A Review of Prevention and Control Methods of *Salmonella* species in Swine Production: The Role of Dietary Non-Nutritional Additives

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**ABSTRACT**

The control of *Salmonella* spp. is still an important issue in pork production. Contamination happens at any stage of the production chain and no stand-alone measure is efficient enough to eliminate this pathogen. In order to minimize *Salmonella* contamination, the farm-to-fork approach is currently used, in which specific measures are implemented in each sector of the production chain. The already known hygiene measures in the abattoir are important to reduce the risk of carcass contamination; however, pre-slaughter conditions have been shown to be high risk factors at slaughter and during processing. *Salmonella* spread at slaughter can be traced back to the pig herd and therefore, there has been increasing focus on the pre-harvest phase. Numerous studies have identified risk factors for *Salmonella* infection in pigs and reviews studies have presented interesting and important comparative analyses on this subject. The use of dietary additives for pig and their effects on *Salmonella* infection have been studied during the last years. Particularly interesting is the use of dietary non-nutritional additives, such as prebiotics, probiotics, phytogenics and essential oils and organic acids. Although, very promising, much research is needed in this field in order to identify the most efficient products and/or compounds and reveal how they act on the bacterium metabolism, aiming to improve the control of *Salmonella* contamination along the swine production chain. In this review, we surveyed the literature to present a compilation of the scientific knowledge currently available about potential uses of dietary non-nutritional additives to prevent and/or control *Salmonella* infection in swine populations.

**Key words:** On-farm control, dietary non-nutritional additives, pig, *Salmonella*, slaughter

**INTRODUCTION**

Serious public health and economic issues are related to foodborne pathogens. Many discussions have been raised on the impact of these micro-organisms of food safety in the last decade, among them the role of *Salmonella* in pork is of major interest (Henao et al., 2010).

Despite technological advances, *Salmonella* is still an important issue to the pork industry worldwide. Out of the estimated 80.3 million cases of food-borne salmonellosis in humans occurring annually in the world (Majowicz et al., 2010), nearly 56.8% were related to pigs and their products (EFSA, 2013). In the US, annual socio-economic costs attributed to pork salmonellosis were estimated at $81.53 million (Miller et al., 2005).

The effectiveness of *Salmonella* control programmes have been proven in poultry in many countries (EFSA, 2010a). Therefore, the same challenge is now faced by the pig industry. In this…
way, to minimize Salmonella contamination, the farm-to-fork approach is currently used, in which specific measures are implemented in each sector of the production chain (O’Reilly et al., 2007). According to the EFSA (2010b), it is estimated the prevalence of the main Salmonella serovars in pigs to be approximately 10.3% at slaughter. Carcass contamination do no exclusively results from pathogen-bearing animals but also from contact with other contaminated carcasses and/or surfaces in the abattoir (Rostagno and Callaway, 2012). Although hygiene measures (or its absence) in the abattoir are important risk factors for carcass contamination (Delhalle et al., 2008; Baptista et al., 2010), pre-slaughter conditions (transportation, lairage, etc) have been shown to increase the risk of contamination at slaughter and during processing (Hurd et al., 2002; Rostagno et al., 2003). In fact, Siekkinen et al. (2006) showed that Salmonella spread at slaughter, by cross contamination, can be traced back to the pig herd rather than be originated from the inherent slaughter plant microflora. Because infection and/or contamination may occur at different levels of the pig production chain, most efforts are made at the level of primary production to minimize the incidence of infected animals (Wierup, 1997).

According to Baptista et al. (2010), reducing Salmonella contamination at the farm level would have major impacts on post-harvest contamination control, due to the lower contamination pressure entering the abattoirs. In fact, some authors (Hurd et al., 2002; Wegener, 2010) have shown that combined pre and post-harvest measures are more effective in reducing the incidence of Salmonella in pork. However, in pork production, the control of Salmonella at the farm level remains a challenge. Because there is no unique strategy for the effective eradication of Salmonella from pig herds, the implementation of biosecurity, sanitation, vaccination, medication and management of known risk factors (Denagamage et al., 2007; Godsey et al., 2007; Baptista et al., 2010) is crucial but often insufficient as stand-alone measures (Mannion et al., 2007).

In this regard, the use of non-nutritional additives, such as prebiotics, probiotics, phytogenics and essential oils and organic acids may contribute to reduce Salmonella at farm level. Although promising, many inconsistencies are still found in the literature leading to uncertainties on the use of some of these additives. This field is a rich area of research and much needs to be done to clarify the contradictory results and therefore improve the control of Salmonella contamination along the swine production chain.

In this review, we surveyed the literature to present a compilation of the scientific knowledge currently available about potential use of dietary non-nutritional additives to prevent and/or control Salmonella infection in swine on-farm. Considering the wide literature on this subject, here it presented an insight of the most promising additives.

**Role of Salmonella spp. in swine, pork and humans:** Salmonella is one of the major foodborne diseases around the world. Besides its relation with a wide variety of food, the endemic and high morbidity make this zoonotic pathogen a public health issue (Greig and Ravel, 2009). In the United Kingdom and other European countries, Salmonella enteritis and Salmonella typhimurium (S. typhimurium) are responsible for most of the human cases of salmonellosis (Anonymous, 2009), causing from mild to fatal foodborne illness (Freitas, 2011). Foods of animal origin are the main responsible for these serious problems and among other meat products, pork is of remarkable interest (Kuhn et al., 2013; CDC., 2014), with some variation among countries (Table 1).

**Genus salmonella:** The term salmonellosis is related to different clinical syndromes that include gastroenteritis, bacteremia and endovascular infections. The incubation period is 6-12 h and the

Table 1: Pig carcasses/meat contamination by Salmonella at the abattoir and confirmed cases of salmonellosis in human in selected countries in 2011

<table>
<thead>
<tr>
<th>Country</th>
<th>Description</th>
<th>N</th>
<th>N positive</th>
<th>Positive (%)</th>
<th>Confirmed cases in humans /100,000</th>
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<tbody>
<tr>
<td>Belgium</td>
<td>Abattoir</td>
<td>0.649</td>
<td>44</td>
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<td></td>
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<td>0.292</td>
<td>06</td>
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<td>0.0</td>
<td>12.3</td>
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<td></td>
<td>Processing</td>
<td>0.705</td>
<td>02</td>
<td>0.3</td>
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<td></td>
<td>Retail</td>
<td>0.203</td>
<td>07</td>
<td>3.4</td>
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<tr>
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<td></td>
<td>Processing</td>
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<td>01</td>
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<tr>
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<td>0.0</td>
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<tr>
<td>Germany</td>
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<td>10</td>
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<td>Processing</td>
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<tr>
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<td>0.400</td>
<td>00</td>
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<tr>
<td>Spain</td>
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<td>0.268</td>
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<td>32.8</td>
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<tr>
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<td>Retail</td>
<td>0.116</td>
<td>06</td>
<td>5.2</td>
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Adapted from: EFSA (2013)

Initial symptoms are nausea, vomiting, bloody diarrhea as well as fever, abdominal pain, headaches and chills (Sanchez-Vargas et al., 2011).

Salmonella infection occurs mainly through the orofecal route. The colonization of the distal portion of the small intestine is the first step in the pathogenesis, followed by invasion of the epithelial tissue (Muller et al., 2012). This bacterium can remain in gut lymph nodes and be excreted intermittently during periods of stress, even if clinical signs are no longer present (Berchieri et al., 2000). This is the reason for the high potential of Salmonella contamination throughout the production chain (De Busser et al., 2013).

Members of the genus Salmonella are gram-negative rod-like shape bacteria, part of the Enterobacteriaceae family (Saif et al., 2008). These bacteria do not form spores and most Salmonella present motility, they grow in oxidase-negative colonies with gas formation, under temperatures ranging from 7-45°C and pH from 4.0-9.5 (Ekperigin and Nagaraja, 1998). They are very thermal resistant, being viable after long periods (months until years) (Hirsh, 2003).

The genus Salmonella is formed by two species, Salmonella enterica and Salmonella bongori (Grimont and Weill, 2007). Salmonella enterica is composed by six biochemical and genomic different sub-species that are S. enterica sub-species enterica, sub-species salamae, sub-species arizonae, sub-species diarizonae, sub-species houtanae and sub-species indica. In contrast, Salmonella bongori has only one sub-species that is bongori (Guibourdenche et al., 2010). Each sub-species is composed by various serogroups and serotypes and respective lineages. Approximately 99% of all the most common isolated serotypes belong to the sub-species enterica (Grimont and Weill, 2007).

Salmonella may also be classified according to the host they parasitize in serotypes host-adapted or non-adapted. Serotypes host-adapted include those that parasitize almost exclusively one single animal species and usually cause clinical disease, i.e., Salmonella enterica serotype typhi in humans, Salmonella enterica serotype choleraesuis in swine, Salmonella enterica serotype dublin in cattle, Salmonella enterica serotype pullorum and Salmonella enterica serotype gallinarum in chicken (Schwartz, 1999). Serotypes non-adapted to host include those that parasitize a wide range of animal species and usually cause self-limiting disease that are restricted to the intestinal tract, i.e., typhimurium and enteritidis.
Swine contamination: Multiples sources of *Salmonella* contamination are present along the swine production chain (Kich et al., 2011) (Fig. 1). The introduction of this pathogen may occur through the purchase of replacement stock or animals from different origins as well as via feed, biological vectors (rats, humans, infected pigs, etc) during transport and even at lairage on the slaughter plant (Kich and Cardoso, 2012).

Among the main sources of infection on-farm, the purchase of replacement stock and feed are of major concern because of the high volume and frequency of arrivals to the farm (Gibert and Jaime, 2010). It was demonstrated that the introduction of *Salmonella* into the herd through infected purchased pigs increases *Salmonella* prevalence at slaughter (Van der Heijden et al., 2005). In gilts, Davies and Hilton (2000) reported an increased *Salmonella* excretion after introduction of animals into a new herd, whereas Quessy et al. (2005) showed increased odds of sero-positivity when purchasing replacement stock was made from multiples supplier.

According to Wierup and Haggblom (2010), *Salmonella* can be introduced into the feed by contaminated ingredients; however, contamination can occur during processing, transport, storage at the farm, distribution and administration (Jones and Richardson, 2004). Furthermore, associations between animal feed contamination and both animal and human *Salmonella* infections has been regularly established (EFSA., 2008; Jones, 2011).

Many biological vectors can act as reservoir of *Salmonella* and their presence in the production system increases the risk of swine contamination (Gibert and Jaime, 2010). The absence of rodents control programs on-farm was shown to be related to high risk of infection in pigs in different countries (Letellier et al., 1999a, b; Kich et al., 2005). No only visitors but also the workers of pig husbandries were reported to be risk factor for *Salmonella* transmission. According to Gibert and Jaime (2010), the habit of frequently washing hands was correlated with a lower serologic
prevalence of this pathogen. These same authors stated that the sanitary status of the animals is also of ultimate importance due to the higher risk of *Salmonella* spread in animals co-infected with enteric pathogens, such as *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and *Escherichia coli*.

At the farm level, the finishing phase was shown to be especially important for the increase of *Salmonella* infection (Funk et al., 2001). According to Garcia-Feliz et al. (2009) and Beloeil et al. (2007), the odds of *Salmonella*-positivity were related to farm size, in which finishing units harvesting 3500 pigs or more per year had a higher risk for *Salmonella* shedding. Residual environmental contamination, after cleaning and disinfection, of finishing pens was reported by Funk et al. (2001) as a common occurrence in *Salmonella* positive herds. In this regard, common infections occurring at this period (i.e., *Lawsonia intracellularis* and PRRS virus) were shown to influence *Salmonella* shedding (Beloeil et al., 2004). Furthermore, the fasting period before transport may be related to alterations in the intestinal microflora leading to greater counting of Enterobacteriaceae in the caecum and *Salmonella* in faeces (Martin-Pelaez et al., 2009). However, a variety of additional risk factors may affect the probability of *Salmonella* infection of finishing pigs.

**Pork contamination at slaughter:** An increase in *Salmonella* prevalence after transport from farm to slaughter has been detected in some studies (Rajkowski et al., 1998; Arguello et al., 2011). Pigs are often healthy carriers of *Salmonella* spp. and the stresses associated with transport may induce these latent carriers to become active shedders, resulting in contamination of the environment. In this way, Mannion et al. (2008) demonstrated the need for more stringent cleaning of transport trucks as a measure to reduce the potential for contamination of pigs.

Considering that pigs can acquire this pathogen following exposure times of 30 min to 2 h (Boughton et al., 2007), transport time may be another important risk factor. Longer transport times were shown to be associated with higher level of *Salmonella* shedding (Kasbohrer et al., 2000); however, Rajkowski et al. (1998) did not observe any differences between short or long transport times. Possibly, other factors are involved with *Salmonella* shedding in long and short transport times. More research should be carried to identify and prevent these potential factors.

The continuous entrance of infected pigs in the slaughter plant is considered the main risk factor for the contamination of pig carcasses and pork by *Salmonella* (Arguello et al., 2013a). Inappropriate cleaning and disinfecting procedures of the lairage pens increases the risk of external (skin) more than internal contamination (intestinal content and lymph nodes) (De Busser et al., 2011) and according to Rossel et al. (2009), carcass contamination is directly related to the pig skin contamination prior to stunning.

After stunning, scalding is the most important source of carcass contamination due to the presence of faeces, feed and microorganisms in the water (De Busser et al., 2013). During evisceration, the main risk of carcass contamination is through a leakage of the intestinal content (Arguello et al., 2013a) and the cross-contamination by equipment and butcher’s hands (Berends et al., 1997). Although, Good Manufacturing Practices may help preventing the cross contamination during slaughter and processing, the most effective way to reduce contamination at slaughter is by lowering the infection pressures at the farm level (Borch et al., 1996).

**TRADITIONAL METHODS FOR THE CONTROL OF Salmonella ON FARM**

The prevention and control of *Salmonella* infections in pigs are difficult, especially at the level of primary production. Although a variety of factor may influence *Salmonella* prevalence in pig
husbandries, such as facility design (Bahnson et al., 2006) and environmental temperature and season (Funk and Gebreyes, 2004), considering that for most strains of Salmonella, after an initial reduction in viability in the first 72 h no further reduction was seen over at least 30 days in stainless steel surfaces at 25°C and 33% humidity (Margas et al., 2014), hygiene and biosecurity on-farm are of great importance in decreasing Salmonella prevalence in carcass at slaughter plants (Hotes et al., 2011).

**Hygiene and biosecurity:** Animal housing environment contamination and poor biosecurity measures has long been implicated in many studies as a source of Salmonella infection (Williams, Jr. and Newell, 1968; Fosse et al., 2009). The importance of biosecurity measures, such as the use of specific clothes and boots when entering the facility were demonstrated (Rajic et al., 2007; Hotes et al., 2010). Also, it is known that most disinfectants based on sodium hypochlorite or quaternary ammonium compounds are able to eliminate Salmonella bacteria. However, inadequate cleaning, dosage or contact time may impair their efficacy (De Busser et al., 2013). In fact, challenges and problems are well documented in this subject (Davies and Wray, 1995; Madec et al., 1999).

Curiously, some studies have reported a lower Salmonella shedding prevalence in non-disinfected facilities (Van der Wolf et al., 2001a, b; Poljak et al., 2008). One speculation is that producers who use disinfectants are less careful with clean, believing that the disinfectant would compensate their inefficient cleaning. In this regard, some studies (Davies and Wray, 1996; Madec et al., 1999) have shown that terminal disinfection (through fogging or fine mist of formaldehyde), decreases Salmonella contamination but does not eliminate. Although there is a rich literature on pig housing contamination, interestingly, little is known about cleaning and disinfection protocols that are most effective against Salmonella (Funk and Gebreyes, 2004). Therefore, new studies on the components of cleaning and disinfection practices in swine housing that are effective and economically feasible are needed.

**Facility-related measures:** The facility design was found to be an important risk factor in Salmonella shedding and contamination. The presence of flush-gutter flooring was associated with higher Salmonella prevalence than slotted floors (Davies et al., 1997a, b). According to Hotes et al. (2010), lower serologic prevalence was observed in pigs housed on fully slotted floors. In an interesting study, Beloeil et al. (2004) reported that the frequency of pigs positive to Salmonella in farrow-to-finish herds was lower when a frequent removal of sows’ dung during lactation and the emptying of the pit underneath the slotted floor were performed. Facilities allowing snout contact through pens was associated with increased Salmonella prevalence (Lo Fo Wong et al., 2004; Wilkins et al., 2010). Also, Funk et al. (2001) had demonstrated that higher pig density per pen was associated with high Salmonella prevalence, suggesting that the transmission or shedding of Salmonella is increased by pig-to-pig contact or stress.

**Management practices:** Common management practices have also been shown to be capable of affecting the risk of Salmonella infection in finishing pigs. The prevalence of Salmonella at slaughter may be reduced by an adequate pig purchase policy (Van der Heijden et al., 2005). Lo Fo Wong et al. (2004) reported that increased odds sero-positivity are observed in herds purchasing replacement stock from more than three supplier and finishers from more than one. Additionally, Zheng et al. (2007) showed that integrated herds were less likely to become infected. The use of all in/all out systems was also reported to be an effective control measure against
Salmonella (Lo Fo Wong et al., 2004). However, Proescholdt et al. (1999) found no significant difference between all in/all out and continuous flow systems, whereas Funk et al. (2001) reported a high prevalence (up to 70%) in a three-site all in/all out production system. Although, not always successful, due to persistent contamination, some countries use depopulation as a method of control (Mogelmose et al., 1999). Due to these contradictory results, this subject deserves further investigation.

Another common practice, the split marketing, was shown to increase bacteriologic and serologic prevalence of Salmonella, immediately prior to shipping, from the first to the last group of pigs moved out of the finishing barns (Rostagno et al., 2009). In contrast, Morrow et al. (2002) reported a lower isolation of Salmonella in caecum contents at slaughter in older marketing groups of pigs, possibly because those pigs had more time to recover from the infection prior to slaughter.

Feed-related measures: The role of feed as a potential source of Salmonella is well established and reviewed (Crump et al., 2002; Davies et al., 2004; Molla et al., 2010). Among the factors that have been identified are feed form (pelleted or meal), feed water content (dry or wet feeding) and heat-treatment. These factors may act on the physiology of the gut, altering some conditions, such as microflora populations. In pigs, pelleted feeds have been reported to increase the risk of Salmonella infection (Garcia-Feliz et al., 2009; Hotes et al., 2010; Wilkins et al., 2010). Although pelleting of feed has long been recommended as a means of decontaminating pig feeds (Edel et al., 1967), according to an European Food Safety Authority (ESFA) study, feeds of commercial compound origin or pelleted feed were found to be risk factors for increased Salmonella positivity (EFSA., 2011). However, Bysted (2003) did not found differences for Salmonella positivity between meal and pellets in sows. Lower risk of Salmonella infection in pigs has also been associated to the use of liquid feeding, when compared to solid feed (Hotes et al., 2010; Poljak et al., 2008). However, it has to be stated that fermented or acidified wet feed (lower pH) does not provide the same results as regular wet feed. This is the reason for the controversial results found in the literature in which some authors showed lower Salmonella prevalence in pigs fed wet than dry feed (Bahnsen et al., 2006; Hautekiet et al., 2008), whereas other authors reported the opposite finding (Rajic et al., 2007; Farzan et al., 2006). Another method of control is the use of heat-treated feed. Salmonella may be eliminated by heat treatment performed at 93°C for 90 sec with 15% moisture; however, the level of contamination is a critical factor (Himathongkham et al., 1996). Even more, in addition to the heat damage to nutrients and the adverse effect on the integrity of pellets (Peisker, 2006), as heat treatment has no residual effect, re-contamination of feed can occur.

Vaccination: Considering that the innate immune response lacks “Memory”, although often successful in controlling the initial growth of Salmonella, it does not ensure a long-term resistance (Dougan et al., 2011). In contrast, the acquired immune system (humoral and cell-mediated immune response) allows the establishment of immunity to re-infections (Mastroeni et al., 2001). Vaccination against Salmonella is currently used successfully in poultry in Europe (EFSA., 2012). In pigs, studies have reported decreases in clinical signs and excretion of Salmonella (Farzan and Friendship, 2010; De Ridder et al., 2013). In a review article, Friendship et al. (2009) reported that from 15 studies evaluated, 14 presented reduction of Salmonella prevalence, ranging from 20-80% in weaned pigs to 86% in sows. However, a limited number of studies have documented swine vaccines that are effective against multiple Salmonella serovars (Roof and Doitchinoff, 1995; Charles et al., 2000; Neubauer and Roof, 2005). According to Christensen and Rudemo (1998), the variety of materials or methods used in manufacturing vaccines may result in different levels of
effectivity, safety or side effects. To date, live vaccines orally administered are believed to provide the best protection and should be considered as a control measure against *Salmonella*. However, most of these studies were conducted with relatively small numbers of pre-weaning (Rosler et al., 2010) or weaned piglets (Leyman et al., 2012; De Ridder et al., 2013) and used a challenge infection protocol. Their relevance for field conditions needs to be verified with large numbers of animals and also with finisher pigs (Wray, 2001).

**Antibiotics:** The use of antibiotics is another tool for the control of *Salmonella* infections in swine herds. However, because of the various factors related to the intestinal microflora, possible resistance of some strain and the route and dose administered to the pig, the literature presents inconsistent results, (Funk et al., 2007; Rajic et al., 2007; Varga et al., 2009; Hotes et al., 2010; Farzan et al., 2010; ). In theory, the use of antibiotics should be effective in controlling *Salmonella* infections and shedding but the review study of Friendship et al. (2009) showed a possible selection for resistant serovars that may be potentially related to more severe infection.

**CONTROL OF CARCASS CONTAMINATION AT SLAUGHTER**

**Transport and lairage:** The stress of transport from farm to abattoir increases the *Salmonella* shedding by carrier pigs (Rostagno et al., 2011). Practices performed prior to transport, such as fasting periods were associated with changes in the gut microbial ecosystem with increasing levels of *Salmonella* excretion in faeces (Martin-Pelaez et al., 2009). After transport, at the lairage area, Rossel et al. (2009) have demonstrated that carcass contamination is related to skin contamination before stunning. Therefore, some measures such as adequate area, keeping small groups, presence of showers, slatted floors and good handling may be performed to reduce stress and consequently, the susceptibility to *Salmonella* infection (Hurd et al., 2001). However, none of these practices are effective if proper cleaning and disinfecting procedures are not implemented on trucks after each delivery at the abattoir and at the lairage area (Swanenburg et al., 2001) but, in practice, this is difficult and expensive to achieve.

**Slaughter process:** During the slaughter process, carcass *Salmonella* contamination may possibly occur in several points (Borch et al., 1996). According to Hald et al. (2003), dehairing was a high risk factor for carcass contamination when scalding water tested positive for *Salmonella*. Scalding water temperatures higher than 62°C were shown to be effective in controlling carcass contamination, as long as the volume of organic material does not protect the microorganisms from heating (De Busser et al., 2013). De Busser et al. (2011) reported that chilled contaminated carcasses were related to the contamination after polishing. Therefore, even if singeing is performed before polishing, adding a second flaming device after would help avoiding contaminated carcasses to enter the clean area of the abattoir (De Busser et al., 2011; Da Silva et al., 2012).

The most important and critical step for carcass contamination by *Salmonella* during slaughter is the evisceration (Berends et al., 1997). According to De Busser et al. (2013), good fasting of the delivered pigs, correct evisceration techniques and proper training of abattoir workers are effective in reducing the risk of accidental cutting through the intestines. Carcass contamination during evisceration can be prevented by ease and simple hygiene and sanitization methods by the evisceration staff (Wheatley et al., 2014). Therefore, particular attention should be given to the cleaning management of knives, especially the temperature variations of the water used to clean evisceration knives. The abattoir workers (Bertrand et al., 2010), splitting saw (Smid et al., 2012) and veterinary inspection agents (Vieira-Pinto et al., 2006) are additional risk factors of
cross-contamination. In this regard, the cleaning and disinfecting of the splitting saw many times daily was shown to reduce carcass Salmonella contamination (Delhalle et al., 2008).

Carcass decontamination: Although, all the previous mentioned measures can be performed to avoid carcass contamination by Salmonella, some few procedures also exist to treat contaminated carcasses. It has been shown that washing carcass using high pressure water (Brustolin et al., 2014), water at 80°C for 14-16 sec and the use of acidified sodium chlorite reduced the prevalence of Salmonella on carcass (Hamilton et al., 2010). According to Goldbach and Alban (2006), to avoid the high costs of hot water decontamination, the use of steam suction and ultra-sound appear as possible alternatives. In fact, the combined effect of steam and immersion in a solution of 1000 ppm of organic acids was efficient in controlling superficial contamination by S. typhimurium (Machado et al., 2013). Considering the importance of those measures, more research should be done on this subject.

DIETARY NON-NUTRITIONAL ADDITIVES FOR THE CONTROL OF SALMONELLA SSP. ON FARM

The demand for reduction of antimicrobial use in animal production and the ban on their use as feed additives in the European Union (Regulation 1831/2003/ EC) has contributed in part to a growing need for alternative control strategies for bacterial pathogens of food-producing animals, including S. typhimurium infection of pigs. In this regard, dietary strategies have focused on the prophylactic application of various in-feed supplements such as prebiotics, probiotics, phytogenics, essential oils and organic acids. Such approaches have been demonstrated to improve gut function; however the response of Salmonella populations to such dietary treatments has been more contradictory (Martin-Pelaez et al., 2010).

Prebiotics: The term prebiotic was defined by Gibson and Roberfroid (1995) as “A non-digestible food ingredient that beneficially affects the host by selectively stimulating the favourable growth and activity of one or a limited number of bacteria in the colon and therefore attempt to improve host health”. Prebiotics are mainly medium to long-chain carbohydrates called oligosaccharides or soluble fibre but can also be proteins, peptides and some types of lipids (Searle et al., 2010; Kim et al., 2011).

These prebiotics feed commensal enteric bacteria or probiotics bacteria, offering them a competitive advantage over potential pathogens, such as Salmonella. Developing prebiotic alternatives to antibiotic growth promoters is especially challenging in the area of prevention of intestinal infections (Brufau, 2003). Prebiotics are believed to combat pathogens using less resources, reducing the use of energy by the innate immune responses (Bailey, 2009) and modulating intestinal epithelial cells and dendritic cells functionality (Gaggia et al., 2010), thus helping to preserve gut homeostasis.

A number of carbohydrates (based on glucose, mannose, galactose and fructose) have been shown to have anti-infective properties. Mannose and its polymers are the most commonly used products as feed additives and have long been shown to reduce Salmonella colonization in chickens (Oyofo et al., 1989) and recently in pigs (Badia et al., 2013). The large majority of Salmonella contain mannose-specific lectins (Type 1 fimbriae) on the bacterial surface that bind to glycoproteins (rich in mannose) on the intestinal surface. Mannose sugars can thus compete with the intestinal glycoproteins for attachment sites and prevent colonization. Similar findings have been demonstrated with mannan oligosaccharide at significantly lower concentrations than that required for purified mannose (Spring et al., 2000).
Callaway \textit{et al.} (2008), using an \textit{in vitro} simulation technique for ruminal fermentation, found that pectin could also significantly reduce the prevalence of \textit{Salmonella}. Although those results were partially confirmed by Pieper \textit{et al.} (2009) \textit{in vitro}, these authors suggested that it is not clear to what extent such results could be transferred to \textit{in vivo} conditions and reduce \textit{Salmonella} colonization and/or the transmission among animals.

In an interesting study of Martin-Pelaez \textit{et al.} (2008), \textit{Salmonella} counts were significantly reduced with lactulose as a substrate. Relatively little is known about the \textit{in vivo} effect of lactulose fermentation on the immune response in pigs. However, one study has shown that IL-6 is increased in the colon of pigs fed fermentable carbohydrates (Pie \textit{et al.}, 2007), suggesting that feeding pigs fermentable carbohydrates, such as lactulose, may increase lactic acid producing bacteria, such as \textit{Lactobacillus}, which may increase IL-6 expression in the pig colon.

These results show that the prebiotic effect not only influence the microbial composition of the gut but also to influence the immune system of the host (Roberfroid \textit{et al.}, 2010). In fact, Naqid \textit{et al.} (2015) reported that total serum IgM and IgA levels against \textit{S. typhimurium} were significantly higher in pigs supplemented with lactulose. These results are similar to Yin \textit{et al.} (2008), in which dietary supplementation with prebiotic galacto-mannan-oligosaccharide or chitosan oligosaccharide significantly increased serum levels of IgG, IgM and IgA antibodies in weaned piglets. The mechanisms by which prebiotics affect the immune system are not fully established, it has been proposed that they may have an indirect action through the alteration of native microbiota of the intestine and possibly the resulting changes in microbial metabolite production (Gourbeyre \textit{et al.}, 2011).

Although, most studies have shown positive effects of prebiotics on \textit{Salmonella} infection, Ten Bruggencate \textit{et al.} (2004) indicated a possible adverse effect with increased colonization of \textit{Salmonella} by using fructo-oligosaccharides and inulin.

**Probiotics:** By definition, probiotics are living microorganisms that are fed to animals to colonise the gut environment to create a better microbial balance (Bello \textit{et al.}, 2001). Probiotics have been shown to stimulate gut mucosal and systemic immunity, increasing protection and inhibiting growth and dissemination of pathogenic microorganisms. Currently, the approved and most used probiotics for pigs include \textit{Bacillus} sp. and Bacillus spores, \textit{Lactobacillus} sp., \textit{Lactococcus} sp., \textit{Bifidobacteria} sp., \textit{Pediococcus} sp., \textit{Enterococcus} sp. and \textit{Saccharomyces} sp. (European Commission, 2011).

\textit{Bacillus subtilis} and \textit{Bacillus licheniformis} have been shown to reduce the aggression of \textit{Salmonella} into swine intestinal epithelial cells \textit{in vitro} (Aperce \textit{et al.}, 2010), although this has not been confirmed \textit{in vivo}. In a pig model, Walsh \textit{et al.} (2012) reported, 5 day post challenge, no \textit{Salmonella} in faeces of pigs fed probiotics. These same authors demonstrated that the combined effects of \textit{Bacillus} and \textit{Enterococcus} for weaned pigs challenged with \textit{S. typhimurium} had no effect on prevalence of the pathogen in organs or digesta. In this sense, the use of \textit{Enterococcus faecium} does not appears to be appropriate for the control of \textit{Salmonella}. Kreuzer \textit{et al.} (2012) reported any protective effects of \textit{Enterococcus faecium} on clinical symptoms, shedding or distribution of \textit{S. typhimurium} into organs, whereas Szabo \textit{et al.} (2009) observed a tendency to increase the shedding of \textit{S. typhimurium} in faeces and the count of \textit{Salmonella} in organs of weaned piglets.

The isolation and characterization of anti-Salmonella lactic acid bacteria from porcine gut has identified probiotics that survive the gut passage (Casey \textit{et al.}, 2004). In fact, studies in pigs have shown that lactic acid bacteria can improve immune responses to \textit{Salmonella choleraesuis}, promoting a faster clearance (Chang \textit{et al.}, 2013). According to Yin \textit{et al.} (2014), \textit{Lactobacillus casei
added to feed was more effective in reducing diarrhea and intestinal burden of *Salmonella typhimurium* in pigs, whereas *Lactobacillus zeae* was able to lower the acute-phase local and systemically inflammatory responses and the invasion of *Salmonella* in organs. Szabo et al. (2009) and Naqid et al. (2015) indicated that supplementation with *Lactobacillus plantarum* into the feed resulted in increased levels of immunoglobulins in weaned piglets challenged orally with *S. typhimurium*. These may be due to the persistence of these probiotic bacteria in the gut, acting as immune adjuvant to the humoral immune system and stimulating antibody production.

*Bifidobacterium choerinum* is a native bifidobacterium species of the pig gut and shows potential probiotic properties (Maxwell et al., 2004). Probiotics including bifidobacteria were shown to be able to down-regulate expression of genes in the *S. typhimurium* pathogenicity (Bayoumi and Griffiths, 2010). Bifidobacteria are associated more with the colon than ileum, which is the major site of *Salmonella* translocation and their beneficial effect is caused rather by their metabolic products and the mechanisms of tolerance they induce (Trebičavsky et al., 2009). According to Splichalova et al. (2011), this microbe may need more time to form an effective biofilm on the intestinal epithelium and this could be the major reason for the absence of protective effect of *Bifidobacterium choerinum* in gnotobiotic pigs 24 h after infection with *S. typhimurium*.

*Escherichia coli* Nissle 1917 is a probiotic strain of *E. coli* (Schultz, 2008). These bacteria produce two microcins which reduces invasion of *Salmonella* into enterocytes (Altenhoefer et al., 2004). As a flagellated bacterium, it also induces IL-8 in enterocytes (Hafez et al., 2009) and this could be one of the mechanisms by which it protects against *Salmonella* infection (Splichal et al., 2005).

Badia et al. (2012, 2013) observed that *S. cerevisiae var. boulardii* perform different actions with respect to inhibition of *Salmonella*-induced mRNA and secretion of proteins containing genes involved in inflammation and activation of immune cells. This probiotic decreased the overall proinflammatory profile induced by *Salmonella*, with least expenditure of resources for innate immune response (Badia et al., 2013). Enteropathogenic *Salmonella* have type I fimbriae containing multiple subunits of bacterial lectins that bind to mannann units on the surface of host cells (Althouse et al., 2003). According to Shoaf et al. (2006), *S. cerevisiae var. boulardii*, as a source of mannans may mimic the host cell receptor to which the pathogen attaches. Additionally, these bacteria have been described to bind *Salmonella* on its surface (Gedek, 1999), preserving the intestinal barrier function by inhibiting pathogen adhesion and invasion (Martins et al., 2010).

**Phytogenics and essential oils:** Phytogenic feed additives (also called phytobiotics or botanicals) are commonly defined as plant-derived extracts (Papatsiros et al. 2013), whereas essential oils are volatile components of plants (Si et al., 2006). Both additives can be incorporated into feed to improve the productivity and/or health status of livestock, presenting prebiotic, probiotic or antimicrobial activity. Although here we present some examples of phytogenics with potential to be used for the control of *Salmonella* in pigs, it has to be stated that the enormous variety of herbs and their compounds makes this a very exciting and promising area of research.

The extract of *Macleaya cordata* is a natural plant-derived supplement and has been used in traditional Chinese herbal medicine for its analgesic, antiedemic, carminative, depurative and diuretic properties (Zdarilova et al., 2008). It contains the major alkaloids sanguinarine, chelerythrine, protopine, allocryptopine and phenolic acids (Kosina et al. 2010). The commercially available extract of *Macleaya cordata* is in the European Food Safety Authority list of plants used as a component of feed additives in livestock (Franz et al., 2005) and has been incorporated into swine, bovine, poultry and fish diets to reduce amino acid degradation, increase feed intake and
promote growth (Tschirner, 2004; Rawling et al., 2009). The antimicrobial (Colombo and Bosisio, 1996; Newton et al., 2002), immunomodulatory (Agarwal et al., 1991; Chaturvedi et al., 1997) and anti-inflammatory properties (Tanaka et al., 1993) of Macleaya cordata has been attributed to the quaternary benzo[c]phen-anthridine alkaloids sanguinarine (Sedo et al., 2003). In broiler supplemented with sanguinarine, Pickler et al. (2013) have demonstrated reduced Salmonella enteritidis isolation in the caecum at 7 day post-inoculation. Although some authors have studied the effects of sanguinarine on growth performance (Blank et al., 2010; Kantas et al., 2015) and fermentation activity in the gut of pigs (Pellikaan et al., 2010), not a single study was already performed to evaluate its potential effects for the control of Salmonella in pigs.

In an interesting study (Chang et al., 2013), the herbal extracts of Scutellariae radix, Gardeniae fructus, Houttuyniae herba, Taraxaci herba, Glycyrrhizae radix, Puerariae radix and Rhizoma dioscoreae were screened for their potential application as antimicrobial agents using a mice model. Scutellariae radix and Gardeniae fructus had the best bioactivities in eliminating bacteria and suppressing inflammation induced by infection. In this same study but using a pig model, after a 10 day supplementation with Scutellariae radix or Gardeniae fructus, combined or not with a mix of probiotics, pigs were challenged with a clinical isolate strain of Salmonella choleraesuis. Although herbs supplementation were effective in reducing the Salmonella shedding in faeces and reducing both IL-8 and TNF-expressions in serum, the combination herb+probiotic had the best results. Additionally, the bioactive compounds of Scutellariae radix (baicalin and baicalein) showed stronger anti-Salmonella choleraesuis activity than the bioactive compounds of Gardeniae fructus (geniposide and genipin). Interestingly, neither baicalin nor geniposide could inhibit Salmonella invasion of macrophages, even at concentration of 200 µM. However, baicalein and genipin could prevent 52 and 44% of bacteria invading cells, respectively, in a dose-dependent manner.

Previous studies indicated that seaweed extracts supplemented in-feed promote gut function (McDonnell et al., 2010; O’Doherty et al., 2010). According to Leonard et al. (2010) and Lynch et al. (2010), these effects may reflect the stimulation of commensal lactic acid bacteria along with the host immunity. Sweeney et al. (2011), studying the seaweed extracts fucoidan and laminarin, reported that dietary laminarin tended to reduce Salmonella counts in mesenteric lymph nodes and tonsils but dietary fucoidan increased the numbers of lactobacilli in the caecum and also increased the molar proportion of butyric acid and decreased valeric acid in the caecum and colon. Although those authors interpreted these results as potential anti-Salmonella properties, dietary laminarin and fucoidan induced negligible effects on Salmonella counts in the distal gut and stimulated faecal shedding of Salmonella spp. throughout the challenge period.

These contradictory effects in improving gut environment and increasing faecal shedding of Salmonella raises the question of rather or not some herb extracts with pre or probiotic activity are potential and effective nutritional strategies for the control of Salmonella. Much research on this subject is needed to elucidate this issue.

A range of essential oils have been shown to have bacteriostatic or bacteriocidal properties against Salmonella in vitro (Burt, 2004). Among the most studied essential oils/components known to have anti-Salmonella activity are rosemary, oregano, lemongrass, clove, sage (Hammer et al., 1999), mustard (Turgis et al., 2009), citrus (O’Bryan et al., 2008), basil (Rattanachaikunsopon and Phumkhachorn, 2010), thyme (Cosentino et al., 1999; Hammer et al., 1999), α-terpineol (Cosentino et al., 1999), carvacrol (Kim et al., 1995; Cosentino et al., 1999), citral, eugenol, geraniol, perillaldehyde (Kim et al., 1995) and thymol (Cosentino et al., 1999). However, not all of them have this activity against Salmonella in vivo. In an animal infection experiment with piglets challenged with S. typhimurium, Si et al. (2006) reported that geraniol, clove oil, carvacrol, eugenol cinnamon
oil and thymol showed no effect on the reduction of *Salmonella* shedding when administrated through diets. It is noteworthy that essential oils/components retain their antimicrobial activity *in vitro* when mixed with the caecal digesta but some lose their activity after mixing with the diets in animal trial. It is known that food compositions, including fat, protein carbohydrates and water, can significantly influence the effectiveness of essential oils. In fact, high levels of fat and/or protein in foodstuffs protect bacteria from the action of essential oils (Tassou *et al*., 1995). In contrast, high water and/or salt level facilitates the action of these additives (Tassou *et al*., 1995; Skandamis and Nychas, 2000).

Studies are currently lacking regarding the applicability of different essential oils as a *Salmonella* intervention in feed.

**Organic acids:** The successful use of organic acids in swine diets requires knowledge of its mechanisms of action to choose the right compound and dose in order to treat animals of a particular age and under a specific level of infection; however, their actions are not fully understood and consequently, inconsistent results can be found in the literature.

Organic acids and their salts are known to be bactericide and bacteriostatic agents. They are effective in reducing the gastric pH, resulting in a lower presence of microorganisms in the stomach (Partanen and Mroz, 1999). Nevertheless, the fast absorption in the small intestine limits their beneficial effects along the gut (Grilli *et al*., 2010). Considering that the ileum and colon are preferential sites for *Salmonella* colonization (Boyen *et al*., 2008), technologies such as the microencapsulation were developed to ensure the low and continuous release of these compounds in the lower gut, increasing their action in the ileum and colon (Meunier *et al*., 2007; Piva *et al*., 2007). The non-dissociated organic acids are lipophilic and pass through the cellular membrane of gram-negative bacteria, such as *Salmonella*. Once inside the cell, under a higher pH, the acids dissociate releasing hydrogen that results in the decrease of intracellular pH. The acidic environment impairs enzymatic activities of bacteria, leading to its death (Suryanarayana *et al*., 2012).

The first acid compound to be approved for use in swine diets, by the European Union was a formic acid salt that was reported to reduce the incidence of *Salmonella* in pigs (Blanchard and Kjeldsen 2003). A considerable number of studies have reported effects of organic acids on *Salmonella* at farm level (Lo Fo Wong *et al*., 2004; Van Immerseel *et al*., 2005; Farzan *et al*., 2006; Creus *et al*., 2007; De Busser *et al*., 2009; Arguello *et al*., 2013b). Although short-chain fatty acids (i.e., formic, acetic, propionic and butyric) have been shown to inhibit *Salmonella* growth and medium-chain fatty acids (i.e., caproic, caprylic and capric) may produce even better results (Van Immerseel *et al*., 2006), these effects vary significantly between studies. In fact, the addition of organic acids to the drinking water in weaners (De Ridder *et al*., 2013) and fattening pigs (De Busser *et al*., 2009) were inconsistent for *Salmonella* prevalence.

Some reasons may explain this lack of consistence, among them the most remarkable are the level of contamination (Davies and Cook, 2008), the length of treatment (De Busser *et al*., 2009) and the “Acid tolerance response” that is the organism adaptation to mild or moderate acid conditions (pH 5.8-4.4) enabling its survival during severe acid stress periods (pH 3.0) (Bearson *et al*., 1998).

Acidification can also be used to decrease the risk of feed contamination by *Salmonella* into feed mills (Wierup and Haggblom, 2010). Although, the treatment with organic acids reduces re-contamination after feed preparation (Ricke, 2005), it can mask the presence of *Salmonella* when assessed by standard culture methods (Carrique-Mas *et al*., 2007). These same authors reported that the best efficacy results and lowest masking effect were achieved with formaldehyde-
containing products. Wales et al. (2010) presented a review on the use of various chemicals to reduce *Salmonella* contamination of feed.

**CONCLUSION**

There are many areas where *Salmonella* prevalence could be reduced throughout pork production and processing. This literature review has presented an insight of how contamination by *Salmonella* occurs throughout the swine production chain, traditional methods for prevention/control and an alternative approach that is the use of dietary non-nutritional additives. Considering the “Farm-to-fork” method, measurements of control at the farm level are strictly necessary. In this way, this review shows that the use of these additives is not just promising but already a reality. Although the current knowledge on this subject is improving quickly, many links are already missing to explain the exact action of each product/compound and how they interact with the metabolism of *Salmonella* to impair its growth and shedding. This will allow the development of more precise and low-cost strategies to ensure the control of this pathogen.

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