

## **SALMONELLA IN THE FEED INDUSTRY: PROBLEMS AND POTENTIAL SOLUTIONS**

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**ABSTRACT:** *Salmonella* is one of the most important foodborne zoonotic pathogens, with significant health and economic impact in both humans and animals. The control of *Salmonella* in animal feedstuffs is important, principally to protect the human food chain from contamination by *Salmonella* derived from infected animals. *Salmonella* can reach into the animal feed by multiple ways and during all production stages. Main sources of contamination are fertilizers on the pasture/fields, ingredients, co-products, dust, wild animals and contaminated equipment. Thus, there is importance for implementation of strategies for preventing feed contamination with *Salmonella*, by minimizing dust, maximizing hygiene of space and processing equipment in feed mills and implementing control measures in each stage of feed production. Elimination of *Salmonella* refers to thermal treatment (pelleting, extrusion) or chemical treatment. Feed additives, such as organic acids, short- and medium-chain fatty acids, prebiotics, probiotics and, more recently, essential oils of plant origin, have the potential to reduce *Salmonella* levels when added to the feed. Therefore, the purpose of this review is to discuss the prevalence and prevention methods for the control of *Salmonella* in the feed industry.

**Key words:** *animal feed, pathogenic bacteria, Salmonella, contamination*

### **INTRODUCTION**

*Salmonella* is a leading cause of foodborne disease in humans worldwide and is a significant cause of morbidity and even mortality and consequently high economic losses (Foley and Lynne, 2008). Although many foods contain *Salmonella*, raw meats, poultry, eggs, milk, and dairy products are often implicated as sources of infection (Jones, 2011). For this reason, consumers are increasingly concerned about *Salmonella* contaminations in their food. There are, in turn, many sources of *Salmonella* contamination in animals, including feed and feed ingredients of plant origins such as seeds and cereal grains (Sanchez et al., 2002).

Although their native habitat is the intestinal tract of humans and animals, *Salmonella* spp. are widely found in nature, where is able to survive, but not multiply, for long periods on material that contain very low levels of moisture (Jones, 2011). Some species exist in animals without causing disease symptoms; others can lead to any of a wide

range of mild to serious infections called salmonellosis in humans. This genus is composed of two species, *Salmonella enterica* and *Salmonella bongori*. *S. bongori* comprises 22 serotypes that are mainly associated with cold-blooded animals, and human infections are uncommon (Lamas et al., 2018). *S. enterica* is divided into six subspecies. The subspecies *enterica* is responsible for more than 99% of human salmonellosis, and it includes 1,531 serotypes among which are *Salmonella* Typhimurium and *Salmonella* Enteritidis. Humans are the only reservoir of typhoid *Salmonella*, produced by *Salmonella* Typhi and *Salmonella* Paratyphi. The rest of *Salmonella* serovars are known as non-typhoid, where the animals are the major reservoir (Eng et al., 2015). According to EFSA (2010) several serovars of *Salmonella* simultaneously occur in different parts of the food chain in many Member States of the EU, therefore it is difficult to evaluate the importance of feed as a source of *Salmonella* infection in animals and its subsequent spread to humans.

*Salmonella* has capability to modify according to the changing environment and it can develop resistance against routine elimination practices of sanitation, chemical treatments and antimicrobial drugs (Butaye et al., 2006; Chen et al., 2004; Vestby et al., 2009). Consequently, *Salmonella* may enter anywhere in the animal production chain and be a potential source of contamination of all feed ingredients. For this reason, in order to control *Salmonella* challenges in the animal production chain, all possible measures for controlling *Salmonella* should be used. This review aims to provide a brief overview of the *Salmonella* contamination issue in animal feeds as well as potential control measures for its limitation.

### ***Salmonella* PREVALENCE IN THE FEED INDUSTRY**

The presence of *Salmonella* spp. in many type of ingredients such as grains, oilseed meals, fish meal has been documented many years ago (Cox et al., 1983; Maciorowski et al., 2006), especially in food sources rich in protein (Wierup and Haggblom, 2010). Feed can serve as both a direct and/or an indirect route of transmission. This depends partially upon whether individual feed ingredients were originally contaminated prior to or during feed mixing or the mixed feed becomes contaminated during feeding (Maciorowski et al., 2006). In compounded feed and even in heat-treated and pelleted feed *Salmonella* may be found due to environmental contamination of feed mills and the high likelihood for cross contamination in the feed mill and during transport and storage at the farm (Berge and Wierup, 2012; Jones, 2011). In the production environment, *Salmonella* tends to form biofilms on both inert and organic surfaces. Recent studies have shown that *Salmonella* are capable of forming biofilm on different contact surfaces like glass, polymer, steel (Čabarkapa et al., 2015; Giaouris and Nychas, 2006; Vestby et al., 2009). In this state, bacteria are better protected against environmental stresses. Once a biofilm is formed, it becomes a source of feed contamination in processing lines, representing a serious concern for the feed industry (Lunestad et al., 2007).

*Salmonella* contamination has been associated with an elevated indicator organism such as *Enterobacteriaceae* counts (Jones, 2011). *Enterobacteriaceae* counts higher than  $10^4$  cfu/g in unprocessed feeds and higher than  $10^2$  cfu/g in processed feeds may be indicators of *Salmonella* presence (Jones and Richardson, 2004). A wide variety of *Salmonella* serovars have been identified in feeding stuffs. Serovars commonly isolated

from animal feed are *S. Typhimurium*, *Salmonella* Montevideo, *Salmonella* Hadar and *Salmonella* Tennessee (Lamas et al., 2018). The surveillance carried out by the European Member States shows that the appearance of *S. Typhimurium* and *S. Enteritidis* in animal feed was low and other *Salmonella* serovars were found mainly. *Salmonella* Senftenberg (11.6 %) was detected more frequently from poultry feed than *S. Enteritidis* (6.2%) and *S. Typhimurium* (4.1%) together (EFSA 2010).

Data on prevalence of *Salmonella* in different parts of the feed chain are difficult to compare as observed prevalence may be biased by difficulties in sampling and cultivation of *Salmonella* from feed sources as well as limitations of the detection methods (Binter et al., 2011). Therefore, it is very difficult to compare data from different studies and several hundreds of samples are sometimes necessary to determine contamination level accurately. In addition to the importance of collecting adequate samples, it is also important to ensure that samples are collected aseptically in order to assess the true contamination rates (Jones, 2011). According to Mitchell and McChesney (1991) at least 30 samples need to be analyzed individually to reliably determine if the given batch of feed ingredients is *Salmonella* negative. On the other hand, Haggblom (1994) found that large amounts of sampling material make expensive testing of *Salmonella* contamination. Furthermore, he suggested that the collection of samples only from feed manufacturing facilities including the raw material receiving pits, dust collection filters, top of pellet coolers, pellet cooling area and the top of finished feed bins is enough to isolate *Salmonella*. Since 1993, Sweden has abandoned the sampling of individual feed samples to detect the *Salmonella* contamination in poultry feed (Jones, 2011).

Dust proved to be the most sensitive monitoring sample for the detection and isolation of *Salmonella* (Haggblom, 1994; Jones and Richardson, 2004). Therefore, it is very important to control dust in feed mills from the very beginning of the feed production, i.e. from the unloading process where the largest quantity of dust is produced (Morita et al., 2006). Grinders, mixers, elevator legs, conveyers, pellet scalpers are additional points of dust formation that must be controlled within the feed mills (McDaniel, 2005). Morita et al. (2006) indicated feed mill operators as the major source of *Salmonella* cross-contamination and pointed out that it is important to designate areas within manufacturing facilities as “dirty” and to limit the flow of personnel, equipment, and “clean” air inbetween these areas.

Fats tend to protect *Salmonella* from environmental or physiological stresses. This is why the reduction of fat accumulation in the feed mill environment is very important in order to decrease *Salmonella* survival and spreading (D’Aoust, 2007; Morita et al., 2006). The control of temperature and moisture conditions is also of primary importance, since *Salmonella* can survive for long periods in dry material and multiply rapidly in presence of moisture (Sauli et al., 2005; Ziggers, 2003). Additionally, rodents and birds have long been recognized as vectors for spreading of *Salmonella* and other pathogenic bacteria (Jones, 2011; Morita et al., 2006). Without adequate control measures, *Salmonella* can become endemic in a feed mill and extremely difficult to eradicate due to formation of biofilms on the surfaces of the equipment, and control measures regarding this must be implemented (Davies and Wales, 2010).

## ***Salmonella* CONTROL MEASURES IN ANIMAL FEED**

Animal feed production is generally considered to be the primary source of *Salmonella* contamination. As already mentioned, *Salmonella* is very difficult to control and every possible tool should be used in the control program. Prevention of entry of *Salmonella* into production facilities and their replication within facilities, methods for eradicating the already present microorganisms are control measures for *Salmonella* spp. during the process of animal feed production. Diverse steps are available in the process of reducing or eliminating *Salmonella* contamination in feed, among which the heat treatment, use of organic acids, and other chemical preservatives are the most widely used (Jones and Richardson, 2004; Sauli et al., 2005).

The effect of heat on *Salmonella* depends on the treatment time, temperature and moist, but considerable resistance to heat is strongly influenced by the strain, the physiological state and the matrix in which the bacterium is found (Lunestad et al., 2007). Different heat treatments (pelleting, extrusion and expansion) can be employed to destroy bacteria in animal feed. It has been also suggested that animal feeds should be heated to 80–85°C to destroy *Salmonella* (Jones and Richardson, 2004), but heat tolerance varies among serovars, with decimal reduction times at 80°C (and 0.8 water activity) ranging from approximately 2 to 12 min (Davies et al., 2004). Although the heat is generally considered to be the most effective decontamination procedure, in some circumstances it is not sufficient and other options are applied. The goal of controlling the *Salmonella* in such cases can be achieved by the addition of chemical compounds including organic acids and formaldehyde (Carrique-Mas et al., 2007). ). Adding organic acids to animal feed changes its pH value (pH 4.5 and lower) and creates unfavorable conditions for the growth and survival of *Salmonella* (Dahiya et al., 2006). Short-chain fatty acids, such as formic, acetic, propionic and butyric acids, have all been shown to have an inhibitory effect on *Salmonella* growth (Van Immerseel et al., 2006). On the other hand, the use of formaldehyde is avoided due to evaporation and its toxicity to humans. Other compounds such as chlorine, peroxides, or ammonium compounds are also used, which also have a residual effect and cause changes in the sensory properties of the final products (Prunić et al., 2017). In addition, chemical treatment helps to reduce recontamination in feed and also helps to reduce contamination of milling and feeding equipment and the general environment (Berge and Wierup, 2012).

Nutritional strategies to minimize *Salmonella* in animal feed production are one of the key components in producing safer food, and can be carried out via general diet formulation or feed additives. According to EFSA (2017) feed additives are defined as substances, microorganisms or preparations other than feed material and premixtures which are intentionally added to feed or water in order to perform one or more functions mentioned in Article 5.3 of Regulation (EC) No 1831/2003 (Table 1). A number of feed additives have gained commercial acceptance in helping to reduce *Salmonella*, including probiotics and prebiotics which can be used to control the microbes in the intestinal tract while use of antibiotics may be prohibitive due to production cost factor (Berge and Wierup, 2012).

Probiotics have been defined as 'live-microorganisms which, when administered in adequate amounts, confer a health benefit on the host' (FAO/WHO, 2002). It have been shown that probiotics stimulate the development of a healthy microbiota, inhibit the growth and dissemination of pathogenic microorganisms, improve digestive capacity

and lowering the pH, improve mucosal immunity, or enhancing gut tissue maturation and integrity (de Lange et al., 2010).

Table 1. Conditions for authorisation of the use of feed additives (Official Journal of the European Union, 2003)

The feed additive shall	The feed additive shall not
<ul style="list-style-type: none"><li>• favourably affect the characteristics of feed,</li><li>• favourably affect the characteristics of animal products,</li><li>• favourably affect the colour of ornamental fish and birds,</li><li>• satisfy the nutritional needs of animals,</li><li>• favourably affect the environmental consequences of animal production,</li><li>• favourably affect animal production, performance or welfare, particularly by affecting the gastro-intestinal flora or digestibility of feedingstuffs, or</li><li>• have a coccidiostatic or histomonostatic effect.</li></ul>	<ul style="list-style-type: none"><li>• have an adverse effect on animal health, human health or the environment,</li><li>• be presented in a manner which may mislead the user,</li><li>• harm the consumer by impairing the distinctive features of animal products or mislead the consumer with regard to the distinctive features of animal products.</li></ul>

Further, many *in vitro* and some *in vivo* studies have suggested that the lactic acid bacteria *Bifidobacterium* and *Lactobacillus* and probiotic yeast *Saccharomyces boulardii* are able to prevent intestinal infections caused by the adherence or invasion of *S. Typhimurium* (Collado et al., 2007; Czerucka and Rampal., 2002; Tomičić et al., 2016). Prebiotics are non-digestible food ingredients which beneficially affect the host by stimulating the proliferation and activities of bacteria associated with a healthy gut, such as bifidobacteria and lactobacilli (Gibson et al., 2004). Commonly used prebiotics in animal feed are oligosaccharides with different molecular structure. Prebiotics may be used alternatively or support the effect of probiotics, whose combination may be even more efficient in the stimulation of intestinal microbiota and protection of animal health (Markowiak and Slizewska, 2018). Recently, there was extensive research on the antimicrobial activity of essential oils against gram-negative *Salmonella* and *Echerichia coli* over gram-positive *Listeria monocytogenes* (Burt, 2004; Tomičić et al., 2018; Puvača et al., 2018), seeking natural and safer means for food hygiene or preservation.

## CONCLUSIONS

Animal feed producers have ethical obligations to reduce the risk of foodborne hazards in animals under their care. Contamination of feed with *Salmonella* can originate from different sources and a variety of routes, and it is one of numerous potential sources of animal and human infection with *Salmonella*. Several improvements in the feed processing industry, including improved hygiene, feed treatments and good

manufacturing practices in the feed mill, might lead to an improved control of this pathogenic organism.

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