1111/j.1863-2378.2008.01222.x/pdf> Accessed June 7, 2010.
- Wall, H., and R. Tauson. 2002. Egg quality in furnished cages for laying hens—Effects of crack reduction measures and hybrid. *Poult. Sci.* 81:340–348.
- Wyeth, P. J. 1975. Effect of infectious bursal disease on the response of chicken *S. typhimurium* and *E. coli* infections. *Vet. Rec.* 96:238–243.
- Yang, A., M. J. Brewster, M. C. Lanari, and R. K. Tume. 2002. Effect of vitamin E supplementation on α -tocopherol and β -carotene concentrations in tissues from pasture- and grain-fed cattle. *Meat Sci.* 60:35–40.