Can Stress in Farm Animals Increase Food Safety Risk?

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Abstract

All farm animals will experience some level of stress during their lives. Stress reduces the fitness of an animal, which can be expressed through failure to achieve production performance standards, or through disease and death. Stress in farm animals can also have detrimental effects on the quality of food products. However, although a common assumption of a potential effect of stress on food safety exists, little is actually known about how this interaction may occur. The aim of this review was to examine the current knowledge of the potential impact of stress in farm animals on food safety risk. Colonization of farm animals by enteric pathogens such as *Escherichia coli* O157:H7, *Salmonella*, and *Campylobacter*, and their subsequent dissemination into the human food chain are a major public health and economic concern for the food industries. This review shows that there is increasing evidence to demonstrate that stress can have a significant deleterious effect on food safety through a variety of potential mechanisms. However, as the impact of stress is difficult to precisely determine, it is imperative that the issue receives more research attention in the interests of optimizing animal welfare and minimizing losses in product yield and quality, as well as to food safety risks to consumers. While there is some evidence linking stress with pathogen carriage and shedding in farm animals, the mechanisms underlying this effect have not been fully elucidated. Understanding when pathogen loads on the farm are the highest or when animals are most susceptible to infection will help identifying times when intervention strategies for pathogen control may be most effective, and consequently, increase the safety of food of animal origin.

Animal Welfare and Food Safety

The constantly increasing demand for animal products (meat, milk, and eggs) around the world represents a serious challenge to the animal production industry. Major contemporary issues in animal production include animal welfare and food safety. While animal welfare is fundamentally an ethical issue, food safety is a defining global market issue as the presence/absence of contamination with foodborne pathogens is considered an additional measure of food quality. In general, the fundamental welfare concerns in animal production systems are based on the common causes of stress, which are mostly focused toward the impact of housing systems and management practices. From the standpoint of food safety, the main source of concern is the potential carriage and transmission of foodborne pathogens and antimicrobial-resistant microorganisms and/or genetic determinants.

All farm animals will experience some level of stress during their lives. Stress reduces the fitness of an animal, which can be simply expressed through failure to achieve production performance standards or targets (Mitlohner et al., 2001; Collier et al., 2006; Estevez, 2007; White et al., 2008), or more drastically, through disease and death (Vecerek et al., 2006; Duff and Galyean, 2007; Ritter et al., 2007; Fitzgerald et al., 2009). Additionally, it has been demonstrated that stress in farm animals can also have detrimental effects on the quality of food products (Hughes et al., 1986; Rosenvold and Andersen, 2003; Ferguson and Warner, 2008). However, although a common assumption of a potential effect of stress on food safety exists, little is actually known about how this interaction may occur. Therefore, this review article was prepared with the objective of compiling the current knowledge and evidence available in the scientific literature examining what it is known about the relationship between stress in farm animals and microbial food safety risk. It is not the intention of this review to discuss all the physiological mechanisms of the stress response(s), although several excellent references were selected and cited throughout the article for the convenience of the readers. Also, it is important to mention that issues related to legislations and regulations dealing with animal welfare and food safety were not included in this review. However, it needs to be kept in mind that they may have mutual impact on each other.
Stress in Farm Animals

Stress, a response to adverse stimuli, is a difficult concept to define because of its fundamental abstract and volatile interpretation. According to Selye (1976), stress is “the nonspecific response of the body to any demand,” and stressor is “an agent that produces stress at any time.” In other words, stress represents the reaction of the body (i.e., a biological response) to stimuli that disturb its normal physiological equilibrium or homeostasis, whereas stressors can be defined as conditions that endanger, or are perceived to endanger, the homeostasis of an individual and, therefore, cause a stress response or reaction.

In general, stressors can be grouped into three broad categories: (1) psychological stressors, based on a learned response to the threat of an impending adverse condition (e.g., fear, anxiety, and exposure to a novel environment); (2) stressors that consist of a physical stimulus and have a strong psychological component (e.g., pain, shock, and immobilization); and (3) stressors that challenge cardiovascular homeostasis (e.g., hemorrhage, exercise, and heat exposure). Whether a stressor can be considered harmful depends on how an organism is able to cope with a threatening situation as it maintains a state of homeostasis (Van de Kar and Blair, 1999). The response to stressors requires a progression of events within the animal’s body beginning with sensing and signaling through various biological mechanisms that a threat exists. These events are followed by activation of neurophysiological mechanisms to mount a biological effort to resist and prevent major damage. The various sensory detectors not only receive the information but also transform that information into neural signals that are transmitted to either or both cognitive and noncognitive centers of the nervous system to generate a coordinated response to the challenge. The hypothalamic–pituitary–adrenal axis and the autonomic nervous system coordinate the stress response through several hormones, neurotransmitters, and neuropeptides regulating the physiological process following a determined time course and specificity for each stressor triggering the response (Minton, 1994; Mostl and Palme, 2002; Carrasco and Van de Kar, 2003; Mormede et al., 2007; Marketon and Glaser, 2008).

Naturally, farm animals will be challenged by different stressors and, consequently, develop varying degrees of stress responses during their lives. Each environment poses its unique set of stressors to which the animal must be able to appropriately respond (Siegel, 1995). Modern animal production systems typically house animals in large and homogeneous groups, in enclosed environments (e.g., barns, rooms, and pens). Often, these production systems place the animals into situations in which it must be able to adapt and respond to a variety of stressors, which are usually confined with one another (i.e., occurring simultaneously or overlapping). Some of the common factors that produce stress when they act excessively within any animal production system include inadequate nutrition, deprivation of water and/or feed, heat, cold, overcrowding, and handling (i.e., interaction with humans, or human manipulation of the animals). Additionally, most farm animals are transported at some stage in their lives, sometimes to places where feed ingredients are more readily available, to a different owner or a different raising location, and sometimes to slaughter. The handling, loading, transporting, and unloading of animals can have substantial detrimental effects on their well-being by causing stress. Moreover, during this process, animals can be exposed to a range of challenging stimuli, including handling and increased human contact, transport (vibration, movement, and jolting), novel/unfamiliar environments, food and water deprivation, changes in social structure (through separation and mixing during transport and/or at the final destination), and changes in climatic conditions (i.e., heat and cold). These challenges perturb the homeostasis of the animals, and an adaptive response is activated in an attempt to restore balance. However, considerable variability exists between animals not only in their perception of the stressor but also in their coordination of the response (Siegel, 1995). According to Dantzer and Mormede (1983) and Ferguson and Warner (2008), both stress perception and response are modulated by several intrinsic animal factors (genetics, species, sex, age, physiological state, and past experiences and acquired learning), and also by characteristics of the stressor (nature, timing, avoidability, frequency, severity, and duration). An additional factor of the individual perception of stressors that contributes to the variability of effects or consequences consists of the hormetic nature of stress. Hormesis is defined as the adaptive response of an organism to an exogenous or intrinsic factor (i.e., any type of stressor) in which the factor induces stimulatory or beneficial effects at low doses, and inhibitory or adverse effects at high doses (Calabrese et al., 2007; Mattson, 2008). This biphasic type of response relative to stress level or intensity is broadly observed, and further contributes to inconsistent results in studies aiming at determining effects of stress on a variety of functions and systems (e.g., growth performance, immunological parameters, and resistance/susceptibility to infection).

Stress and the Gastrointestinal Tract

The enteric nervous system (ENS) is an integrative network located within the wall of the gastrointestinal tract. It contains approximately 100 million neurons, which is close to the same number found in the spinal cord. The ENS controls the microcirculation, motility, and exocrine and endocrine secretions of the gastrointestinal tract. The ENS is bidirectionally linked to the central nervous system (CNS) by the sympathetic and the parasympathetic pathways forming the brain–gut axis (Goyal and Hirano, 1996; Costa et al., 2000). Although we are only beginning to understand the complex physiology of brain–gut interactions involved in stress-related gastrointestinal alterations, there is evidence that stress may not only be responsible for functional disorders, but may also contribute to inflammatory disorders and infections of the gastrointestinal tract (Soderholm and Perdue, 2001; Bhatia and Tandon, 2005).

Neurotransmitters play a key role in animal responses to challenges (i.e., stressors). Norepinephrine, a major neurotransmitter in the peripheral nervous system, is present naturally in the gastrointestinal tract. The gastrointestinal tract is highly innervated and neurons secrete norepinephrine into the mucosa (Konaka et al., 1979; Hart and Kamm, 2002), the primary habitat of a variety of bacteria, including foodborne pathogens. According to Hart and Kamm (2002), concentrations of norepinephrine are usually high at mucosal surfaces and in covering mucin.
The crosstalk between the neuroendocrine and immune systems is now well established, with these systems utilizing a number of similar ligands and receptors to provide an intrasystem and intersystem network of communication. It is known that communication between these systems is essential for maintaining physiological homeostasis and health. A number of hormones, neurotransmitters, and neuropeptides are known to participate in various aspects of immune development and function. Lymphocytes, monocytes, and various other immune cells subsets express receptors for many of these mediators. Similarly, receptors for immune-derived cytokines, chemokines, and growth factors have also been identified on neuronal cells and within endocrine organs under normal physiological conditions, and under stress responses. Disturbances within these systems may lead to immune activation or suppression, depending on the systems being affected and the nature of the stimuli. Moreover, the autonomic nervous system also communicates with the lymphoid compartment through the release of norepinephrine and acetylcholine from sympathetic and parasympathetic nerves. Thus, it appears that multidirectional communication networks exist within the body that permit the transmittal of signals between these various systems during times of stress and infection (Steinman, 2004; Marques-Deak et al., 2005).

Physical and psychological stress can result in neuroendocrine mediators being released from the CNS and endocrine organs that directly impact immune function. An imbalance in any of these systems in response to stress can lead to significant changes in immune responsiveness, and consequently, in susceptibility to infection. Through the action of these stress mediators (particularly, glucocorticoids hormones and catecholamines—epinephrine and norepinephrine), significant effects on immune functions have been observed, including reduced natural killer cell activity, alterations in peripheral lymphocyte subsets and reduced cell proliferation, impaired antibody production, and the reactivation of latent infections (Markton and Glaser, 2008; Taub, 2008). As previously mentioned, the immune system and CNS are involved in functionally relevant crosstalk to maintain homeostasis under normal and disease conditions. In fact, this intimate connection (anatomical and functional) of immune and nervous systems could simply and mechanistically explain why stress can influence susceptibility to infection. However, although this interaction has until recently been the central pillar to support the hypothesis on the effects of stress on infections, it does not provide a complete and failure-proof explanation of what really occurs.

A significant amount of effort has been dedicated to study the potential effects of stress on the gastrointestinal tract of laboratory animals as a means to develop knowledge to be applied in human medicine. From years of research, it is known that during stress, the release of catecholamines results in a decreased gastric acid production, delayed gastric emptying, and accelerated intestinal motility and colonic transit (Moon et al., 1979; Enck et al., 1989; Tache et al., 1999; Monnikes et al., 2001). As a consequence, the increase of the pH in the stomach leads to a greater probability that foodborne pathogens (such as *Escherichia coli*, *Salmonella*, and *Campylobacter*) will survive gastric passage and colonize the gastrointestinal tract. Additionally, as previously mentioned, hormones released in response to stress also act at the intestinal mucosa to alter interactions between luminal microorganisms and epithelial cells (Wang and Wu, 2005). For instance, Saunders et al. (2002) observed increased permeability of the intestinal mucosa in rats subjected to psychological and physical stress. This functional change in the intestinal mucosa permeability leads to increased microbial invasion ability in the gastrointestinal tract (i.e., increased translocation rate). Consequently, animals under stress become more susceptible to new (and more severe) infections, and may carry more pathogens in the gastrointestinal tract and associated lymphoid tissue. Moreover, animals that already excrete bacteria can shed more with an increased defecation frequency, because of increased intestinal motility (Lenz et al., 1988; Williams et al., 1988; Barone et al., 1990).

Early studies (Miraglia and Berry, 1962; Previte et al., 1970, 1973) showed enhanced *Salmonella* infection (frequency and persistency) in mice exposed to cold temperature. Tannock and Savage (1974) observed that deprivation of food, water, and bedding for 48 hours caused increased intestinal population levels of *Salmonella Typhimurium* and coliforms, and reduced levels of lactobacilli, in mice. Dreau et al. (1999) showed that stress caused by social conflict favored *E. coli* O157 in a closed chamber model in mice (2.26 log increase in bacteria numbers). Nettelbladt et al. (1997) showed that starvation increases the number of bacteria in the cecal contents and increases bacterial adherence to the cecal epithelium, and Nettelbladt et al. (2003) showed that bacterial translocation from the gastrointestinal tract is substantially increased in rats subjected to mild and severe stressors. According to Velin et al. (2004), chronic psychological stress in rats causes increased antigen and bacteria uptake through the intestinal epithelium. Additionally, Nazli et al. (2006) suggest that increased intestinal epithelium permeability, both paracellular and transcellular permeability, occurs in metabolically stressed epithelium (i.e., a decrease in barrier function, with increased access of bacteria and their products to the mucosa and submucosa). A recent study by Bailey et al. (2006) showed that psychological components of social stress (i.e., response to stressors that occur when individuals live in groups) facilitate the translocation of indigenous bacteria into the host (mouse), as evidenced by a significant increased occurrence of total aerobic and facultatively anaerobic bacteria in mesenteric lymph nodes.

The gastrointestinal tract harbors a dense and metabolically active microbiota comprised primarily of bacteria. Bacterial populations can reach around $10^{12}$–$10^{14}$ organisms/g of intestinal contents, comprising many hundreds of species (Zhu and Joerger, 2003; Rastall, 2004; Richards et al., 2005). A pathogen invading and colonizing the gastrointestinal tract must overcome the competition of the local microbiota, in addition to a variety of innate and adaptive host immune defenses. As previously mentioned, the gastrointestinal tract is extensively innervated with sympathetic nerve terminals, which are distributed throughout the ENS (Goyal and Hirano, 1996; Costa et al., 2000). In fact, according to Freestone et al. (2008), half of the norepinephrine present within the mammalian body is synthesized and used within the ENS. Therefore, the gastrointestinal tract is an environment in which, normally, there is a significant presence of catecholamines (particularly, norepinephrine). Further, during episodes of stress, catecholamines are released by the ENS, or spill over from the systemic circulation (Aneman et al., 1996; Eisenhofer et al., 1997), causing significant local increases. These changes
can then markedly affect the status and behavior of the resident microbiota and colonizing pathogens, as discussed above (i.e., indirectly, through suppression of the immune system, and promoting physiological alterations in the gastrointestinal tract). However, a new area of scientific knowledge has recently emerged investigating the direct effects of the ENS on the gastrointestinal tract microbial populations, including foodborne pathogens.

**Microbial Endocrinology: Host Stress and Bacteria**

Until recently, the concept of how stress in animals influences the outcome of infections was primarily based on the immune response, and on other potential indirect effects, as discussed above (i.e., physiological changes in the gastrointestinal tract). However, a few years ago, the first studies were conducted to examine the direct influence of stress-related neuroendocrine hormones, such as the catecholamines, on bacterial growth (Lyte and Ernst, 1992). The idea that bacteria could directly respond to the neuroendocrine outflow that resulted from the stress response has led to a series of studies that demonstrated significant increase in growth of Gram-negative bacteria exposed to norepinephrine in vitro (Lyte and Ernst, 1992; Lyte and Ernst, 1993; Lyte et al., 1996). Additionally, virulence-associated factors, such as adhesions in enterotoxigenic *E. coli* and toxins in enterohemorrhagic *E. coli* (Lyte et al., 1996, 1997), were shown to increase in norepinephrine-stimulated bacteria. Such direct effects of catecholamines on the bacteria provided evidence of a new pathway for host stress-induced alterations of infections, and provided a theoretical framework for the role of microbial endocrinology in the pathogenesis of infectious diseases (Lyte, 1993).

According to Lyte (2004), microbial endocrinology is a new transdisciplinary area that represents the intersection between microbiology and neurophysiology. It is based on a holistic approach, with bacteria seen as an active participant in the process of understanding how stress affects the progression of infections. Research in this area is rapidly emerging and providing critical knowledge on how bacteria can actively respond and use to their own advantage the neurohormonal products of the stress response of their host (Freestone et al., 2008).

According to Aneman et al. (1996) and Alverdy et al. (2000), expression of virulence factors increases in enteropathogens in response to environmental signals indicating host stress. In fact, Cogan et al. (2007) and Dowd (2007) showed that *Campylobacter jejuni* and *E. coli* O157:H7 (respectively) increase the expression of virulence factors when exposed in vitro to norepinephrine. Moreover, Chen et al. (2003, 2006) have shown that catecholamines modulate *E. coli* O157:H7 adherence to the cecal epithelium, whereas according to Dunn et al. (2003), translocation from the gastrointestinal tract of *Salmonella* Typhimurium is associated with hypothalamic–pituitary–adrenal axis activation, and noradrenergic and indoleaminergic responses.

Although it is widely accepted that bacteria are very dynamic microorganisms constantly adapting to changes in their environment, little is known about the mechanisms used in these responses, particularly in vivo. Most studies composing the knowledge base in this area were conducted in vitro and/or with laboratory animals. To date, very little is known about the potential effects of stress and its mediators on the carriage and virulence of bacterial pathogens in vivo, particularly in farm animals.

**Stress in Farm Animals and Foodborne Pathogens**

Although much of the bacterial contamination of animal products (pork, beef, and poultry) occurs within abattoirs (along the slaughter and processing line), infected animals leaving the farm are considered as the original source of abattoir contaminations. It has been shown that a substantial number of pigs, cattle, and poultry are constantly carrying foodborne pathogens into the abattoirs (Rostagno et al., 2003, 2006; Wesley et al., 2005; Woerner et al., 2006; Arsenault et al., 2007c, Fox et al., 2008), with the gastrointestinal tract frequently contaminated/infected and providing a source from which bacteria may be spread in the abattoir contaminating carcasses. According to Berends et al. (1996), live pigs carrying *Salmonella* are three to four times more likely to produce positive carcasses than *Salmonella*-free animals. Correlations have been reported between fecal and hide prevalence of *E. coli* O157:H7 in beef cattle, and the frequency of carcass contamination within lots (Elder et al., 2000; Woerner et al., 2006; Fox et al., 2008). Arsenault et al. (2007a, 2007b) and Reich et al. (2008) have shown positive correlations between cecal prevalence of *Salmonella* and *Campylobacter*, and carcass contaminations in broiler chickens and turkeys.

The gastrointestinal microbiota may be disturbed by many forces, including antimicrobial drugs and other feed additives, dietary changes, and a variety of stressors. As a consequence, levels of pathogens in the gastrointestinal tract and shedding from unapparent carriers (i.e., subclinically infected animals) may be affected by a long list of factors. For instance, during the process of being transported from production farms to abattoirs, animals are exposed to a variety of potential stressors before slaughter (Warriss, 2003; Averos et al., 2008). Consequently, many believe that the number of animals carrying and shedding foodborne pathogens, as well as the levels of the bacteria in the gastrointestinal tract will be increased in response to stressors. It is also believed that their susceptibility to new infections will increase. However, although these assumptions are widely accepted, definitive proof still lacks, as most of the current knowledge is based on limited scientific evidence.

An early small study by Williams and Newell (1970) suggests that transportation of pigs leads to increased shedding of *Salmonella*. Isaacsen et al. (1999a) showed that pigs experimentally infected with *Salmonella* Typhimurium exhibited increased shedding after transportation. The same authors, however, reported conflicting results in another study to determine the effect of feed withdrawal and transportation on the shedding of *Salmonella* Typhimurium in experimentally infected pigs (Isaacsen et al., 1999b). Collectively, the results of these two studies (Isaacsen et al., 1999a, 1999b) suggest that there is an interaction between feed withdrawal and transportation that can lead to increased shedding of *Salmonella* by pigs. However, further research is required to clarify these observations. Marg et al. (2001) suggested that transportation for 8 hours resulted in increased shedding, but no effect on the distribution in inner organs in a small group of pigs inoculated with a high dose of *Salmonella* Typhimurium DT104. In a study conducted with young pigs naturally infected with
Salmonella, Rostagno et al. (2005) did not find difference in prevalence estimates, based on pretransportation and posttransportation fecal samplings. Scherer et al. (2008) also did not observe any effect of transport-induced stress on Salmonella shedding rates in feces and distribution patterns in organs of pigs infected with Salmonella Typhimurium DT104. Additional research suggests that stress may cause changes in bacterial species and biotypes, and antimicrobial resistance phenotypes in pigs. Jones et al. (2001) and Mathew et al. (2003) showed that changes occur in the intestinal populations of E. coli upon exposure to various stressors. According to Molitoris et al. (1987), a moderate transit time (6 hours) was associated with an increase in the diversity of antimicrobial resistance patterns, when compared with a short transit time (2 hours). Moreover, according to the same authors, increased proportions of resistant strains and strains capable of resistance transfer occurred after long holding times in E. coli. Studies conducted in a swine herd, in which the prevalence of antimicrobial drug–resistant E. coli decreased progressively after 13 years of discontinuing subtherapeutic feeding, suggest a significant increase in antimicrobial resistance after transportation of these animals (Dawson et al., 1984; Langlois et al., 1986). Changes in antimicrobial resistance have been reported after simply moving animals into and out of their pens (Hedges and Linton, 1988). Moro et al. (1998) investigated the effects of cold stress on the prevalence of antimicrobial resistance in E. coli from the intestinal tract of swine, and showed that cold stress caused significant increases in ampicillin and tetracycline resistance. Additionally, Moro et al. (2000) reported that heat stress in pigs increased levels of single and multiple antimicrobial resistance in E. coli cultured from feces. According to the authors, only 25% of the prestress isolates showed multiple antimicrobial resistance patterns, in contrast to 85% of isolates from poststress. Moreover, a significant difference was observed for tetracycline resistance between isolates obtained from the carcasses of the control (40%) versus the stressed group (80%), suggesting that stressed animals were shedding higher numbers of resistant bacteria that contaminated the carcasses. According to Dowd et al. (2007), a mild handling stressor (single daily weighting) was sufficient to markedly alter the balance of the intestinal microbiota of pigs. Profiling of shed enteric bacteria showed a significant increase in numbers of E. coli and other coliforms in feces of test groups compared with controls. Callaway et al. (2006) showed that social stress of weaned pigs increased susceptibility and/or fecal shedding of Salmonella. An interesting study by Toscano et al. (2007) examined the effects of in vitro pretreatment of Salmonella Typhimurium with norepinephrine before infecting young pigs. Examination of the tissue distribution revealed that norepinephrine-treated bacteria were present in greater numbers and more widely distributed in gastrointestinal tissues than control bacteria.

Farm animals are sometimes subjected to feed withdrawal before transportation to slaughter to clear the gastrointestinal tract of fecal contents, and thus reduce the potential fecal contamination of carcasses. While investigating the effect of dietary stress (feed restriction or withdrawal) on fecal shedding of E. coli O157 in calves, Cray et al. (1998) observed that stressed calves were more susceptible to infection and shed significantly more bacteria than calves maintained on a normal diet. According to Reid et al. (2002), feed withdrawal for 24–48 hours causes increased numbers of E. coli shed by cattle. In pigs, there has been very little research conducted to determine if preslaughter feed withdrawal affects prevalence and/or levels of intestinal bacteria, although withholding feed before slaughter is commonly practiced. Morrow et al. (2002) reported results from a field study in which no feed withdrawal effect on Salmonella prevalence was observed. Recently, Martin-Pelaez et al. (2008a, 2008b) reported that increasing preslaughter feed withdrawal and lairage leads to cecal fermentation changes with increased pH and decreased concentrations of short chain fatty acids, and consequent increased numbers of Enterobacteriaceae and Salmonella in market pigs. In broiler chickens, Byrd et al. (1998) and Corrier et al. (1999) showed that feed withdrawal causes a significant increase in Campylobacter and Salmonella in the crop, but has no effect in the ceca. Stern et al. (1995) and Whyte et al. (2001) demonstrated that transport increased the isolation rate of Campylobacter from broiler chickens. Likewise, the isolation rate of Salmonella from broiler chickens has been reported to increase after subjecting the birds to the stressful preslaughter practices of feed withdrawal and transportation (Line et al., 1997; Ramirez et al., 1997; Corrier et al., 1999). In a recent study, Burkholder et al. (2008) showed that stressors such as 24-hour feed withdrawal and 24-hour exposure to high temperature (heat stress) can cause changes in the normal intestinal microbiota and epithelial structure in broilers, which may lead to increased attachment of Salmonella. Rostagno et al. (2006) conducted a study under commercial conditions, and reported that Salmonella prevalence in market-age turkeys was not affected by common preslaughter stressors, including feed withdrawal, catching, transportation, and holding. On the other hand, Wesley et al. (2009) observed a significant increase in Campylobacter prevalence in market-age turkeys subjected to similar preslaughter stressful conditions. Dutta et al. (2008) showed that Listeria monocytogenes colonizes liver and synovial tissues of cold-stressed turkeys, and may constitute a source of contamination of processing plants.

Feed deprivation is the most commonly used method by the layer industry (i.e., egg industry) to induce molting and stimulate multiple egg-laying cycles in aging hens. However, research has demonstrated that feed removal during forced molting decreases the resistance of hens to Salmonella Enteritidis infection (Holt, 1993; Durant et al., 1999), resulting in increased severity of infection (Holt and Porter, 1992a; Porter and Holt, 1993), increased intestinal shedding of Salmonella (Holt and Porter, 1992a, 1993), increased horizontal spread of infection to molted hens in neighboring cages (Holt and Porter, 1992b, 1993; Holt, 1995), and, finally, increased egg contamination (Holt, 2003; Golden et al., 2008) and consequent food safety risk to consumers. According to Humphrey (2006), molting of hens through feed removal increases the severity of Salmonella infection and facilitates crossinfection between groups. Feed withdrawal alters the environment of the crop as well as of the rest of the intestinal tract, causing alterations in the indigenous microbial populations along with lactate and short chain fatty acid concentrations leading to an increase in pH. The altered crop environment is accompanied by an increase in Salmonella colonization of the crop and ceca along with invasion of the spleen and liver.

Recently, Humphrey (2006) reviewed and discussed the interaction between poultry welfare and infection susceptibility, including food safety. It was concluded that birds
become more susceptible to *Salmonella* and *Campylobacter* when they are in a poor environment, fed a poor diet, and/or under physical or psychological stress. Humphrey (2006) also concluded that egg contents contamination *in ovo* may be linked to transient stress in laying hens.

In cattle, according to Hancock et al. (1997), Fairbrother and Nadeau (2006), and Chase-Topping et al. (2007), weaning and movement of the animals constitute risk factors for *E. coli* O157:H7 infection and shedding. A study conducted by Corrier et al. (1990) suggests that marketing stress (i.e., transportation of feeder calves) may induce fecal excretion of *Salmonella*. Also, Barham et al. (2002) reported that transportation may be a potential stressor for cattle, causing increased shedding of *Salmonella*. However, in both studies, the possibility of new infections acquired from contaminated environments, trailers, and other animals may have acted as confounders. Additional studies in cattle have shown that *E. coli* O157:H7 and *Salmonella* prevalence and levels increase in hide samples when the animals are transported from the feedlots to the abattoir, and also when subjected to pre-slaughter lairage (Reicks et al., 2007; Arthur et al., 2007; Dewell et al., 2008a, 2008b). However, it is important to consider that increased hide contamination does not directly imply an increased infection frequency, although it suggests increased shedding with subsequent environmental and hide contamination. According to Edrington et al. (2004), environmental factors and production demands (i.e., physiological stress) may influence shedding patterns of *E. coli* O157:H7 and *Salmonella*. According to Renter et al. (2008), higher *E. coli* O157:H7 prevalence in summer months and with warmer temperatures is common in feedlot cattle. Fitzgerald et al. (2003) reported differences in the shedding pattern of foodborne pathogens due to stage of the milking production cycle in dairy cattle, with the prevalence of cows shedding *E. coli* O157:H7 being higher in lactating and primiparous cows compared to nonlactating and multiparous.

**Concluding Remarks**

Colonization of farm animals by enteric pathogens such as *E. coli* O157:H7, *Salmonella*, and *Campylobacter*, and their subsequent dissemination into the human food chain are a major public health and economic concern for the food industries. The aim of this review was to examine the current knowledge of the potential impact of stress in farm animals associated with food safety risk. As shown, there is increasing evidence showing that stress can have a significant deleterious effect on food safety through a variety of potential mechanisms. However, as the impact of stress is difficult to precisely determine, it is imperative that the issue receives more research attention in the interests of optimizing animal welfare and minimizing losses in product yield and quality, as well as food safety risks to consumers. As it is common in any evolving scientific discipline, the research discussed in this review often poses many more questions than it provides answers. While there is some evidence linking stress with pathogen carriage and shedding in farm animals, the mechanisms underlying this effect have not been fully elucidated. In fact, there is a lack of research evidence demonstrating a direct cause and effect relationship between what happens physiologically during a stress response and what happens to growth and shedding of foodborne pathogens. As shown, research in the area of microbial endocrinology has helped provide *in vitro* evidence, but our current knowledge of what really happens *in vivo* is very limited, at best.

Stress can occur in a variety of forms, physical or psychological, acute or chronic, and individual or concurrent. A major problem of applied stress research in farm animals relates to the methodology of stress assessment or to the lack of specificity of the selected parameter(s) to measure. A lot of research has been developed and published in this exciting and evolving scientific area (i.e., stress physiology) with a variety of physiological (e.g., hormones, neurotransmitters, heat shock proteins, heart rate, and others) and behavioral parameters being explored as potential stress markers or indicators. However, confounding is common and challenging often leading to contradictory or misleading results, probably also reflecting the differences in the quality, intensity, frequency, and duration of the impinging stressor(s). The response of an individual animal to a stressor also depends on genetic factors and early experiences. Therefore, within species, and even within a defined population or group, individuals differ in their neuroendocrine response to challenges/stressors. Obviously, research in animal stress and welfare is complicated *per se*, but it becomes much more complicated when aiming to determine the effects of stressors on other also complex biological variables, such as microbial populations in the gastrointestinal tract. Therefore, studies under controlled conditions, based on solid experimental designs are of critical importance and necessary to advance our knowledge in this area, particularly focusing on the effects of common stressors, individually and combined (i.e., simultaneously occurring), on the susceptibility of farm animals to infections with different foodborne pathogens, and on the carriage and shedding levels of foodborne pathogens as well as of bacteria resistant to antimicrobials.

Understanding when pathogen frequency and load (or level) on the farm are the highest or when animals are most susceptible to infection will help identifying times when prevention or control measures may be most effective. Some of the potential intervention measures to be strategically applied include changes in management practices that minimize stress and promote animal well-being, and manipulation (or stabilization) of the gastrointestinal microbiota through the use of probiotics, prebiotics, symbiotics, and other products (via feed and/or water).

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