Review

Review of induced molting by feed removal and contamination of eggs with Salmonella enterica serovar Enteritidis

Neal J. Golden a,*, Harry H. Marks b, Margaret E. Coleman c, Carl M. Schroeder a, Nathan E. Bauer Jr. a, Wayne D. Schlosser a

a USDA, Food Safety and Inspection Service, Office of Public Health Science, Risk Assessment and Residue Division, 1400 Independence Avenue S.W., Aerospace Building, Washington, D.C. 20250, United States
b USDA, Food Safety and Inspection Service, Office of Policy, and Program Development, Risk Management Division, 1400 Independence Ave, S.W., South Building, Washington, D.C. 20250, United States
c Syracuse Research Corporation, 301 Plainfield Road Suite 350, Syracuse New York 13212, United States

Received 1 October 2007; received in revised form 27 February 2008; accepted 18 March 2008

Abstract

As laying hens age, egg production and quality decreases. Egg producers can impose an induced molt on older hens that results in increased egg productivity and decreased hen mortality compared with non-molted hens of the same age. This review discusses the effect of induced molting by feed removal on immune parameters, Salmonella enterica serovar Enteritidis (SE) invasion and subsequent production of SE-contaminated eggs. Experimental oral infections with SE show molted hens are more susceptible to SE infection and produce more SE-contaminated eggs in the first few weeks post-molt compared with pre-molt egg production. In addition, it appears that molted hens are more likely to disseminate SE into their environment. Molted hens are more susceptible to SE infection by contact exposure to experimentally infected hens; thus, transmission of SE among molted hens could be more rapid than non-molted birds. Histological examination of the gastrointestinal tracts of molted SE-infected hens revealed more frequent and severe intestinal mucosal lesions compared with non-molted SE-infected hens. These data suggest that induced molting by feed deprivation alters the normal asymptomatic host–pathogen relationship. Published data suggest the highest proportion of SE-positive eggs is produced within 1–5 weeks post-molt and decreases sharply by 6–10 weeks and dissipates to the background level for non-molted hens by 11–20 weeks. Appropriate treatment measures of eggs produced in the first 5 weeks post-molting may decrease the risk of foodborne infections to humans.

Published by Elsevier B.V.

Keywords: Molting; Salmonella; Enteritidis; Feed removal

* Corresponding author. Tel.: +1 202 690 6419; fax: +1 202 690 6337.
E-mail address: neal.golden@fsis.usda.gov (N.J. Golden).

0378-1135/S – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetmic.2008.03.005
1. Introduction

The presence of Salmonella in poultry and eggs is a public health concern for regulatory agencies, food industries, and consumers. It is estimated that there are approximately 1.3 million illnesses in the United States each year from foodborne Salmonella (Mead et al., 1999), of which approximately 180,000, are estimated to result from eating eggs contaminated with Salmonella enterica serovar Enteritidis (SE) (Schroeder et al., 2005). In the late 1970s, an increase in human sporadic illness and outbreaks due to SE was first observed in the U.S. A decade later, this relatively unknown serotype had become the leading Salmonella serotype associated with human illness (Angulo and Swerdlow, 1998). Today, with annual production of more than 77 billion table eggs (USDA, 2007b) and per-capita egg consumption at approximately 250 eggs (USDA, 2007a), the need to better understand SE and employ mitigating strategies is important to reduce human illness from foodborne salmonellosis.

A potential target for mitigating the number of SE-positive eggs is the practice of induced molting. Molting can be characterized as a considerable, but temporary, change in hen physiology. As laying hens age, egg production and quality decrease. Industry producers impose an induced molt on hens that results in increased egg productivity and decreased hen mortality (Alodan and Mashaly, 1999). Hens experience a loss of primary feathers and an involution of the reproductive system that results in rejuvenation of the egg laying potential. Hens allowed to enter this process naturally initiate molting at different ages and continue molting for varied lengths of time. This arbitrary start and stop associated with molting is not economically desirable and producers will often molt their hens to synchronize and renew the egg laying cycle. Hen flocks are typically molted once or twice resulting in 1 or 2 additional laying cycles (Bell, 2001).

Induced molting has become a predominant practice among egg producers in the U.S. Nationwide survey data suggest that 83% of farms routinely induce molting of their hens by feed removal and up to 97% in some areas of the country routinely practice induced molting (USDA, 2000). Others estimate 75–80% of commercial U.S. flocks molted annually (Bell, 2001, 2003). In addition, 1996–2006 survey data from the National Agricultural Statistics Service demonstrate that the percentage of hens molted at any given time in the U.S. is increasing (USDA, 2006). However, these data are not specific to induced molting by feed removal. Updated estimates of the percentage of the U.S. egg industry are needed given the United Egg Producers efforts to discourage induced molting by feed removal (United Egg Producers, 2004). As molted hens represent a portion of the egg-producing hens, the effect of molting on the production of SE-positive eggs may impact the risk of SE to consumers.

Given the importance of feed deprivation induced molting as a flock management practice and its implications to public health, this review was...
developed. Its purpose was twofold: (1) to collate published data describing the changes in the normal hen and SE host–pathogen relationship in regard to induced molting by feed removal and (2) develop an analysis of SE egg contamination data to analyze the effect of molting on the prevalence of SE-contaminated eggs produced by recently molted hens. Factors that could affect this relationship are discussed below include the hen’s innate and adaptive immunity, pathogen susceptibility, intestinal and systemic colonization, shedding, transmission, and recurrence. Based on the evidence considered in this paper, the applicability of data on induced molting to better understanding human exposure to SE and potential mitigation strategies are discussed.

2. Immunity and fasting

Though there are many methods employed to induce molting of commercial hens, feed removal is used most frequently (Brake, 1994; Ruszler, 1998; USDA, 2000). Withdrawal of feed for up to 10–14 days is commonly used (North and Bell, 1990), resulting in a temporary state of fasting. Popular molting regimens that employ fasting recommend a loss of 25–30% of the hen’s previous body weight to ensure adequate post-molt performance. A large proportion of hens are likely to experience many of the effects of prolonged feed withdrawal, including (1) weakened host immune defenses, (2) altered intestinal tract microecology and (3) diminished normal intestinal function (Latshaw, 1991; Berry, 2003; Ricke, 2003). Fasting also could result in immunosuppression combined with alterations in gut microecology and physiology leading to a dissolution of the normal host–pathogen relationship. These mechanisms and their role in the production of contaminated eggs are discussed below.

Non-molted hens exposed to and subsequently colonized with SE do not typically demonstrate clinical signs of illness, suggesting an active immune response toward infection. However, induced molting by feed removal may result in suppression of the immune system, thereby enhancing the ability of SE to spread and cause disease (Holt, 1992a, Holt and Porter, 1992a,b). Immunosuppression of hens induced to molt by fasting is evidenced by the presence of stress hormones in hens following feed removal. For instance, fasting can provoke an increase in the blood levels of corticosteroids of poultry (Freeman et al., 1981; Harvey and Klandorf, 1983; Etches, 1990; Braw-Tal et al., 2004). Corticosteroid levels have been observed to remain significantly high at least 3 weeks post-feed return following a 14-day feed removal (Davis et al., 2000) and Hoshino et al. (1988) found numerically higher corticosterone levels, a corticosteroid, 17 days post-feed return following an 8-day feed removal. Stress hormones are known to possess anti-inflammatory properties (Compton et al., 1987; McFarlane and Curtis, 1989; Isobe and Lillehoj, 1992; Burton et al., 1995) that could reduce immune effectiveness.

In addition, removal of feed and water for 48 h was shown to lower serum antibody titers of birds following intravenous dosing with Escherichia coli compared to chickens fed ad libitum (Ben-Nathan et al., 1977). Clearance of Staphylococcus aureus from the blood of chickens was also slowed in birds deprived of feed and water (Ben-Nathan et al., 1981). Hens fed every other day also have significantly poorer cell-mediated immune responses (see below) compared with those fed daily (Latshaw, 1991), and higher protein diets have been shown to lower mortality rates of E. coli inoculated chicks (Boyd and Edwards, 1963). Deficiencies of vitamins A and E (Panda and Combs, 1963; Marsh et al., 1981; Davis and Sell, 1989) as well as sodium and chloride (Pimentel and Cook, 1987) have been shown to lower immune responsiveness. Panda and Combs (1963) demonstrated Salmonella Pullorum exposed chicks fed a diet deficient in vitamin A produced lower titers of S. Pullorum immunoglobulin compared to chicks for whom vitamin A was readily available. Alternatively, some studies suggest that lower or higher than adequate protein diets reduce mortality when birds are challenged with E. coli or coccidia (Boyd and Edwards, 1963; Britton et al., 1964). These data suggest that optimal nutrient levels are needed to maintain proper hen immunologic physiology. Fasting could therefore alter the natural intestinal microecology and ability of the hen immune system to respond to an infectious agent.

2.1. Innate immunity and fasting

Innate immunity provides the first line of defense against many common microorganisms. This branch
of the host defenses includes physical barriers such as the epithelial layer of mucosal surfaces (e.g., oviduct, intestine), antibacterial peptides (e.g., defensins), serum complement, and inflammation and recruitment of non-specific immune cells such as macrophages and heterophils (the avian equivalent of neutrophils).

Inflammation involves local accumulation of fluid, immune cells and other inflammatory infiltrate triggered by infection or physical injury. During exposure of SE in the gastrointestinal tract, epithelial cells mediate the beginnings of an inflammatory response. Heterophils, phagocytic cells capable of ingesting and destroying microorganisms, are first recruited to the infection site as part of innate immunity. These cells are essential effector cells for both chick and adult *Salmonella* immunity (Kogut et al., 1993, 1994) and are typically recruited to the site of intestinal infection. The number of SE colony forming units (CFUs) required to infect chickens with reduced circulating heterophils was significantly lower than controls (Kogut et al., 1994) suggesting a role of the innate immune system in protecting hens against SE.

To evaluate further the role of heterophils in molting hens, Kogut et al. (1999) examined the number and functional responsiveness of heterophils from molted hens challenged with SE. Following a 2-day feed removal, significantly higher numbers of circulating heterophils were observed. This suggests a higher heterophil-to-lymphocyte ratio and indicates increased stress likely due to fasting. However, despite the increased numbers, significantly fewer heterophils from these birds phagocytized bacteria compared with fed hen counterparts. Chemotaxis, number of engulfed bacteria per heterophils, and oxidative burst were significantly reduced in heterophils from fasted hens. In addition, none of 525 eggs or egg shells were SE-contaminated from hens inoculated with heterophil-susceptible SE compared to 12 of 479 (2.5%) eggs and eggshells from wild-type SE challenged hens (Kramer et al., 1998). These data suggest that heterophils may be important in preventing internal contamination of eggs by SE. In addition, increased circulating corticosteroids, often observed following feed removal, appear to have immunosuppressive effects on bovine neutrophils (Gilbert et al., 1992; Burton et al., 1995). These data suggest that the effectiveness of heterophils to mediate clearing of an infection would be impaired during feed removal.

The importance of innate immunity is not unexpected. Commercial adult hens identified as SE-positive or those experimentally exposed to SE typically do not demonstrate clinical signs of infection. Signs such as ruffled feathers, dehydration, and drooping of wings can be observed from hens administered $8.0 \log_{10}$ SE orally (Kogut et al., 1994); however, morbidity or mortality associated with SE is seldom observed. Histological examination of the gastrointestinal tracts of SE-infected hens rarely show signs of colitis (Holt and Porter, 1992a,b; Porter and Holt, 1993) and hens can harbor SE for extended periods (Gast and Beard, 1990b; Holt and Porter, 1993; Saeed, 1999), in some cases, without fecally shedding the bacteria at culture detectable levels (Holt and Porter, 1993).

Though healthy adult hens do not suffer severe clinical symptoms during SE infection, SE-infected hens deprived of feed exhibit a range of pathology. Histopathology of SE-infected intestinal tissue from molting hens showed more severe and frequent intestinal mucosal lesions in the distal ileum and ceca compared with non-molting hens (Holt and Porter, 1992a; Porter and Holt, 1993; Macri et al., 1997). Blood was detected in feces or alimentary secretions in up to 42 and 50%, respectively, of molted infected hens orally dosed with SE (Holt and Porter, 1992a; Holt et al., 1994, 1995). In addition, Moore and Holt (2006) showed significantly increased in vitro invasion of cecal tissue from molted vs. non-molted hens. Acute phase proteins synthesized in response to bacterial infection and inflammation were significantly elevated in fasted compared with fed hens (Holt and Gast, 2002). In addition, Arnold and Holt (1996) measured an intestinal spike of tumor necrosis factor alpha (TNF-\(\alpha\)) approximately five times greater following oral inoculation of fasted compared to non-fasted hens. This is noteworthy because TNF-\(\alpha\) can activate various immune cell types. Yet, overproduction can have physically detrimental effects on intestinal tissue, suggesting SE-exposed fasting hens are more likely to develop intestinal tissue damage due to the cytotoxic effects of TNF-\(\alpha\). Overall, these data suggest innate immunity is critical to control an infection, perhaps by establishing an equilibrium between host–pathogen interaction.
2.2. Adaptive immunity and fasting

The adaptive immune response comprises the cell-mediated and the humoral (antibodies) branch of the immune system. This response is triggered when pathogens evade innate defenses and produces a threshold level of infectious agent. Unlike innate immunity, adaptive immunity is antigen specific and has delayed onset. Adaptive response is critical for clearing pathogens and for memory response. Below, both arms of adaptive immunity are discussed in terms of molting.

The cell-mediated branch of the immune system is critical in activating type 2 T-cell dependent B-cells to produce antibodies, stimulating CD4+ T-cell macrophage mediated destruction of extracellular and intracellular pathogens, and activating cytotoxic CD8+ T-cell-mediated intracellular pathogen destruction (Janeway, 1997). Holt (1992b) reported a significant decrease in the numbers of a critical set of T-cells in blood, CD4+ T-cells, 3 and 10 days after feed removal; however, CD8+ T-cells were not different from controls. CD4+ T-cells are a central part of cellular immunity suggesting that this branch of the immune system of molting hens is impaired. Holt (1992a) and Holt and Porter (1992a) demonstrated the delayed type hypersensitivity (DTH) response was depressed in molted hens 3 and 7 days post-feed removal. This immunological reaction is mediated by CD4+ T H1 T-cells. CD4+ T-cells differentiate into T H1 and T H2 T-cell subtypes upon antigen stimulation. Differentiation into the T H1 cell subtype results in macrophage stimulation and macrophage recruitment to the site of infection as well as B-cell stimulation (Janeway, 1997).

The results of the DTH experiment suggest that T H1 cells are either numerically depleted or functionally suppressed in molting hens. T H1 cells are involved in controlling bacterial intracellular infections; thus molting hens might be more susceptible to infection due to this attenuated immune compartment. Salmonella spp. are intracellular pathogens and are capable of growing within the vesicles of macrophages; they survive because the vesicles they occupy do not fuse with the macrophage lysosome, a vesicle containing antimicrobial agents. T H1 cells can activate the macrophage to induce vesicle and lysosome fusion, thereby increasing the likelihood of pathogen destruction. At the same time, the macrophage activates other antimicrobial mechanisms and the T H1 cell releases cytokines that attract immune cells to the infection site. The role of T H1 cells to control intracellular bacteria suggests the increased susceptibility and pathology associated with SE infection in molting hens is a direct consequence of depressed T H1 numbers or function during the molting process.

The humoral immune response, the other branch of acquired immunity, is primarily responsible for antibody production by B-cells. Protection by this branch of the immune system appears to be important during SE infection. Hens experimentally inoculated with SE typically produce a SE-specific serum antibody response that peaks within 1–3 weeks post-inoculation (Gast and Beard, 1990c; Humphrey et al., 1991; Bichler et al., 1996; Gast and Holt, 2001). In experimentally inoculated hens, the majority of SE-positive eggs are produced during the first and second week (Gast and Beard, 1990a; Gast and Holt, 2001). Once the antibody response has been established, fecal shedding of the bacteria and production of SE-positive eggs decreases. These observations suggest formation of an antibody response is important for clearance of SE. In addition, hens incapable of making B-cells (bursectomized hens) have been shown to shed statistically greater numbers of SE in their feces at 7–14 weeks (Arnold and Holt, 1995) and 13–41 days post-inoculation and be cecally colonized with statistically greater SE numbers 21–42 days post-inoculation (Desmidt et al., 1998) compared with hens that have B-cells.

To investigate the role of the humoral immune response of molting hens, researchers first measured B-cell levels within molting hens. Holt (1992b) reported no difference in B-cell numbers among molted and non-molted hens. This evidence was subsequently supported by Holt and Porter (1993) who discovered no differences in serum antibody titers between the two groups of hens. The ability of molted hens to respond to an antigenic challenge was not found to be significantly different (Holt, 1992a; Alodan and Mashaly, 1999), suggesting molting does not effect the humoral branch of the immune response. However, when investigators looked for antibodies within specific locations, they found a significant reduction in levels of immunoglobulin IgA within the alimentary secretions of molted hens at 24 and 31 days.
post-feed withdrawal (Holt and Porter, 1993). These data suggest IgA might have a role in clearing intestinal SE infection (Seo et al., 2002).

Therefore, both the cell-mediated and humoral branch of the immune system are activated during an SE infection. Given that SE are intracellular pathogens, reduced effectiveness of the cell-mediated branch may play a more critical role in control of SE compared to antibody production.

2.3. Intestinal microecology and fasting

Fasting may alter the natural microecology, physiology, and function of the hen’s intestinal tract, contributing to a disease state. In the crop of laying hens, typically dominated by resident lactobacilli, feed deprivation reduced the total viable levels of bacteria and altered crop physiology by increasing the pH (Humphrey et al., 1993). This was associated with increased survival of SE in the crop of fasted hens. A similar phenomenon of decreased lactobacilli and other indigenous microflora and increased colonization of \( S. \) Typhimurium was observed in the intestinal tract of food, water, and bedding deprived mice (Tannock and Savage, 1974). Maintenance of the resident microbiota in the gastrointestinal tract has been shown important in reducing gastrointestinal colonization and growth by \( Salmonella \) spp. in mice and poultry (Bohnhoff and Miller, 1962; Bohnhoff et al., 1964). An altered gastrointestinal tract microecology due to fasting may therefore affect the colonization of SE. Durant et al. (1999) found withdrawing feed from hens induced a reduction in crop microbiota and altered crop physiology (pH). These hens showed significantly increased crop and cecal colonization and spleen and liver invasion by SE compared to non-fasted counterparts. Corrier et al. (1997) found molting hens fed lactose to make up for absent lactobacilli were significantly less cecum, spleen, and liver-colonized than fasting birds. Holt et al. (1995) observed that SE could colonize both the colon and the cecum in feed-deprived hens compared to primarily the cecum with fed counterparts. These data suggest fasting alters the resident microbiota and physiology along the gastrointestinal tract of hens thereby increasing the likelihood of SE colonization.

3. Susceptibility of molted hens to SE

With data suggesting feed deprivation of hens can alter the host–pathogen relationship, it remained unclear whether this might impact prevalence of SE within and among flocks and production of SE-contaminated eggs. The effect of fasting on hen susceptibility to SE infection was assessed by oral inoculation of fasted or fed hens with serial dilutions of SE to determine the dose required to infect 50% of birds tested (ID\(_{50}\)). Non-fasted hens aged 52 and 58 weeks had an ID\(_{50}\) of 3.8 and 3.4 log\(_{10}\) CFU compared with an ID\(_{50}\) of <3.2 and 0.1 for fasted hens, respectively (Holt, 1993; Holt et al., 1994). For non-fasted hens aged 67 and 78 weeks, authors observed an ID\(_{50}\) of 4.7 and 4.5 log\(_{10}\) CFU compared with an ID\(_{50}\) of <1.0 and 0.5 for fasted hens, respectively (Holt, 1993). These data suggest feed removal can increase the susceptibility of feed-deprived hens to SE colonization as compared to fed hens. These data are relevant for commercial industry practices as 92% of farms are reported to molt flocks at \( \geq 62 \) weeks of age (USDA, 2000).

4. Shedding of SE by molting hens

Several authors have reported increased levels of SE shedding into their environment in molting hens (Table 1). Holt and Porter (1992a) observed statistically significant differences in SE levels within alimentary secretions from 69- to 84-week-old molted hens 3 and 10 days post-infection (PI) (Trial 1) and 10 and 17 days PI (Trial 2) as compared to non-molted counterparts. This suggested that molted hens carried a greater intestinal load of SE (Holt and Porter, 1992a,b, 1993) than non-molted hens, and thus would be more likely to disseminate SE into their environment at higher levels. To determine if molted hens fecally shed more SE, Holt and Porter (1992b) contact exposed fasted and fed hens to hens experimentally inoculated with SE. Molted vs. non-molted contact exposed hens were observed to shed statistically significant greater SE levels at 3 days PI for 20-week-old hens, 10 days PI for 20-, 40- and 74-week-old hens and 17 days PI for 74-week-old hens (Holt and Porter, 1992b, 1993). Fasted hens shedding greater levels of SE into their environment among a susceptible
population suggests SE could easily be transmitted within a molted flock and result in greater difficulty of removing SE from the hen house environment.

5. Transmission of SE by molting hens

Infected hens can transmit SE to other hens by physical contact, sharing infected feed and water (Lahellec et al., 1986; Nakamura et al., 1994, 1997), and aerosols (Baskerville et al., 1992; Nakamura et al., 1997; Holt et al., 1998). Table 2 summarizes published data that show increased frequency of SE horizontal transmission. Fasted hens placed in adjacent cages to SE-infected hens were infected at higher rates compared with fed counterparts. To discern if fasting hens were more susceptible to aerosol transmission by SE-infected hens than non-molted hens, Holt et al.
(1998) placed SE-infected hens 1 m away from fasting hens that were housed in adjacent cages. Of fasting hens, 54% became infected by airborne transmission compared with 4% of non-molted hens. These data suggest aerosols containing SE or a combination of aerosol and contact transmission can infect a larger proportion of feed-deprived hens more frequently compared to fed counterparts.

6. Recurrence of SE in molting hens

Hens that are experimentally infected with SE often shed the bacteria for 1–2 weeks. However, even when large doses of SE are administered, hens often shed limited numbers or stop shedding SE within 3–4 weeks (Holt and Porter, 1993; Saeed, 1999; Seo et al., 2001). Such hens have either cleared the organism completely or harbor SE that is neither cleared nor results in overt infection. The observation that hens may harbor the bacteria for up to 22 weeks post-exposure (Gast and Beard, 1990b; Holt and Porter, 1993; Saeed, 1999) supports the latter.

In regard to molting hens, investigators posed the question whether fasting hens previously inoculated with SE would lead to a reappearance of SE shedding. Holt and Porter (1993) orally inoculated hens aged 59-weeks with approximately 7.0 log10 SE. Initially, 71% of hens shed SE; however, by day 24, the bacteria could not be detected in alimentary secretions of non-molted hens. For a portion of these hens, feed was removed 21 days post-inoculation and returned 14 days later. On day 24, 25% of fasting hens were readily shedding SE. By day 38 and 45 post-inoculation, the frequency of SE-shedding hens increased to 62.5% (Holt and Porter, 1993). Similarly, Nakamura et al. (1994) orally inoculated 16 of 32 hens aged 28 weeks with 5.0 log10 SE. The authors placed inoculated hens adjacent to non-inoculated and observed the infection spread to 89% of previously uninfected hens within 14 days. However, by 28 days, approximately 20% of all hens were actively shedding SE. When feed and water were removed on day 30, approximately 65% of hens were now observed to shed SE on day 32. Though these two studies do not negate the possibility that re-infections might account for the reappearance of fecal shedding, these results suggest that feed removal and short term feed and water removal can negatively alter the host–pathogen equilibrium.

7. Systemic SE infection

As mentioned in earlier sections, fasting of hens increases susceptibility, prolong fecal shedding, transmission, and recurrence of SE. There also appears to be a greater likelihood of internal contamination of eggs by SE from molted hens. SE can contaminate the internal contents of eggs by two modes of transmission. First, by shell penetration, SE deposited on the eggshell surface by contaminated feces (Gast and Beard, 1990a) can translocate the outer shell of a fresh, un-cracked egg thereby gaining access to internal egg contents (Schoeni et al., 1995). Second, eggs can become vertically contaminated through transovarian infection. That is, SE colonization of extraintestinal organs such as ovary and oviduct tissue can contaminate egg yolk or albumen prior to the deposition of the outer shell (Humphrey et al., 1989; Timoney et al., 1989; Gast and Beard, 1990a; Thiagarajan et al., 1994, 1996; Keller et al., 1995, 1997). Fasting could result in increased contamination of feces and/or extraintestinal organs thereby potentially affecting egg contamination by either route.

The effect of feed withdrawal on SE colonization for intestinal and extraintestinal organs is given in Table 3. Fasting significantly increased the presence of SE in the cecum of commercial and specific pathogen free (SPF) hens, suggesting SE-contaminated feces of molting hens could contaminate eggs more frequently. SE was present also in the livers and/or spleens of commercial and SPF molting birds more frequently than non-molting birds, suggesting molting increases the likelihood SE can move from the intestinal compartment to other organs. One such target organ appears to be the ovary. Seo et al. (2001) found significantly more ovarian tissue samples from non-fed birds positive for SE than in non-molted counterparts. Interestingly, these authors found little difference in ovarian contamination using commercial molted and non-molted hens. Furthermore, gentamicin invasion assays did not show increased SE invasion into ovarian tissues from non-fed vs. fed birds (Moore and Holt, 2006). Clearly, further investigation is needed as well as a comparative analysis of other
extraintestinal organs, such as the oviduct, between fasted and non-fasted hens. Nonetheless, increased ovarian tissue contamination by SE from experimentally infected molting hens suggests this phenomenon is possible in naturally infected hen populations.

8. SE contamination of egg contents from molted hens

Table 4 summarizes studies on effects of molting on frequency of SE contamination. These studies, conducted in both experimentally and naturally infected hens, suggest molted hens are more likely to produce SE-positive eggs than non-molted hens.

A study conducted by the USDA identified the frequency of SE-positive eggs before and after molting of commercial hens (Schlosser et al., 1998). These authors found molted hens produced 4.5 times more SE-positive eggs than non-molted birds 20-weeks post-molt. However, as the age of molted and non-molted hens were not matched, the increase in SE-positive eggs may reflect age differences. To investigate this, a second trial was performed tracking when SE-positive eggs were produced relative to molting (Table 5). These data suggest age could not exclusively account for the increase in SE-positive egg production. Additionally, neither could a clear age effect be discerned regarding increased SE susceptibility and transmission by 20–78-week-old molted hens (Holt and Porter, 1992b; Holt, 1993) nor severity of intestinal lesions of 18–72-week-old molted hens (Holt and Porter, 1993).

To determine the magnitude and significance of the increase of the percentage of SE-positive eggs laid by post-molted hens, it is necessary to establish a model for describing the percentage of positive eggs occurring prior to molting (Schroeder et al., 2006). The difficulty is associated with the results from period /C0 to /C16 weeks pre-molt. For this period the percentage is higher than those of the other pre-molt periods. Therefore, it was necessary to determine if this result can be considered an outlier, perhaps due to a period-specific effect, and therefore excluded from the other pre-molt percentages. Testing for a period effect on the percentage of SE-positive eggs, a chi-square test over the four pre-molt periods was not significant (P = 0.10). Using a likelihood ratio test also demonstrated non-significance (P = 0.15). Comparing the first period percentage of positive eggs (0.057%) with the percentage obtained pooling the results over the last three periods (0.017%) a chi-square test with one degree of freedom is significant at the 0.026 level, again a marginal significance considering that this comparison represents the most extreme of all multiple comparisons that are possible.
A Bonferroni approximation for contrasting each of the four periods with the other three was not significant ($P > 0.10$). The above statistical tests, when taken together, do not provide sufficient evidence for discarding the data from the first period. Hence, in the analysis below, the data of the first period are included.

The average of the four pre-molt period percentages of positive SE eggs is 0.026%; if the results are pooled, the percentage of positive eggs is estimated at 0.021%. Using this latter value as a basis of comparison, for the 0–5 weeks post-molt the percentage of SE-positive eggs increased significantly by a factor of nearly 7, with a standard error of 2.6 based on a regression assuming that the period-specific number of positive eggs was distributed as a binomial distribution. Using the former value the factor increase was still significant (5.5 with standard error of 2.3). An analysis of variance using the arcsine transformation of the square root of the percentage of positive eggs weighted by the number of eggs also indicated a significant difference ($P = 0.05$) between the average percentage of positive eggs pre-molt and the percentage for the eggs in the period 0–5 weeks post-molt. For the periods covering 6–20 weeks post-molt, the (pooled) percentage of SE-positive eggs was 0.028%, only a slight increase over either baseline estimates.

This analysis of commercial layer flocks demonstrates that eggs from molted hens are statistically more frequently contaminated with SE as compared to non-molted hens. Interestingly, the percentage of SE-positive eggs decreases rapidly after an initial increase over the baseline level. These data suggest that increased internal egg contamination by molted hens is temporary, not lasting much beyond 5 weeks post-molt.

9. Conclusions

The commercial egg industry relies on molting to minimize rearing and replacement of pullets and depopulation of spent hens (Bell, 2001). Economically, induced molting as a flock management practice has increased the profitability of egg production (Bell, 2001). The above assemblage of data suggests that induced molting can alter the pathogen–host relationship to the disadvantage of the layer. This, in turn, may be responsible for the increased egg contamination by SE observed among commercial flocks (Schlosser et al., 1998).

---

**Table 4**

<table>
<thead>
<tr>
<th>Publication</th>
<th>Study type</th>
<th>% SE-positive eggs by non-molted hen</th>
<th>% SE-positive eggs by molted hen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holt and Porter (1992a)</td>
<td>Experimental oral inoculation</td>
<td>0 (0/13)</td>
<td>18 (2/11)</td>
</tr>
<tr>
<td>Holt and Porter (1993)</td>
<td>Experimental oral inoculation</td>
<td>0 (0/163)</td>
<td>1.4 (3/212)</td>
</tr>
<tr>
<td></td>
<td>Contact exposed to inoculated hens</td>
<td>0 (0/112)</td>
<td>1.3 (2/158)</td>
</tr>
<tr>
<td>Schlosser et al. (1998)</td>
<td>Naturally infected commercial hens</td>
<td>0.014 (14/100,000)</td>
<td>0.063 (97/154,000)</td>
</tr>
</tbody>
</table>

**Table 5**

<table>
<thead>
<tr>
<th>Pre- or post-molt flock status</th>
<th>Range of weeks egg collected</th>
<th>% SE positive eggs (egg/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-</td>
<td>−20—16</td>
<td>0.057 (4/7,000)</td>
</tr>
<tr>
<td>Pre-</td>
<td>−15—11</td>
<td>0.006 (1/16,000)</td>
</tr>
<tr>
<td>Pre-</td>
<td>−10—6</td>
<td>0.017 (4/23,000)</td>
</tr>
<tr>
<td>Pre-</td>
<td>−5—0</td>
<td>0.024 (5/21,000)</td>
</tr>
<tr>
<td>Post-</td>
<td>0—5</td>
<td>0.144 (13/9,000)</td>
</tr>
<tr>
<td>Post-</td>
<td>6—10</td>
<td>0.026 (5/19,000)</td>
</tr>
<tr>
<td>Post-</td>
<td>11—15</td>
<td>0.011 (2/18,000)</td>
</tr>
<tr>
<td>Post-</td>
<td>16—20</td>
<td>0.039 (11/28,000)</td>
</tr>
</tbody>
</table>
The majority of reports studying the effect of induced molting have been performed on SPF hens. This is important because commercial hens may be exposed to different *Salmonella* serotypes over the course of their egg-producing life (Schlosser et al., 2000); therefore, data generated by use of SPF hens may not necessarily represent that of commercial flocks. Exposure to other serotypes may affect the ability of SE to infect hens because of competition and immunologic memory from shared *Salmonella* serotype immunogens. Birds previously exposed to other *Salmonella* spp. may be therefore better adapted to resist infection by SE. For SPF hens, these birds should be practically naive to *Salmonella* surface antigens and might develop an altered immune response compared to their *Salmonella*-exposed counterparts. If so, SPF hens would be expected to be relatively more susceptible to SE infection and therefore might produce more SE-positive eggs. However, the effects of previous exposure to other *Salmonella* serotypes on the protectiveness to SE infection and egg contamination are unclear (Hassan and Curtiss, 1994, 1997; Holt et al., 2003; Babu et al., 2004; Holt and Gast, 2004).

Studies that used commercial hens to investigate feed withdrawal and SE infection of hens and contamination of eggs (Schlosser et al., 1998; Durant et al., 1999; Seo et al., 2001) found shedding and colonization of the cecum and spleen/liver by SE were significantly increased in layers that were withheld feed compared to fed counterparts. These data were similar to those data obtained using SPF hens. Additionally, the SE Pennsylvania Pilot Project found that molted hens produced more internally SE-contaminated eggs than non-molted birds (Schlosser et al., 1998). The increased prevalence of SE in eggs was significant for 0–5 weeks post-molt compared to 20 weeks of pre-molt conditions suggesting that in a commercial setting, layer hens can produce a significantly greater number of SE-positive eggs from birds of a similar age. As noted previously, by 6 and 20 weeks post-molt, molted hens were not producing increased numbers of eggs internally contaminated, suggesting the observed increase was temporary.

Mitigation strategies can be implemented along the farm-to-table continuum to reduce the exposure of the public to SE-contaminated eggs. Alternative molting procedures that may lessen the risk to SE are available and should provide producers with economical options to feed removal-induced molting. Options include altered diet regimes such as specific nutrient restrictions or feed additives and vaccination (Holt, 2003; Moore et al., 2004; Woodward et al., 2005). The above evidence suggests that induced molting by feed removal may shift the host–pathogen relationship resulting in more SE-contaminated eggs from molted birds. Immediately following the molting process, hens produce the greatest frequency of contaminated eggs, yet this declines several weeks later (Table 5). This scenario suggests that eggs produced immediately following emersion from the molt are at a greater risk of being internally contaminated with SE. To reduce the exposure by consumers to SE-contaminated eggs, shell eggs produced immediately following molt could be designated for in-shell pasteurization. Shell eggs from molted hens directed to liquid egg pasteurization could also be pasteurized at a higher level. Such practices should reduce the public’s exposure to SE-contaminated eggs, while allowing further development and implementation of alternative molting procedures.

**Acknowledgments**

We thank Drs. W. T. Disney, R. K. Gast, P. S. Holt, and T. J. Humphrey for their critical review of the manuscript and Sharyn Lavender for her technical work.

This manuscript has been reviewed by the Office of Public Health Science, Food Safety and Inspection Service, U.S. Department of Agriculture and approved for publication. Approval does not signify that the contents necessarily reflect the views or policies of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

**References**


