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Review

Advances in Enteropathogen control throughout the meat chicken production chain

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Abstract: Enteropathogens, namely *Salmonella* and *Campylobacter*, are a concern in global public health and have been attributed in numerous risk assessments to a poultry source. During the last decade a large body of research addressing this problem has been published. The literature reviewed contains review articles on certain aspects of poultry production chain, however in the past decade there hasn't been a review on the through production chain, farm to fork, production of poultry. This review, a pool of 514 articles were selected for relevance via a systematic screening process (from >7500 original search articles). These studies identified a diversity of management and intervention strategies for the elimination or reduction of enteropathogens in poultry production. Many studies were laboratory or limited field trials with implementation in true commercial operations being problematic. Entities considering using commercial anti-enteropathogen products and interventions are advised to perform an internal validation and fit for purpose trial as *Salmonella* and *Campylobacter* serovars and biovars may have regional diversity. Future research should focus on non-chemical application within the processing plant and how synergistic through chain intervention may contribute to reducing the overall carcass burden of enteropathogen, coupled with increased consumer education on safe handling and cooking of poultry.

Keywords: Salmonella; Campylobacter; poultry; review; vaccines; processing; farm-to-fork; broilers; meat birds; production

1. Introduction

Poultry meat globally has been associated and attributed with human enteric disease, especially *Campylobacter* and *Salmonella* which are the number 1 and 2 bacterial causes of gastroenteritis in the developed world respectively[1,2]. Even though many bacterial enteric outbreaks in recent years have been linked to fresh produce and fruit, enteric disease is still often attributed to poultry meat [3].

As consumption of poultry meat is increasing throughout the developed world [4], the amount of research into growing and producing a safe product for human consumption is a large body of work currently. In 2016, *Salmonella* and *Campylobacter* human case rates were 74.7 and 146.9 notified cases per 100,000 individuals in Australia[5] indicating they are ongoing risks. The Australian Agrifutures research body produced a research proposal to write a best practice guideline for the Australian poultry industry to reduce prevalence and load of the said pathogens. Prior to writing an Australian best practice, current literature in the topic matter needed to be read and reviewed and the most efficient approach was to perform a literature review based upon known ingress points of *Salmonella* and *Campylobacter*.

This review, from farm to fork, selected relevant published original research articles using a systematic search strategy and resulted in 514 relevant articles from a total of 7806 publications returned in the initial search. The search strategy was designed to select primary research articles that described an intervention strategy for reducing *Campylobacter* or *Salmonella* in the poultry meat production chain. In order to focus on recent advances,

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the search only looked for publications from the past 10 years since the last review, 2010, by the current author[3]. A detailed description of the search and screening process can be seen in Appendix A. Some additional articles were included in this review when suitable to provide context and background, including topic specific reviews and relevant government documents. The objective of this review is to summarise the advances made in bacterial enteric diseases control in the farming and processing of meat birds in a narrative-style, and to identify further areas of research.

2. Primary Production

Poultry is produced via pyramidic growth, with three generations of Pedigree and three generations of commercial production. These generations are grandparent, parent and production[3]. The Australian model is to purchase Pedigree broilers, predominately from Cobb or Ross for meat birds and ISA browns for table egg layers [3]. The progeny of these pedigree broilers are hatched in quarantine stations and become the great grandparents of the Australian poultry operation and using multiplier houses eventually produce the broiler or egg layer flocks.

The following sections summarise the intervention strategies identified in literature at each relevant stage in the primary production chain. The sections are ordered chronologically, starting with the hatchery, then farm biosecurity, litter, feed, drinking water and immunisation, and finally chicken transport. The nature of enteropathogen risk at each stage will also be described.

2.1. Hatchery

Commercial hatcheries are ideal environments for contamination, biofilm formation and dissemination of enteropathogens [3,6,7]. A comprehensive review by Wales and Davies (2020) stated 'Studies of *Salmonella* contamination and colonisation that employ subtyping techniques can provide good evidence of hatcheries acting as conduit and multiplier for such organisms present in breeder flocks'[8]. Ideally the incoming eggs should be enteropathogen free however in reality this is not commercially practical in many countries. Controlling *Salmonella* at the hatchery is of particular importance as it is a point where vertical transmission from hen to egg and subsequent cross contamination of naïve hatchlings can occur. Therefore, recently described strategies employed to prevent *Salmonella* and *Campylobacter* spread and contamination of broiler hatching eggs are summarised in the following paragraphs.

Interventions that can be applied to eggs at the hatchery stage typically looked at either egg-surface sanitation treatments or i*n-ovo* injections. One egg surface treatment in recent literature is hydrogen peroxide application followed by UV-C irradiation (coined "advanced oxidation process"), which can significantly reduce *Salmonella* on the surface of eggs by up to 6 log and significantly reduce prevalence in treated eggs and birds hatched from treated eggs[9,10]. Treatment with only hydrogen peroxide solution has also been shown to reduce the prevalence of *S.*Typhimurium on inoculated eggs when delivered via immersion[11] or spray[11,12], but it appears that immersion is the more effective method[11].

A range of other antimicrobials can reduce *Salmonella* prevalence when sprayed on the surface of broiler eggs, including hydrogen peroxide, ammonium chloride, quaternary ammonium compounds, a bronopol and a biguanide[11,12]. Bacteriophage surface treatment may also be effective: in one study, chicks hatched from eggs inoculated with *S.*Enteritidis then sprayed with an anti-*S.*Enteritidis bacteriophage had significantly lower cecal *Salmonella* prevalence at 8 days post-hatch[13].

Some interventions also involve injecting a substance into the egg prior to hatch (*in ovo*). *In ovo* injections can be automated using machinery so are suitable for large-scale operations, but an important consideration for the use of *in ovo* treatments is the effect they may have on hatchability [14]. There is evidence that probiotics delivered *in ovo* can provide protection against *Salmonella*. An *Enterococcus.faecium* probiotic added to feed and delivered *in ovo* lowered *Salmonella* prevalence from 81% to 53% in hatched birds, while a *Bacillus subtilis* probiotic was ineffective[15]. A commercial probiotic (Protexin) delivered

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in ovo significantly reduced *S.*Enteritidis at 1 d post-challenge (2 days post-hatch) but not at 7 d, while the same probiotic delivered post-hatch via spray, oral gavage or deposited on vent lip reduced counts by 2.2-3 $\log_{10}$ at 7 d post-challenge[16]. Another study identified a commercial competitive exclusion product that when delivered *in ovo* significantly reduced cecal S.Heidelberg counts at 3 days of age (1 day post-challenge) while not affecting hatchability, but no significant effect was seen from 7 days onwards[17]. When delivered *in ovo*, a commercial lactic acid bacteria probiotic mixture (Floramax B11) was able to protect against *S.*Enteritidis challenge, reducing cecal prevalence from 100% to 45% and reducing count by 1.68 $\log_{10}$ CFU/g at 24 h post-infection[18]. *in ovo* administration of threonine significantly reduced cecal *Salmonella* counts in hatched birds at 9 days of age (7 days post-challenge), and did not reduce hatchability[19].

Vaccinations can also be delivered *in ovo*, with recent studies describing vaccines that may be able to reduce *Campylobacter* [20] and *Salmonella* [21] post-hatch when delivered in this manner, while a different vaccine had no effect on *Campylobacter* [22]. Induction of the innate immune response through *in ovo* injection of CpG oligodeoxynucleotides significantly reduced *S.*Enteritidis in hatched birds at 16 days of age (6 days post-challenge) in one study[23] and similar results were seen in another[21].

A simple method to limit *Salmonella* in broilers may be to increase egg incubation temperature: in one study, increasing incubation temperature from 37.7°C to 38.7°C from day 11 of hatch onwards significantly reduced cecal *S.*Enteritidis prevalence and count at 10 days of age (8 days post-challenge) relative to birds hatched from eggs incubated at 37.7°C for this time[24].

Some studies looked at treatments that could be applied to live birds shortly after hatch (d 0 or 1 of life). Spraying of these treatments is one method that is practical in commercial conditions. Probiotics sprayed on day 1 of age reduced *Salmonella* carriage of broilers in two studies[25,26], and could be effective against *Salmonella* when sprayed after chicks have been placed into their shipping containers[16,27].

While potentially less practical, probiotics can also be delivered to chicks orally shortly after hatch. Some probiotic treatments have been seen to provide protection against *Campylobacter* colonisation to varying degrees, but overall evidence is mixed. In one study a *B.subtilis* probiotic selected for enhanced motility given as a single oral dose on day 1 of life significantly reduced cecal *C.jejuni* at 14 days of age by 1-2 $\log_{10}$ CFU/g in broiler chicks challenged on day 7 of age[28]. A *Lactobacillus salivarius* probiotic treatment every three days was also successful at reducing *C.jejuni* in challenged birds early in life[29]. A commercial product (Broilact) containing a mixture of gut bacteria isolated from a hen provided orally on day of hatch consistently lowered cecal *C.jejuni* in broilers in a pilot-scale trial by 1.4-5 $\log_{10}$ CFU/g [30]. Other trials were inconclusive on the effect of orally-dosed probiotics on *Campylobacter* [31-36]: no clear effect on *Campylobacter* was seen in trials looking at early-life, multi-dose treatment with *Enterococcus* [31-33] or *Lactobacillus*[34] probiotics, and other studies saw no effect from a single, day 1 oral dose with *Bacillus, Enterococcus* or *Lactobacillus* probiotics [35,36]. A study did find that 15 d of daily oral treatments with a *Bacillus* probiotic later in life could reduce *Campylobacter*, so perhaps more than one dose is necessary for an effect on *Campylobacter*[37].

A number of studies found that single oral doses of probiotics on days 0 or 1 of life can protect against *Salmonella* early in life, where they led to reductions in *Salmonella* prevalence in the spleen and liver[38] and ceca[39-43], and reduced cecal counts by 0.9-3 $\log_{10}$ CFU/g[43-47]. Treatments consisting of multiple oral doses of a *Lactobacillus* probiotic starting from day 1 of life were found to reduce cecal *Salmonella*[48,49]. Overall, although many studies saw reductions in *Salmonella*, there is a lack of evidence if these effects can last until later in life, and on the effect of treatment in birds naturally exposed to *Salmonella* rather than challenged.

2.2. Biosecurity and Farm Management

Biosecurity is defined as the prevention and/or reduction of the spread of microbial disease prior to detection and is a collection of rules and procedures that minimise exposure (security) of a susceptible population to an infectious (biological) agent [3]. There are

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numerous industry and government standards, guidelines and protocols published around the world that underline the importance of biosecurity[50,51]. A recent review by Sibanda et al (2018) comprehensively described procedures and protocols that will decrease the colonisation of *Campylobacter* into a flock[52], and the reviewed practices also may prevent horizontal spread of *Salmonella*. The following paragraphs will describe the latest research in biosecurity.

Some studies identified farm management practices that can have an effect on *Salmonella* and *Campylobacter* colonisation in broilers. High stocking density [53], lighting schedule[54] and heat stress[55,56] are factors that may increase *Salmonella* in broilers, although two other studies did not see a significant difference in *Salmonella* between high and low stocking density broiler groups[57,58]. In one study [59], a 24h feed withdrawal at 42 days of age (DOA) increased ileal *S*.Enteritidis count by nearly 2 $\log_{10}$ CFU/g compared to the *ad libitum* fed control group., However, when birds were fed a feed restricted diet early in life (d 4-6 DOA), the increase was limited to only 0.5 $\log_{10}$ CFU/g, showing that early feed stress may give resistance to feed stress later in life[59]. Contact with workers is a likely source of *Campylobacter* contamination: in one study, birds isolated in a flock by a 'biosecure cube' (such that they did not come into contact with staff but shared the same feed and water as the rest of the flock) took 7-21 d longer to be colonised with *Campylobacter* than the rest of the flock, with some remaining *Campylobacter* negative until slaughter[60]. The practice of flock-thinning can increase the chance of flock *Campylobacter* colonisation, perhaps due to the increased contact with workers[61,62].

Even bird breed may have an effect on *Campylobacter* colonisation: while one study found that between a range of Aviagen broiler genotypes, there was no significant difference in at-slaughter *Campylobacter* loads[63]; another suggested that feed additive interventions may be effective at reducing *Campylobacter* in some breeds but not others[64]. The rearing method also may affect the risk of *Campylobacter* and *Salmonella* colonisation, but more research is needed. A UK study found that conventionally-raised flocks may be *Campylobacter*-positive less frequently than free range[65], but a Greek study found no difference in *Campylobacter* prevalence between conventional and free-range broiler meat[66]. One study comparing organic "antibiotic free" broilers with conventionally raised broilers found that although there were some differences in *Campylobacter* count and prevalence at various points in processing there was ultimately no significant difference between the two in post-chill carcasses[67]. In one challenge study, no significant difference in *C.jejuni* prevalence was seen when broilers where fed from 10 days of age either *ad libitum* or intermittently[68].

Some biosecurity-based interventions may help reduce *Campylobacter* in broiler flocks. A UK study found that a financial incentive, rewarding farm managers and stockpersons for broiler houses considered "not highly contaminated", reduced the chance of a broiler house being highly contaminated with *Campylobacter* at 1-3 d prior to final depopulation by 54%[61]. A study conducted over a three year period demonstrated that the use of fly screens in commercial broiler sheds can significantly reduce the prevalence of *Campylobacter* positive flocks by approximately 26% relative to sheds not using fly screens[69]. A proposed alternative cleaning protocol involving dry and wet cleaning and the use of detergents and disinfectants saw reduced *Campylobacter* counts on the floor and drinkers of broiler pens compared to conventionally cleaned pens where only water and detergent was used, but no difference in the counts on the birds at slaughter[70]. Treatment of clothes and medical kits in a disinfection channel with slightly acidic electrolysed water spray can reduce *Salmonella* counts by up to 2.36 $\log_{10}$, but this may be not practical in a poultry production environment[71].

Some *Salmonella* species can form biofilms, and two lab-scale studies looked at the effect of antimicrobials on these biofilms on primary-production relevant surfaces. One trial found that a foam or liquid form treatment with a mixture of levulinic acid and sodium dodecyl sulfate could significantly reduce *Salmonella* biofilms on a range of surfaces by 5-6 $\log_{10}$ CFU/cm^2 [72]. The other looked at the ability for a range of disinfectants to reduce *S*.Typhimurium biofilms on galvanized wire and PVC coupons, being relevant to

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cage and drinking line surfaces respectively. Clorox, Pril + Clorox, calcium hypochlorite and Formalin were the only disinfectants to completely remove biofilm after 10 mins treatment on the wire, while the majority of treatments were effective against biofilms on PVC coupons after a 24 h treatment[73].

2.3. Litter

Litter is an absorbent material used to line the floor of poultry houses, and depending upon local availability, consists of nonsterile; straw, wood shaving, peanut shells, rice hulls or other similar material [3]. As such litter is a potential source of *Salmonella* and *Campylobacter* contamination from previous flocks, and/or can provide an environment for pathogens to reside[74].

When reusing litter from a previous flock, a range of litter treatments can effectively lower *Salmonella* and *Campylobacter*, although litter reuse itself may increase *Campylobacter* prevalence in broilers relative to replacement[75].The use of acidifiers, sulfuric acid or aluminium sulphate as litter additive treatments have been shown to reduce *Salmonella* in litter[76,77]. A study found that the addition of sodium bisulfate to litter increased *Salmonella* levels, while also lowering the pH[78]. Heat or steam treatments also may be effective against *Salmonella* in litter: studies have shown them to reduce *Salmonella* levels, however effects are strongly dependent on temperature (50 to 120^{0}C) and duration (hours to days) of the treatment [79-84]. These experiments were not performed at a commercial scale, but demonstrate procedures that could be further developed into something suitable for large-scale use.

A trial in an experimental broiler shed found that between flocks, litter treatment with quicklime, shallow fermentation or windrowing can reduce *Salmonella* in the litter[85], but in a live bird trial, shallow fermentation quicklime, or a combination of the two all had no significant effect on the spread of *Salmonella* from litter to seeder birds when used as a between-cycle treatment[86]. While composting of litter can reduce pathogen load, one study suggested that it does not help prevent future pathogen recolonization[87]. Quicklime treatment has also been shown to reduce *Salmonella* in litter in conditions simulating built-up litter depth, and was more effective in combination with steam treatment using a soil steamer cart[80]. One study found that use of a rubber mat bedding instead of wood shavings may reduce *C.jejuni* spread post-challenge[88]. Litter aeration, a practice used to improve litter quality, was found to have no significant effect on *Salmonella* counts when trialled[89].

2.4. Breeder Flock.

An important area of research is in the prevention of *Salmonella* in Broiler breeders as they are longer lived than the standard meat bird, and can pass *Salmonella* on to their progeny. A large number of studies describing live-bird treatments (such as feed additives) that can reduce *Salmonella* or *Campylobacter* were identified, but relatively few of them specifically looked at broiler breeders as the treatment population. It is likely however that treatments proven for broilers will also be effective when used with broiler breeders. The studies that did specifically look at broiler breeders are summarised in the following paragraph.

An organic acid/probiotic drinking water additive [90], a prebiotic feed additive [91], and vaccinations [92-94] were all found to reduce *Salmonella* in broiler breeders. In addition, some of these treatments were shown to also reduce *Salmonella* in the progeny of the treated breeders [91-94], indicating a reduction in horizontal transmission. The practice of skip-a-day feeding, where birds are fed double the feed every two days rather than daily feed provision, was found to contribute to higher *Salmonella* and *Campylobacter* colonisation in broiler breeders in one study[95].

2.5. Feed and water additives

The largest area of research identified in this review was into feed and water additives. Feed and water can both serve as a convenient vehicle for large-scale treatment of broilers, so additives that can reduce *Salmonella* or *Campylobacter* when delivered via this method are desirable. The main types of additives trialled were organic acids, phytogenic

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additives, direct fed microbials and prebiotics, but others were also described including bacteriophage, inorganic compounds and digestive enzymes. There was also research into combination treatments with different types of these additives together (the largest category of these being synbiotics), however there is a lack of studies comparing a combination treatment with groups given diets containing its individual components, which would help indicate if the treatments are truly synergistic.

Feed may also act as a vehicle of *Salmonella* transmission. Some literature looked at the use of additives to reduce *Salmonella* in the feed itself: microencapsulated lemongrass essential oil addition can lower *Salmonella* counts in feed[96]; formaldehyde, essential oils, organic acids and sodium bisulfate can reduce *Salmonella* in a range of protein-meals used in poultry feed, although the effect varied between meals[97]. A commercial product containing a mixture of organic acids, prebiotics and spice extracts significantly reduced *Salmonella* counts in the feed relative to an untreated control during a live bird trial[98].

2.5.1. Organic acids

Organic acids are organic compounds that function as acids, most commonly due to the presence of a carboxylic acid group, and include fatty acids (which can be further categorised based on chain-length) among other compounds [99]. The hypothesised mode of action is that organic acids inhibit *Salmonella* and *Campylobacter* in the gut by both lowering the pH and via interactions with the cellular functions of gram-negative bacteria unrelated to the pH reduction[100]. Organic acids are easily absorbed by the gut, limiting their ability to reach the lower gastrointestinal tract[100], and are sometimes used in an encapsulated form in an attempt to mitigate this[101]. One study found that encapsulation of an organic acid additive may improve its effects against *Salmonella* and *Campylobacter*[101], but another found no significant difference[102]. Studies investigating the effects of organic acid-based additives on *Salmonella* and *Campylobacter* are summarised in **Error! Reference source not found.** and **Error! Reference source not found.** respectively.

Table 1 *Salmonella* novel and commercially available organic acids in-bird trials (No. [citations]) summary through different delivery routes (feed or water).

Organic acid	Feed	Water	Commercial products
Short chain fatty acid based (C1-C5)	14[25,101,103-114]	8[25,109,115-120]	Adimix Precision[105], , Gustor XXI B92[101], Gustor XXI BP70[101], Biotronic Se Forte[113], ButiPEARL[103], Galli-acid®[110], Aciflex®[120]
Medium chain fatty acid based (C6-C12)	5[121-125]	2[90,121]	Aromabiotic[125]
Organic acid with other additive type	8[98,106,107,110,126-128]	5[25,90,116,128,129]	Amaril[107], Gallimix[106], FormaXOL[106], Bi-acid™[110], Novacid[98], Acid Pak 4-Way®[25], Acti-vate®[116]
Combinations of different types of organic acid	3[106,110,130]	2[117,121]	Optimizer[90,117], Fysal[106]
Organic acids other than fatty-acids	2[131,132]	0	

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Table 2: *Campylobacter* novel and commercially available organic acids in-bird trials (No. [citations]) summary through different delivery routes (feed or water).

Organic acid	Feed	water	Commercial products
Short chain fatty acid based (C1-C5)	6[111,133-137]	1[134]	Adimix Precision[135], Excential Butycoat[135], Biotronic Se Forte[113], Selko®-pH[133], BabyC4®[133]
Medium chain fatty acid based (C6-C12)	9[64,135,138-144]	2[145,146]	Lodestar C8-C10[143] Fortibac®[141]
Organic acid with other additive type	7[135-137,147-150]	3[134,147,151]	Power Protexion[135], Biotronic®Top3[135], Campylostat[135], Forticoat[151], Auranta 3001[150]
Combinations of different types of organic acid	2[113,147]	1[152]	Biotronic® SE Forte[113], Selko® 4Health[152]
Organic acids other than fatty-acids	0	0	None

Recent literature indicates that *Salmonella* can be reduced in broilers through the addition of short-chain fatty acid (SCFA) additives to their feed or drinking water, including butyric acid, citric acid, formic acid, lactic acid and SCFA mixtures[101-105,107,108,110,112,114,116]. While they are effectively the same active ingredient, SCFAs are often added to feed in their salt form due the volatility of the free acid[103,112].

Butyric acid feed additives, in encapsulated and non-encapsulated forms, have been shown to significantly reduce *Salmonella* prevalence in the ceca, liver and spleen[101], and to reduce cecal count by 0.6-0.9 $\log_{10}$ CFU/g[103,104]. Conversely, some studies did not see a significant reduction when butyric acid was added to feed[105,112]. Butyric acid additives also may reduce *Salmonella* when added to drinking water[118].

There is also evidence that formic acid additives can significantly reduce *Salmonella* in broilers ceca when added to drinking water during feed withdrawal [119], and in the ileum and ceca when added to feed throughout life[107]. Other studies found no effect on *Salmonella* when formic acid was added to feed[109] or drinking water[109,115].

Acetic acid added to feed significantly reduced cecal S.Pullorum by 2.8-4.1 $\log_{10}$ CFU/g in broilers challenged 21 days prior to sampling in one study[108], and when added to drinking water significantly reduced cecal S. Enteritidis counts[118].

Lactic acid trialed as a water additive had no significant effect on cecal S.Heidelberg, but did reduce crop prevalence[116]. A study showed no significant effect on ileal *Salmonella* when citric acid was used as a feed additive[111], however a combination of citric acid and hydrogen peroxide added to drinking water was found to significantly lower S.Heidelberg crop prevalence in another study[129].

Additives containing mixtures of different SCFAs have also been seen to be effective against *Salmonella* in the gastrointestinal tract of broilers when delivered via feed[102,110] or drinking water[117,120,121,153]. A study found that a combination of benzoic acid, fumaric acid and 2-hydroxy-4-methylthio-butanoic acid significantly reduced S.Typhimurium prevalence in the crop, ceca and spleen of challenged broilers when added to the feed[130].

Some studies found that medium chain fatty acids (MCFAs) can significantly reduce *Salmonella* in broilers when provided via feed for a short period before slaughter[123,124]and when provided throughout life[122,125]. MCFA also lowered *Salmonella* in broilers when added to drinking water[121].

There is less research into the effect of organic acid feed additives on *Campylobacter*, but some evidence indicates that MCFAs can reduce *Campylobacter* in broilers[138,139,142-144,147], with two other studies finding no effect, one of these suggesting that chicken intestinal mucus can protect *C.jejuni* from the action of organic acids in the gut[140,141]. Studies on the effect of SCFA additives on *Campylobacter* saw mixed results and it is unclear if there is a beneficial effect[111,134-136,147].

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One production-scale trial found a mixture of SCFA and MCFAs added to drinking water may be effective against *Campylobacter*[152], and another found that MCFAs added to drinking water could increase the initial dosage of *C. jejuni*: required for colonisation[145], while some other drinking water organic acid trials found no significant effect[134,146].

Additives containing monoglycerides of organic acids may also have a reductive effect on *C. jejuni*: in one study Monoglycerides of MCFAs added to drinking water led to a significant reduction, while a parallel MCFA treatment had no significant effect[147]. In another, A mixture of organic acids, mono and diglycerides added to drinking water was found to significantly reduce *C. jejuni*: transmission between birds in adjacent cages separated by 0.75 m, but not between birds sharing the same cage[151].

Overall there was more research into the effect of organic acids on *Salmonella* than on *Campylobacter*. A wide range of organic acids were seen to reduce *Salmonella* in meat chickens, while for *Campylobacter* MCFAs in particular seem promising and would be a good candidate for further research.

2.5.2. Phytogenic additives

Phytogenic feed additives are broadly defined as plant products and extracts added to feed, and are of interest as they are generally non-toxic, tend not to affect beneficial gut microbes, may improve broiler performance and gut health and can stimulate appetite and digestion[154-156]. The antimicrobial activity of these additives is generally due to natural bioactive molecules in the additive [155]. In the literature reviewed, this was commonly the phenolic compounds found in essential oils (e.g. carvacrol, eugenol, and thymol), but some other types of additives were also assessed. Tables 3 and 4 summarise these studies investigating novel and commercially available phytogenic additive effects against *Salmonella* or *Campylobacter*. r.

Table 3 *Salmonella* novel and commercially available phytogenic additive in-bird trials (No. [citations]) summary through different delivery routes (feed, water or orally).

Phytogenic additive category	Feed	Water	Oral	Commercial products
By-product	6[157-162]	1[163]	0	None
Active ingredient	11[126,154,164-172]	1[173]	0	Enviva®EO 101[164], Sangrovit® WS[172]
Extract	10[154,165,174-181]	5[116,182-185]	4[186-189]	Digestarom®[181], Natusol®[180], Oregain®[116], Mix-Oil®[184]
Combination treatments	10[102,106,107,110,126-128,165,186,190]	1[128]	0	Amaril®[107], FormaXOL®[106], Biacid™[110]

Table 4 *Campylobacter* novel and commercially available phytogenic additive in-bird trials (No. [citations]) summary through different delivery routes (feed, water or orally).

Phytogenic additive category	Feed	Water	Oral	Commercial products
By-product	2[191,192]	0	0	
Active ingredient	5[193-197]	0	1[198]	
Extract	3[135,147,195]	2[199,200]	0	Excential Alliin Plus[135]
Combination treatments	5[133,135,147-149]	1[150]	0	Biotronic® Top3[135], Power Protexion[135], Anta®Phyt[135]

Feed and water additives containing phenolic compounds were seen to reduce *Salmonella* [164,166,167,181,183,184] or *Campylobacter* [193,196,197,199] in numerous studies. Other phytogenic additives that may reduce *Salmonella* are quercetin [169,201], ginger root extract [185], sanguinarine [173], oridonin [170], curcumin[165,186], tetramethylpyrazine [202,203], pine bark extract [177], Achyranthes japonica extract [178], turmeric [162],

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pomegranate by-products [158], mushroom extract[182] and black cumin seeds [159,161]. Some other phytogenic additives that may reduce *Campylobacter* are tea-tree oil, eucalyptus compounds or lemon myrtle oil[195], β-resorcylic acid[194], *Galla rhois* and *Cinnamomum* cassia extracts[200], Propyl propane thisulfonate[168], seaweed extract [191] and olive mill wastewater extract[192]. Some phytogenic additive studies did not find there was a significant effect on *Salmonella*[157,160,172,204] or *Campylobacter*[147,205]. There is evidence that phytogenic additives as a whole can reduce *Salmonella* and *Campylobacter* in broilers, but no specific additive stood out as being the most effective. Commercial-scale trials would be important in determining if they are suitable for large-scale use.

Some studies trialled oral doses of phytogenic compounds and could be followed up with feed trials for a more practical application method. A thymol conjugate (thymol-b-D-glucopyranoside) but not thymol itself was seen to reduce *Campylobacter* in broilers [198]. A study showed that daily doses of carvacrol essential oil could reduce *Salmonella* in naturally challenged broilers at slaughter[187], but in a different study, similar treatment with oregano essential oils reduced *Salmonella*, while thyme or carvacrol essential oils did not have an effect[189].

This is an interesting area of research and the effects on enteropathogens prevalence and populations can be significant. With commercial products already available it would be beneficial research to see the outcome of these inhibitors not just in bird but on the final carcass post processing.

2.5.3. Direct fed microbials (DFMs)

Direct-fed microbial (DFM) additives are live microbes provided to broilers, and can be delivered via feed or drinking water. They can provide broilers resistance to *Salmonella* and *Campylobacter* colonisation through competitive exclusion, modulation of immune function and the production of growth-inhibiting metabolites (including organic acids)[100,206]. Studies identified in this review that investigated the effect of DFMs on *Salmonella* or *Campylobacter* are summarised in

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Table and Table 6 respectively.

Table 5 *Salmonella* novel and commercially available direct fed microbial (DFM) in-bird trials (No. [citations]), summary from different delivery routes (feed, water, sprayed or orally).

DFM	Feed	Oral	Water	Sprayed	Commercial products
Bacillus	21[15,58,207-226]	0	0	0	Toyoserin[218], Norum™[207], Pro.B®[212], Sporulin®[222], GalliPro®[58,223], Calsporin®[225]
Lactobacillus	8[180,208,227-231]	6[38-40,48,49,232]	1[233]	0	LactoPlan™[229], Lactofeed[230]
Pediococcus	3[26,103,234]	0	0	0	Bactocell PA10[26], Sim®Lac (Simbiyotek)[234], Pedi Guard[103]
Enterococcus	3[15,210,235]	0	0	0	Cylactin®ME20[235]
Mixture of LAB	5[25,44,57,210,236]	4[39,46,47,237]	4[233,237-239]	3[16,25,27]	Primalac [57,182], FloraMax-B11[27,39,46,47,236,237], Protexin[16,240]
Bacillus with LAB	1[241]	0	0	0	
Saccharomyces	5[26,242-245]	0	0	0	Levucell SB20[26,242,243]
Saccharomyces with bacteria	4[231,240,246,247]	0	1[248]	1[248]	Lavipan®[246], GRO-UP[249], Mircoguard[240]
Synbiotics	4[43,44,238,250]	2[43,44]	0	0	Biomin PoultryStar®[238,250,251], Biomin®IMBO[113]
Fermented products	5[247,252-255]	0	0	0	
Undefined or partially defined	1[44]	2[44,237]	1[233]	1[26]	Broilact[237], Colostrum Liquido[237], Aviguard[26,44]
Other organisms	2[160,256]	1[257]	0	0	

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Table 6 *Campylobacter* novel and commercially available direct fed microbial (DFM) in-bird trials (No. [citations]), summary from different delivery routes (feed, water, sprayed or orally).

DFM	Feed	Oral	Water	Sprayed	Commercial products
Bacillus	4[135,147,210,219]	4[28,35-37]	0	0	Calsporin®[135], Ecobiol[135]
Lactobacillus	1[258]	6[29,34-37,259]	0	0	
Pediococcus	0	0	0	0	
Enterococcus	1[210]	4[31-33,35]	0	0	
Mixture of LAB	2[133,210]	1[35]	0	0	Primalac®[133]
Bacillus with LAB	0	1[35]	0	0	
Saccharomyces	2[147,242]	0	0	0	Levucell SB20[242]
Saccharomyces with bacteria	1[260]	0	1[248]	1[248]	Lavipan®[260]
Synbiotics	5[36,113,135,261,262]	1[36]	1[263]	0	Biomin PoultryStar®[135,263], Biomin®IMBO[113]
Fermented products	1[254]	0	1[134]	0	
Undefined or partially defined	1[134]	1[30]	0	0	Broilact[30]
Other organisms	1[264]	1[259]	0	0	

There is a large number of studies with data showing that addition of *Bacillus*[58,208,210-218,220-224,226] and lactic acid bacteria (LAB) based DFM additives[26,46,47,103,180,182,208,210,228-230,234,235] to feed can be effective against *Salmonella* in broilers, while *Saccharomyces*[26,242-244] and combinations of *Saccharomyces* and bacteria[240,246,247,249] also may be effective. *Bacillus*-based DFMs are particularly suited to use as feed additives as *Bacillus* is a spore-forming organism, giving it resistance to stress during storage and the high temperatures used in the feed pelleting process[206]. There is also evidence that *Clostridium butyricum* can be effective against *Salmonella* when added to feed[256], or when provided orally[257]. These DFM additives may not always be effective however, with some studies seeing no significant effect on *Salmonella* when *Bacillus*[207,209,217,219,225], LAB[25,57,227,231,236], *Saccharomyces*[245], or *Saccharomyces*/bacteria combination[231,241] additives were used. It is unclear precisely why these contrasting results exist, but it could be due to differences in the DFM strain or species, or in some other aspect of the study design. When considering the use of said commercial products it is highly advisable that an internal validation and fit for purpose trial is conducted by the individual entity.

Various fermented agricultural by-products containing live microbes have been found to reduce *Salmonella* when added to feed[230,247,252-255], but the practicality of their use will likely depend on the availability of the specific feedstock to be fermented. Some DMFs may reduce *Salmonella* when added to broiler drinking water[233,239], while others did not have a significant effect[237,248].

Relative to *Salmonella*, there is less research into the effects of DFMs on *Campylobacter* and overall evidence indicates DFMs can be an effective intervention against *Campylobacter* with certain microbes and not others, with some additives causing a reduction[134,135,147,226,258,260,264] and others having no effect[147,219,242,248]. Overall there is evidence that DFM additives can effectively reduce *Salmonella* in broilers, and while they may also be effective against *Campylobacter*, the evidence is mixed and further research is necessary to determine what specific conditions and organisms are ideal. Further trials would be warranted, in particular for *Bacillus* because of the factors making it more practical to use, and the large volume of evidence supporting its ability to reduce *Salmonella*.

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2.5.4. Prebiotics

Prebiotics are non-digestible carbohydrates, typically oligosaccharides, that can promote the growth of desirable gut microflora[265]. In addition to promoting the growth of beneficial gut microbes by serving as a source of nutrients, oligosaccharides can increase organic acid production in the gut of chickens[100] and may also agglutinate gram-negative bacteria[91]. These properties give prebiotics the potential to reduce *Salmonella* and *Campylobacter* in broilers when used as additives. Studies identified for this review on the effect of experimental and commercially available prebiotic additives on *Salmonella* are summarised in **Error! Reference source not found.**.

Table 7 *Salmonella* novel and commercially available in-feed Prebiotics live bird trials (No. [citations]).

Description/treatment category	Total Feed trials	Commercial products
Mannan-oligosaccharides	10[25,103,179,208,234,249,266-269]	AgriMOS[268], ActiveMOS[103,269], Bio-MOS®[25,208], oligomanno®[249]
Fructo-oligosaccharides	4[43,121,208,270]	None
Beta-glucan	2[271,272]	None
Refined functional carbohydrates (Enzymatically hydrolysed yeast products)	5[91,273-276]	Aviator™ SCP[91,273], CELMA-NAX™SCP[275,276],
Mixture of carbohydrates or incompletely defined	8[241,245,270,277-281]	Biolex MB40[277], Safmannan[245], Original XPC™[280]
Other	7[230,234,268-270,282,283]	XOS 95P[268], Longlive 95p[230]

There is evidence that mannan-oligosaccharides added to fed can reduce *Salmonella* in broilers[25,103,179,208,234,249,266-269,279], while other oligosaccharides including fructo-oligosaccharides[208], xylo-oligosaccharides[230,268], arabinoxylan oligosaccharides[234] and sodium alginate oligosaccharides[282] may also be effective, but more commercial-scale research is needed to make any conclusions. A study saw a significant reduction in *S.*Typhimurium intestinal colonisation when birds were supplemented with galactoglucomannan oligosaccharides[269], while a galactoglucomannan oligosaccharide-arabinoxylan complex had no effect[270]. Refined functional carbohyrates (RFCs), produced through the enzymatic hydrolysis of yeast oligosaccharides, caused large, significant reductions in *Salmonella* when used as feed additives in a number of studies, and were added at low concentrations relative to other prebiotics [91,273-275]. Some other prebiotic additives with evidence of *Salmonella* reduction in broilers are inulin[278], β-glucans[271,272], a commercial "prebiotic-like" additive (Original XPC) [280,281] and *Aspergillus* meal (which contains a mixture of prebiotic compounds)[283]. No studies looked at the effect on *Salmonella* of prebiotics added to drinking water.

There was less research on the effects of prebiotics on *Campylobacter* and results were inconsistent: some prebiotic additives reduced *Campylobacter*[135,276,284] while others had no effect[134,261,284] and it is unclear what factors distinguish the effective and ineffective treatments.

Overall there is evidence that prebiotic dietary additives can reduce *Salmonella* in broilers, but research into the effect on *Campylobacter* is limited. Mannan-oligosaccharides and RFCs in particular have a volume of supporting research that warrants validation and consideration for use.

2.5.5. Synbiotics

Synbiotics are a combination of DFMs and prebiotics , with the aim to simultaneously provide broilers beneficial microbes, and nutrients to enhance the growth of these microbes in the gut[285]. Synbiotics may be effective against *Campylobacter* when added to feed[135,262] or drinking water[263]. Synbiotics were also shown to reduce *Salmonella* when added to the feed of broilers[43,44,103,250,251], but other studies saw no significant effect[113,238,241]. A commercial additive (Biomin Poultrystar) was the only synbiotic

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seen to reduce both *Salmonella* and *Campylobacter* among the studies mentioned [135,250,251,263]. There is less research on synbiotic additives relative to other categories, but evidence indicates they may be effective against *Salmonella* and *Campylobacter* in broilers. This, combined with the evidence supporting use of the prebiotic and probiotic components individually, indicates the use of synbiotic additives could be an effective intervention against *Salmonella* and *Campylobacter*.

2.5.6. Bacteriophage

Bacteriophage treatments are a relatively emerging field and had mixed results as feed and water additives. Studies have identified bacteriophage additives which may reduce *Salmonella*[286] and *Campylobacter*[287,288], but others saw no effect from treatment[289,290]. Some trials identified orally dosed bacteriophage treatments successful at reducing *Salmonella*[40,291-294] and *Campylobacter*[295-298], and may be followed up in the future with feed and water additive trials. A barrier to the effective use of bacteriophage treatments is the host-specificity of bacteriophage, so cocktail mixtures with a range of bacteriophage are often used. Bacteriophage will also not grow effectively when below a threshold host bacteria population so effects may be limited when *Campylobacter* and *Salmonella* counts in the gut of broilers are already low[288,297]. While bacteriophage-based additives show promise for use against *Campylobacter* and *Salmonella*, more research is needed and specific products should be developed before considering for commercial use.

2.5.7. Metals and inorganic additives

Studies which looked at various metal-based and inorganic additives saw varied effects. Zinc supplementation in feed was not found to have a significant effect on *Salmonella* in broilers[299], but a zinc-bearing zeolite compound lowered *Salmonella* [300]. Zeolite alone may also be effective against *Salmonella* as a feed additive[301]. Addition of copper to feed had no significant effect on *Salmonella* or *Campylobacter* in broilers[111]. Inorganic selenium added to feed had no effect on *Salmonella*, but there was a significant reduction when organic selenium was used[302], whereas in a similar experiment for *Campylobacter* there was no significant effect [303]. Addition of manganese to feed can actually increase *Salmonella* in broilers[304]. A supplement containing a range of ionised and chelated minerals saw limited (0.2 log reduction) or no significant effect on *Salmonella* when added to the drinking water of unchallenged broilers from 1-28 days of age [305]. There is evidence that a commercial ferric tyrosine product (TYPLEX®) can significantly reduce *Campylobacter* in broilers when added to feed[306-308]. In a trial under commercial conditions, an ion-exchanged-clay-based feed additive had no significant effect on cecal *Campylobacter* load in broilers at slaughter, despite being effective in smaller experimental trials[309]. This area of research requires more work generally, and in particular into large commercial scale trials around reduction in broiler cecal *Campylobacter* populations using ferric tyrosine.

2.5.8. Digestive enzymes

It is unclear if digestive enzymes (including xylanase, cellulase and phytase) have an effect *Salmonella* and *Campylobacter* when added alone to feed[111,310,311], but there are studies that showed that xylanase can be effective against *Salmonella* in combination with DFMs[227] or phytogenic additives[164]. A mixture of digestive enzymes combined with a DFM in feed significantly reduced *Campylobacter* populations relative to the DFM alone[219]. There is not a large amount of research into the effect of enzyme supplementation on *Campylobacter* and *Salmonella* in broilers, but it seems that when added alone the effect is limited, but in combination with other treatments the reduction of *Campylobacter* and *Salmonella* may be enhanced.

2.5.9. Feed Structure Modification

The modification of feed structure, specifically the substitution of ground components with unground equivalents, may also have an effect on *Salmonella* and *Campylobacter* colonisation. In one study the inclusion of oat hulls or whole wheat in the feed of broilers significantly reduced the spread of *C.jejuni* from seeder birds to contact birds after

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challenge on day 31, compared to a basal diet containing only ground wheat[88]. A later study by a different group[312] where birds were treated from day 1 to 42 and challenged on day 14 of age found that on day 42 while there was no significant change in cecal *C.jejuni* in a group treated with whole wheat only, there was a significant 1.4 $\log_{10}$ CFU/g reduction in the group treated with both whole wheat and oat hulls[312].

A whole wheat feed was found to protect contact birds from colonisation of *S.*Enteritidis significantly relative to a basal diet containing finely ground wheat, while a coarsely ground feed had no significant effect[313]. A study comparing ground corn to whole corn in feed found that at 21 d of age there was a significant linear decrease in cecal *Campylobacter* counts with increasing whole corn substitution, but no significant reductions in individual groups[314]. Broilers fed a diet containing whole grain rice in place of ground corn from 14 days old had significantly lower *C.jejuni* prevalence two days after the challenge on day 30 of age, indicating this diet may have provided resistance to colonisation[315]

In broilers fed either coarse or fine feed and challenged on day 25 with *C.jejuni*, there were significantly less *C.jejuni* positive birds in the coarse feed group than the fine feed group from days 1-5 post-challenge of seeder birds, with no data collected past day 5[68]. The studies reviewed show that the use of unground components in feed may result in lower populations of cecal *Camplyobacter* and should be further investigated in larger commercial field studies.

2.5.10. Substitute Feed Components

Some studies trialled alternative feed components. Whey added at 1% in the feed had no effect on *C.jejuni* counts in one study[316]. In a group of broilers fed a maize-based diet, *Salmonella* prevalence was significantly lower than in the group fed a wheat/rye based diet at 4 days post-challenge with *S.*Enteritidis (15 days of age)[317]. There was no effect on *C.jejuni* colonisation when birds were fed a diet in which maize was substituted by crimped kernel maize silage, relative to a basal diet with 58% maize[318] and in another study, no significant difference in *C.jejuni* cecal load was seen between broilers fed a wheat based diet or a maize based diet containing 10% less protein for 42 days[63]. There was no significant effect on *Salmonella* spp. in the ileum when broilers were given feed containing gamma-irradiated rice bran, relative to feed containing untreated rice bran[311].

Wheat bran, which contains a range of prebiotic compounds, was found to provide broilers protection against *S.*Enteritidis challenge when first ground to a reduced particle size of 280 um, with *S.*Enteritidis cecal counts 2.3 $\log_{10}$ CFU/g lower than the control group at 4 days post-challenge, but no effect at 18 days post-challenge onwards[319]. In heat-stressed, unchallenged broilers, diets containing 5% molasses and soy oil substituting for tallow significantly increased cecal *Salmonella* count by 1.4-1.8 $\log_{10}$ CFU/g relative to the heat-stressed basal diet control[55]. Glycerol in the feed of unchallenged broilers at 4 or 8% may reduce *Salmonella* prevalence[320]. Substituting soybean meal with canola meal in broiler diets had no significant effect on S.Typhimurium populations in challenged broilers[252].

Modifying the protein components of the feed may also have an effect on *Salmonella* and *Campylobacter*. One study developed a feed with reduced crude protein but supplemented with the key essential amino acids needed for broiler growth, with the aim of lowering *C.jejuni* colonisation by limiting available amino acids and mucin, two things important for the growth of *C.jejuni* in the gut[321]. The diet, provided from day 7 to 42 of life, significantly reduced *C.jejuni* in excreta of seeder birds on day 23 (2 days post-challenge of seeder birds), but had no significant effect on cecal count in contact and seeder birds at slaughter[321]. In a study trialling four different self-made feeds containing alternative protein sources (soya bean, rapeseed meal, haemoglobin powder or *Chlorella vulgaris* algae powder), all significantly increased cecal *Campylobacter* count at slaughter age (44 or 45 days old) by at least 4.2 $\log_{10}$ CFU/g compared to a commercial feed with soya-bean as a protein source[322]. The authors proposed that this increase may have been because of the higher crude protein in the self-made feeds compared to the commercial feed rather than the nature of the protein source itself[322]. A third study from the same

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group found that providing broilers with a choice of a high or low protein feed from day 14 of age did not provide protection against *C.jejuni* colonisation relative to a conventional diet, but interestingly, *C.jejuni* challenge did increase the consumption of the high protein diet compared to the unchallenged birds[323].

In another study, a high-crude protein diet did not affect cecal *Campylobacter* in unchallenged broilers when fed from day 1 to 21, but a diet supplemented with essential amino acids (methionine, lysine and threonine) reduced count to below detection (control count was 5 $\log_{10}$ CFU/g)[324]. Increasing threonine content in feed by 12% had no significant effect on cecal *S.*Enteritidis in challenged birds[24] and no significant effect on cecal *S.*Typhimurium was seen in broilers given feed containing added glutamine at 4, 10 or 11 days post-challenge[325]. In a different study, threonine supplementation of 3 g/kg was found to significantly reduce cecal *Salmonella* spp. by 1 $\log_{10}$ CFU/g in broilers when treated from day 1 to 21 of age[326].

2.5.11. Antibiotics

Antibiotic additives may be effective against *Salmonella* and *Campylobacter* but should not be used routinely. Many alternatives exist with a similar or greater effect against *Salmonella[98,110,180,231,244,249,275,327]* and *Campylobacter*[181,205] when trialled in parallel, and incorrect usage of antibiotics can negatively impact the health of broilers and contribute to antibiotic resistance[327,328].

Avilomycin added to feed from day of hatch reduced cecal *Salmonella* in challenged broilers by 2.5 $\log_{10}$ CFU/g relative to the basal diet at three days post-challenge[157]. In a group of broilers given chlortetracycline in feed and challenged with S.Pullorum at 3 days post-hatch, cecal *Salmonella* was reduced by 1.2 $\log_{10}$ CFU/g on day 7 post-hatch but was increased by 0.85 $\log_{10}$ CFU/g on day 21 relative to an untreated control[300]. Zinc bacitracin did not have an effect on *Salmonella* colonisation in broilers challenged with *S.*Enteritidis[317]. Tetracyclin reduced ileal *Salmonella* counts by 0.9 $\log_{10}$ CFU/g in unchallenged broilers[102]. Virginamycin had no significant effect on *Salmonella* spp. in unchallenged broilers when added to feed for 35 days[231], or from d 4 to 49 of age[195]. Flavomycin added to feed from day 1 of age reduced fecal *Salmonella* by 0.8 $\log_{10}$ CFU/g on day 21 and 1.4 $\log_{10}$ CFU/g on day 42[329].

There is some evidence that bacitracin methylene disalicylate may reduce *Salmonella* spp. when added to broiler feed[225], but another paper found no significant effect when it was added to feed at the same concentration[161]. There is evidence enroflaxyn may reduce *S.*Enteritidis in challenged broilers[105]. Vancomycin can increase *S.*Typhimurium count and frequency in challenged broilers, and has been proposed for use as part of a *Salmonella* challenge model[330]. Prophylactic enrofloxacin treatment via drinking water was found to increase organ invasion and intestinal colonisation of *S.*Enteritidis in challenged chicks[327].

Although research into antibiotic feed additives may be beneficial academically (e.g. as a comparison to other treatments), in the current era of antibiotic resistance and responsible use of antibiotics in the poultry industry their use should not be encouraged on a commercial level.

2.5.12. Other additives

Some additives were not heavily studied but further research could confirm if they are truly effective. Chlorine dioxide has been found to reduce *Salmonella* in broilers when used as a feed[331] or water[332] additive. Lactulose (4-O-β-D-galactopyranosyl-D-fructose), an isomer of lactose, was found to significantly reduce *S.*Typhimurium excretion at 10 days of age when added to the drinking water in: chicks treated and challenged simultaneously, treated then challenged, or challenged then treated in the first 2 days of life[333].

A *Lactobacillus* derived bacteriocin added to drinking water was found to significantly reduce *C.jejuni* and *S.*Enteritidis in the ceca of broilers that were challenged then treated shortly before slaughter age (40-43 days of age)[334]. In a challenge study, broilers were fed cationic peptides produced by *Brevibacillus texasporus* for the first 4 days of life, after which the birds were challenged with *S.*Enteritidis and fed a basal diet until the end

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of the experiment: in treated groups at 10 days old, cecal SE was reduced by 1-1.7 $\log_{10}$ CFU/g relative to the control[335]. No significant effects on *Salmonella* were noted when high-temperature stressed birds were provided ascorbic acid alone in the feed[132]. Broilers given the option to consume milk in addition to normal tap water had significantly reduced *Salmonella* counts in the feces in one study[182]. Humic acid extracted from compost added to feed had no significant effect on *S.*Enteritidis in challenged birds[131]. Vitamin E, alone[302], and in combination with MOS and L-Arginine[336] may reduce *Salmonella* in broilers.

Boric acid added to feed did not protect broilers from *S.*Enteritidis challenge when provided for 6 days beforehand[165], but did significantly reduce *S.*Enteritidis when given to chicks challenged at 1 DOA for 10 days following challenge, with reductions of 2.6 and 5.3 $\log_{10}$ CFU/g in the crop and ceca respectively[337].

Sodium bisulfate added to feed did not have a significant effect on cecal and fecal *S.*Enteritidis counts in a challenge trial[338], but can decrease *Salmonella* counts in the litter of broiler pens[339]. A commercial sodium bisulfate based product had no effect on cecal *Campylobacter* when added to broiler drinking water at recommended times throughout life or for 24 h before slaughter, but did decrease *Campylobacter* counts in the drinking water itself[340].

Chitosan added to feed from day of hatch significantly reduced cecal *S.*Typhimurium count by nearly 3 $\log_{10}$ CFU/g in 10-day old broilers challenged on day 3 of life[341]. In unchallenged broilers, provision of feed containing shrimp meal from day 8 to 35 of age significantly reduced *Salmonella* spp. count in the ceca by up to 0.6 $\log_{10}$ CFU/g (10% shrimp meal), while chitin supplementation did not have an effect at any concentration (0.9-3.8%)[342].

These studies into novel feed and water additives all demonstrate potential avenues for further research.

2.5.13. Combination Additives

Combinations of different additive types have the potential to create an enhanced effect compared to the components separately. A commonly trialled combination was that of organic acids and phytogenic products. Multiple studies found that a combination of organic acid and phenolic plant products could be effective as feed or water additives against *Salmonella* in broilers relative to untreated controls[106,110,126-128,174,190]. It is unclear if the combination effect is synergistic however: two studies[102,107] did not find any further reduction in *Salmonella* when broilers were treated with a phytogenic /organic acid combination additive relative to treatment with the organic acid alone. One looked at a combination of cinnamaldehyde with formic acid[107] and the other at garlic and a herbal compound with SCFAs[102]. There is also evidence that additives containing organic acids and phytogenic products can reduce *Campylobacter* in broilers relative to untreated groups[135,137,148,150], but other trials saw no significant effect[147,149]. The mixed findings could be because of the specific combinations used or because of the trial conditions but it is difficult to make conclusions due to the large number of variables at play.

Combinations of organic acids and probiotics added to drinking water have been shown to reduce *Salmonella* in broilers[25,90,182]. Finally, some miscellaneous combinations with organic acids were trialled. Levulinic acid with sodium dodecyl sulfate added to drinking significantly reduced *S.*Heidelberg prevalence in the crop of challenged birds[116]. A clay based additive combined with a SCFA mixture was more effective against *Campylobacter* than either additive alone[134]. Additives containing organic acids with monoglycerides can reduce the spread of *Campylobacter* between pens[151], and reduce cecal count[135].

The combination of additive types in feed has the potential to create a powerful synergistic effect, however no combination treatment described above clearly demonstrated this. Research in this area could further explore the combinations that appear effective at reducing *Salmonella* or *Campylobacter*, and should trial the individual components parallel to the combination treatment to measure their contributions to any effect seen.

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2.6. Immunisation

Vaccination has been used to control *Salmonella* infection/colonisation since first used in 1956 to control host specific serovars Pullorum and Gallinarum [3]. A review by Acevedo-Villanueva (2021) went into depth about the current commercial vaccines available for *Salmonella* and also included a concise review of the chicken immune response[343]. Pumtang et al (2021) performed a systematic review on poultry vaccines for *Campylobacter jejuni*[344]. The findings presented here are based upon screening of original research and summarising those results.

Table 1: A summary of different types of vaccines used in poultry trials for *Salmonella* and *Campylobacter* (No. [citation).

Enteropathogen	Inactivated	Subunit	Live attenuated	Live recombinant	Passive	Combination
Salmonella	4[21,92,93,239]	0	8[345-352]	2[353,354]	1[355]	1[94]
Campylobacter	2[356,357]	7[20,22,358-362]	0	3[259,363,364]	1[365]	0

2.6.1. Salmonella immunisation

A range of *Salmonella* vaccines and vaccine types were trialled in recent literature and are summarised in Table 2. Two studies trialled multivalent, inactivated *Salmonella* vaccines: one vaccine provided protection against heterologous *Salmonella* challenge [92], while another provided unchallenged broilers some protection in a production-scale trial [93]. Other inactivated vaccines were found to protect against *S.*Typhimurium [21] and *S.* Enteritidis[239]. Two studies found that a commercially available live-attenuated *S.*Typhimurium vaccine can also protect against *S.*Heidelberg [345,349], and a live-recombinant *S.*Enteritidis vaccine was also seen to protect against *S.*Heidelberg[353]. In two live vaccine studies, there was no protection against heterologous challenge[351,354]. Numerous studies described other live-attenuated vaccines that protected against *S.*Typhimurium and/or *S.*Enteritidis [346-348,350,352]. In one study, passive immunisation using in-feed IgY had no effect on *Salmonella*[355].

There is evidence that immunisation of breeder flocks can provide protection against *Salmonella* to the progeny of those flocks[92-94], which is important in cases where vaccination of the broiler flock itself may be impractical.

CpG oligonucleotides can be used as an adjuvant and have been shown to reduce *Salmonella* without a paired *Salmonella*-specific antigen [366,367]. A study and a follow-up found that a CpG oligonucleotide injection protected 2 day old broilers from S.Enteritidis challenge 3h post-treatment, but was ineffective in 5 and 20 day old broilers, indicating this treatment may only be effective early in life[366,367].

Table 2: Broiler *Salmonella* immunization studies, with summary of vaccine type, delivery route, challenge, and the findings.

Vaccine type[1]	Description	Route[2]	Challenge details	Findings	Ref
I	Trivalent vaccine with serogroups B, C and E	IM	Birds challenged with serogroups autologous or heterologous to those used in the vaccine	Reduced cecal *Salmonella* prevalence in serogroups B and C (up to 50% reduction relative to control) with no evidence for reduction of E. May protect progeny of vaccinated breeders from *S.*Typhimurium colonisation.	[92]
I	*S.*Enteritidis bacterin	IM	Challenged with *S.*Enteritidis	Significant reduction (up to 35%) in *S.*Enteritidis prevalence across all organs measured	[239]
I	Killed vaccine with *S.*Typhimurium,	SC	No challenge	No significant reduction in treated broiler breeders, but there was a reduction in	[93]

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	*S.*Enteritidis and *S.*Kentucky			*Salmonella* prevalence in samples taken from their broiler progeny flocks.	
I	Electron-beam irradiated *S.*Typhimurium	IO	*S.*Typhimurium challenge on d 18 post-hatch	Approximately 2.5 log_{10} CFU/g reduction of the challenge strain in the ceca in 23 d old chicks	[21]
LA	*S.*Typhimurium with downregulated *mviN*	O	*S.*Typhimurium challenge	Approximately 3.5 log_{10} CFU/g reduction of the challenge strain in the ceca	[352]
LA	*S.*Typhimurium with genes aroA and serC deleted (Poulvac®ST)	S then DW	*S.*Heidelberg challenge	Approximately 2 log_{10} CFU/g reduction of *Salmonella* spp. In the ceca, no significant change in the liver.	[345]
LA	Combination of *S.*Typhimurium and *S.*Enteritidis hilAssrAfliG deletion mutants	O	Challenge with *S.*Enteritidis, *S.*Typhimurium or S.paratyphi B var. Java	Significant reduction in *S.*Enteritidis (~2 log_{10} CFU/g) and *S.*Typhimurium (5.5 log_{10} CFU/g) in the ceca, no significant effect on *S.*paratyphi B var. Java. No effect on any challenge strain in the spleen.	[346]
LA	*S.*Enteritidis hilAssrAfliG deletion mutant	O	Challenge with *S.*Enteritidis	Up to ~2.5 log_{10} CFU/g reduction in the cecum and ~1 log_{10} CFU/g in the spleen of the challenge strain.	[347]
LA	*S.*Enteritidis hilAssrAfliG deletion mutant	DW or S	Challenge with *S.*Enteritidis	Up to ~3.5 log_{10} CFU/g reduction of challenge strain in the cecum by both delivery methods .No significant effect in the spleen.	[348]
LA	*S.*Typhimurium with genes aroA and serC deleted (Poulvac®ST)	S	Challenge with *S.*Heidelberg	No significant reduction of challenge strain in the ceca when challenged 3 days after intial vaccination, approximately 1.5 log_{10} CFU/g reduction in when challenged 28 days after (also after booster at d 20)	[349]
LA	*S.*Enteritidis with deleted fliD and cured pSEV	O	Challenge with *S.*Enteritidis 147	Reduction in cecal, liver and spleen colonisation prevalence for up to 3 weeks post-challenge compared to control group (Around 1 log_{10} reduction and up to 100% reduction in prevalence)	[350]
LA	S.gallinarum with deleted cobS and cbiA	O	Challenge with *S.*Enteritidis	No significant reduction of *S.*Enteritidis in cecal contents	[351]
P	Egg yolk powder containing anti-*Salmonella* IgY	F	Simultaneous challenge with *S.*Enteritidis and *S.*Typhimurium	No significant reduction in *Salmonella* spp.	[355]
LR	*S.*Enteritidis or *S.*Typhimurium expressing PAL, CJ0113, and HMGB1 in different orders	O, S	Challenge with *S.*Heidelberg	The spray-delivered *S.*Enteritidis vaccine significantly reduced population of the challenge strain in the ceca at 4 and 7 days post-challenge.	[353]
LR	*S.*Enteritidis attenuated vector expressing fliC	O	Challenge with wild-type *S.*Typhimurium	No significant protection against challenge.	[354]
LA then I	*S.*Typhimurium with cya and crp deleted then	S then IM	No challenge, study, looked at two commercial	Significant reduction in *Salmonella* prevalence on the carcasses of broiler breeders (32%), and in the carcasses of their progeny (10%).	[94]

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	bacterin prepared from *S.berta* and *S.kentucky*	poultry companies			

[1]I: inactivated, LA: live-attenuated, LR: live-recombinant, P: passive.
[2]IM: intramuscular injection, SC: subcutaneous injection, IO: in ovo injection, O: oral, S: spray, F: feed, DW: drinking water

2.6.2. Campylobacter immunisation

The majority of *Campylobacter* vaccine studies looked at subunit vaccines or recombinant live vaccines expressing a subunit (summarised in Table 3). Unlike *Salmonella*, there were no production-scale trials. All studies looked at protection against *C.jejuni* challenge, with some papers specifying that the challenge was heterologous. Most subunit and recombinant vaccine papers looked at proteins either in the *C.jejuni* flagella[22,358,361] or in the group of CjaA, CjaC, and CjaD, proteins[20,363,364],which are highly conserved between *C.jejuni* strains[368] and were originally identified because of their immunodominance[369].

Three studies found that there was no significant protection provided by the vaccine(s) trialled; one using homologous challenge[22], one using heterologous challenge[363] and one looking at both[358]. In two studies, vaccination was able to reduce *Campylobacter* levels in the ceca to below the detection limit[357,364], while the remainder saw reductions of 0.64 to 3 $\log_{10}$ CFU/g cecal contents [20,356,359-361]. Notably in one study, the combination of probiotics with a vaccine candidate led to an average ~7 $\log_{10}$ CFU/g reduction, with *C.jejuni* below detection levels in the majority of birds, significantly more effective than separate use of the vaccine or probiotics alone (2 $\log_{10}$ CFU/g reduction and no change at all respectively)[259]. One study trialled passive immunisation using egg yolk containing anti-*C.jejuni* IgY added to feed, and found that transmission from challenged to sentinel birds was completely stopped in the treated group but not the control group[365].

A study that tested multiple routes of vaccination found that while the subcutaneous injected vaccine was highly effective, when delivered orally it had no effect[357]. It is unlikely that vertical transmission is a major source of *Campylobacter*[370,371], so vaccination of parent flocks may not be as effective of a strategy as when dealing with *Salmonella*. For this reason a vaccine for production-scale use against *Campylobacter* should be practical to administer to large numbers of birds, which injections and oral gavage are currently not. Ideally, a broiler based vaccine needs to either be administered *in ovo* or ingested by live birds through water or spray.

Table 3: Broiler *Campylobacter* immunization studies, with summary of vaccine type, delivery route, challenge, and the findings.

Vaccine type[1]	Description	Delivery route[2]	Challenge details	Findings	Ref
I	*C.jejuni* lysate with encapsulated CpG oligodeoxynucleotides, or these components individually.	O	Challenge with *C.jejuni* strain 81-176, unclear if homologous	Significant *C.jejuni* reduction of 2.42 $\log_{10}$ CFU/g in the cecal contents at 22 days post-infection (37 days of age). Encapsulated CpG and lysate alone reduced counts by 1.46 and 2.14 $\log_{10}$ CFU/g respectively. No reductions in prevalence.	[356]
SU	Chimeric *C.jejuni* flagellin protein with TLR5 activating domain from *S.*Enteritidis	IO	Challenge with homologous *C.jejuni* at 18 d post-hatch	No significant reduction of *C.jejuni* in the ceca	[22]

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SU	*C.jejuni* flaA homologous DNA vaccine or heterologous DNA/protein vaccine	SC or IM	Challenge with heterologous or homologous *C.jejuni* at d 21 or 15 post-hatch	No significant reduction of *C.jejuni* in the ceca by any combination of vaccine and injection route	[358]
SU	*C.jejuni* hcp (Part of the type IV secretion system)	O	Challenge with homologous *C.jejuni* BCH71 at 28 d post-hatch	Significant reduction of up to ~1 $\log_{10}$ CFU/g ceca, no reduction in prevalence.	[359]
SU	Conjugate of diphtheria toxoid CRM197 and *C.jejuni* capsular polysaccharide	SC	Challenge with homologous *C.jejuni* at 29 d post-hatch	Significant reduction of 0.64 $\log_{10}$ CFU/g 9 days post-challenge. No prevalence data.	[360]
SU	*C.jejuni* CadF, FlaA, FlpA, CmeC or combination of CadF, FlaA and FlpA	Injection	Challenge with *C.jejuni* at 20 d post-hatch. Unclear if homologous.	Combination vaccine gave the best protection against cecal *C.jejuni* overall with a significant ~3 $\log_{10}$ CFU/g reduction in count and 7/9 birds colonised compared to 12/12 in control group. Individual subunits were also effective to varying degrees.	[361]
I then SU	Extracted *C.jejuni* outer membrane proteins, encapsulated or unencapsulated	O or SC	Challenge with homologous *C.jejuni* 81-176 at 35 d post-hatch	Subcutaneous encapsulated vaccine was the most effective, reducing *C.jejuni* below detection levels in the cloaca and cecum 7 days post-infection (>5 log reduction). Prevalence data not shown	[357]
SU	Hybrid protein of CjaA presenting three CjaD epitopes on the surface, carried by either liposomes or gram-positive enhancer matrix particles	IO	Challenge with heterologous wild-type *C.jejuni* at 14 d post-hatch	Liposomes were the more effective carrier, with a significant ~3 $\log_{10}$ CFU/g reduction of *C.jejuni* in cecal contents at 14 d post-challenge. 3/6 birds in this group were below the detection limit (3 $\log_{10}$) compared to 6/6 in control group.	[20]
LR	Attenuated *S.*Typhimurium expressing *C.jejuni* CjaA	O	Challenge with heterologous *C.jejuni* at 28 d post-hatch	No significant effect on cecal *C.jejuni*	[363]
LR	*E.coli* expressing *C.jejuni* protein glycosylation locus without or in combination with probiotics	O	Challenge with *C.jejuni* 81-176 at 28 d post-hatch. Unclear if homologous.	The vaccine alone significantly reduced C.jejuni prevalence compared to the control, with the addition of probiotics leading to a further significant reduction. Average ~2 log reduction in vaccine only group and 7 log reduction in vaccine + probiotic groups.	[259]
LR	Attenuated *S.*Enteritidis expressing *C.jejuni* CjaD, CjaA or Cj0420	O	Challenge with cocktail of three wild-type *C.jejuni* (PHLCJ1 to -3) at 21 d post-hatch	The CjaD vaccine significantly reduced *C.jejuni* to below the detection limit in all (10) birds treated at 32 day of age(~4.5 $\log_{10}$ CFU/g reduction) this occurred in two independent experiments.	[364]
SU	Various DNA or protein vaccines from *C.jejuni* strain 81-176	IM	Heterologous challenge with *C.jejuni* C97Anses640	Four antigens were identified that may be able to reduce C.jejuni count	[362]

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				in broilers, but further confirmatory research is required.	
P	Egg yolk containing anti-*C.jejuni* IgY	F	Homologous challenge with C. jejuni strain KC40	In the treated group, *C.jejuni* transmission to sentinel birds was completely stopped, while the control group had an average count of 7.3 log_{10} CFU/g. The overall count was reduced by over 5 log_{10} CFU/g, and a second trial yielded similar results.	[365]

[1]I: inactivated, LR: live-recombinant, SU: subunit, P: passive
[2]IM: intramuscular injection, SC: subcutaneous injection, IO: in ovo injection, O: oral, S: spray, F: feed

Vaccination for *Salmonella* in meat chickens is now a well-established process however *Campylobacter* vaccination is still in trial phase. *Campylobacter* vaccines ideally would target both human pathogenic stains *C. jejuni and coli* with an ideal vaccine candidate targeting the genus.

2.7. Chicken transport

Transport crates serve as a potential source of *Salmonella* and *Campylobacter* cross-contamination from previous positive flocks [372]. Poor transport conditions can increase fecal shedding, increasing the chance of *Salmonella* and *Campylobacter* spread [373].

Treatment of transport crates with spray washing followed by dry-stress via the addition of corn-starch [374] or use of hot air [375] may be an effective and practical method for limiting carriage of *Campylobacter* on crates. The use of a compressed air foam system to apply disinfectants to transport crates may be effective against *Salmonella* but has only been shown to work in the context of table egg layers [376]. A foam spray with levulinic acid and sodium dodecyl sulfate significantly reduced *Salmonella* prevalence on the doors of chicken cages used for transportation[72]. Transport duration may play a part in enteropathogen spread but more research is needed: one study found no difference in *Campylobacter* prevalence between birds transported for 0 h and 4 h[377].

Flocks can be sampled and tested for *Salmonella* and/or *Campylobacter* as close as possible to transportation, between 21-28 days of age, such that management process (such as late processing, freezing or commercial cooking) can be instigated if human pathogenic *Salmonella* are present[3].

The major areas of concern with transport are the flock status for *Salmonella* and *Campylobacter* prior to pick-up and the potential to cross-contaminate farms with 'dirty' transport crates and these two factors need to be addressed by individual processors.

3. Meat bird (broiler) Processing

Poultry processing plants globally use very similar processing steps with some variation at critical points that may impact the rate of carriage and population of pathogens remaining on the carcass [3]. A comprehensive guide was produced recently by the United States Department of Agriculture Food and Safety Inspection Service in 2021 which described intervention strategies and best practices for the control of *Campylobacter* and *Salmonella*[378,379]. The following sections of this review are more globally inclusive and include current research approaches which have been identified through systematic screening.

3.1. Scalding

Scalding is a process where chicken carcasses are either immersed in hot water or sprayed with hot steam to open the feather follicles of the skin to facilitate the removal of feathers [3]. In a recent study, a pre-scald cloacal wash with lactic acid (5%) was found to significantly reduce *Campylobacter* on carcasses in a commercial processing plant by 0.66 log_{10} CFU/cm^2 relative to untreated carcasses. No significant reduction was seen in trisodium phosphate (5-20%), citric acid (1-10%) and lactic acid (1% and 10%) treatments although they were effective in preliminary experiments[380].

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Prescald brushing is another intervention that may be used, but a recent study did not find it to significantly reduce *Salmonella* or *Campylobacter* as a singular step[381]. An overall significant reduction in both was seen when this was included in a series of wash-steps throughout processing however, so while this demonstrates that a series of interventions may be necessary for effective *Campylobacter* and *Salmonella* reduction rather than a single 'magic bullet', it is unclear if pre-scald brushing contributed to the reduction[381]. Pre-scald brushing was found to significantly reduce *Escherichia.coli*, *Enterobacteriaceae* and dry-matter on carcasses in another study, but there was no measurement of *Salmonella* or *Campylobacter* specifically[382].

The use of high pH during scalding (pH 9.89) can significantly reduce *Campylobacter* relative to conventional pH (pH 6.88), but had no effect on *Salmonella*[383]. Increasing scald temperature can also enhance the reduction of *Campylobacter* during scald, but may cause the formation of skin lesions with the potential to harbour bacteria[384,385]. A lab scale study found that the use of pulsed electrical fields could reduce *Campylobacter* in scald water[386].

One post-scald intervention was trialled: immersion in electrolysed-oxidising-water after scalding was found to significantly reduce *Campylobacter* on carcasses[387]. The general conclusion to be made from the aforementioned studies is that the stunned birds should be clean as possible prior to entering the scalder and keeping the scald water clean of debris and bacteria will reduce the potential of carcass cross contamination.

The scald tank may act as a 'nutrient' broth for enteropathogens if not managed properly. Ideally, the scald tank should be kept clean with an optimum overflow rate. Also beneficial would be a pre-scald cleaning step (either brushes or sprays) and a post scald spray system to replace scald water film with clean water prior to defeathering.

3.2. Defeathering

In large commercial processors the removal of feathers is performed using an automated system where the carcass, post scald, is defeathered using banks of mechanical rubber picker fingers. Some studies investigated interventions to reduce *Salmonella* and *Campylobacter* at this stage. The rubber picker fingers used in defeathering can contribute to carcass cross-contamination, especially in cases where they are worn and have developed cracks able to harbour bacteria[388]. A recent study found that an iodine-based compound may be suitable for reducing *Salmonella* on rubber picker fingers, but production-scale use was not evaluated[388]. Another study found that a chlorine dioxide spray during defeathering significantly reduced *Campylobacter* and *Salmonella* on carcasses relative to conventional defeathering[389].

Plugging of the cloaca prior to defeathering also may be a strategy to limit contamination between carcasses: in one trial there was no significant effect when expanding foam was used to plug the cloacae of broiler carcasses for defeathering[390], but a later study found that similar use of shredder sponge significantly reduced *Campylobacter*[391]. A commercially-practical method for cloacal plugging was not described however. The addition of a post-defeathering outside water washer to a processing line had no effect on carcass *Campylobacter* contamination relative to untreated carcasses processed earlier in the day[384].

In conclusion, while plugging the cloaca may prevent fecal spillage during plucking it also may interfere with evisceration and introduce foreign bodies into a processing plant, which need to be contained. The addition of chemical sprays either during or after plucking may contribute to the reduction of enteropathogens from the skin or the prevention of them being entrapped within the feather follicle.

3.3. Evisceration

Evisceration is a point where crop and gut-contents can contaminate the rest of the carcass, and many studies trialled post-evisceration decontamination interventions (summarised in **Table 4**).

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Table 4: Post-evisceration carcass treatment trials and their findings on the effect on *Salmonella* and *Campylobacter* populations.

Type of processing aid	Treatment	Findings
Water	• Water immersion or spray[392-394] • Hot water immersion or spray[395,396]	• Hot water treatments reduced *Salmonella*. • Room temperature treatments had mixed effects on *Salmonella* and *Campylobacter*
Chlorine-based or other inorganic compounds	• Acidified sodium chlorite [397-399] • Chlorine dioxide [398,399] • Trisodium phosphate [380,395,397-400] • Sodium hypochlorite [392] • Water/hot water before or after the addition of trisodium phosphate[395]	• Acidified sodium chlorite and trisodium phosphate can reduce *Campylobacter* and *Salmonella* • Chlorine dioxide and sodium hypochlorite had mixed effects on *Salmonella*.
Organic acid	• Citric acid [398-400] • Citric and lactic acid combination[401] • Peracetic acid [397-399,402] • Glycerol monocaprate emulsion[403]	• Citric acid, the citric and lactic acid combination, and peracetic acid all can reduce *Salmonella*. • Citric acid, the glycerol-monocaprate emulsion and peracetic acid can reduce *Campylobacter*
Miscellaneous/Combination treatments	• E-polysine then acidic calcium sulfate [394,404] • Lauric arginate then acidic calcium sulfate [394,404] • Peracetic acid with aqueous ozone[402]	• E-polysine followed by acidic calcium sulfate and Lauric arginate then acidic calcium sulfate can reduce *Salmonella*. • Peracetic acid with aqueous ozone was similar in ability to reduce *Salmonella* and *Campylobacter*, but resulted in lower ambient peracetic acid vapor.

High pressure water spray and carcass trimming were found to be equally effective methods for reducing *Salmonella* and *Campylobacter* in visibly contaminated post-slaughter carcasses[393].

The use of post-evisceration spray cabinets to deliver disinfectant treatments was the focus of many studies: A combination of aqueous ozone (Viriditec™) and peracetic acid saw significant reductions in *Campylobacter* and *Salmonella* compared to control groups[402]. Successive sprays of E-polylysine then acidic calcium sulfate or lauric arginate then acidic calcium sulfate both significantly reduced *Salmonella* by more than 2 $\log_{10}$ CFU/mL[394] and in addition remain effective for up to 6 days through storage at 4.4°C[404]. A study comparing dipping and spraying of post-evisceration carcasses with trisodium phosphate (14%) or citric acid (5%) in a commercial processing plant found that dip significantly reduced *Campylobacter*, while the spray had no significant effect[400]. A solution of peracetic acid (500 ppm) and aqueous ozone, delivered via a post-evisceration spray cabinet, significantly reduced carcass *Campylobacter* and *Salmonella*[405]. Similar results were observed in a peracetic acid-only group, however the inclusion of ozone resulted in significantly less ambient peracetic acid vapour, improving safety[405].

Chlorine-based treatments may not be suitable as post-evisceration treatments due to excess organic material present at this stage: the use of a 500 ppm chlorine drench for carcasses after evisceration had no significant effect on *Salmonella* prevalence compared to untreated and water-treated groups [392].

Sonosteam®, a technique that uses steam and ultrasound on whole carcasses (both inside and outside) in a chamber, is another possible post-evisceration carcass treatment. A study using a proof-of-concept setup found an average 2.51 $\log_{10}$ CFU/carcass reduction

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in *Campylobacter*[406], and a trial in a commercial processing plant saw reductions of 0.87-0.95 log_{10} CFU/g carcass[407].

The use of a post-evisceration hot water spray (71°C) rather than unheated water may reduce *Salmonella* prevalence on carcasses, but had no significant effect on *Campylobacter* and resulted in a partially cooked appearance[396]. Post-evisceration immersion in water containing a glycerol monocaprate emulsion consistently reduced *Campylobacter* on carcasses by up to 1.9 log_{10} CFU/100g relative to untreated carcasses[408]. Immersion of post-evisceration carcasses in trisodium phosphate significantly reduced *Salmonella* prevalence relative to tap water treatment, but brushing of the carcass had no significant additional effect[395]. The use of a post-evisceration spray cabinet to treat carcasses with acidified sodium chlorite or trisodium phosphate was found to significantly reduce *Campylobacter*, while peracetic acid had no significant effect[397]. Post evisceration spray or immersion treatment with a citric and lactic acid mixture can reduce *Salmonella* on carcasses by 1.3 and 2.3 log_{10} CFU/mL carcass rinsate respectively[401]. Lactic acid, cetyl pyridinium chloride and trisodium phosphate immersion treatments all were all able to significantly reduce *Salmonella* on carcasses relative to tap water immersion, by 2.4-4.8 log_{10} CFU/mL carcass rinsate[409]. Combination of these treatments with the surfactant Tween 20 did not lead to a further reduction[409].

One study found that the timing of evisceration can be modified to limit the increase in *Campylobacter* during defeathering. Although pre-defeathering counts were higher, there was a small decrease in post-defeathering *Campylobacter* counts (0.63 log) on carcasses when evisceration was done pre-defeathering relative to conventionally treated carcasses yet to undergo evisceration[390]. A follow up trial found that pre-scald evisceration was even more effective, with a post-pick count over 2 log lower than conventionally treated carcasses yet to undergo evisceration, perhaps due to anti-bacterial action of the hot scald water[390].

There was a lot of research into post evisceration treatments of carcasses with dips and sprays using processing aids, and treatments were generally able to reduce carcass loads of *Salmonella* or *Campylobacter*. Many of these were treatment/control studies and there was limited data shown on carcass enteropathogen populations/prevalence at the end of processing (post chill) rather than immediately after treatment, which would be a useful indicator of how effective a treatment is in a commercial context.

3.4. Inside Outside Washer

No studies specifically looked at an inside-outside bird washer (IOBW) step, possibly because it's already a well-established practice. Many post-evisceration interventions were trialled however, but did not specify if they were intended as in place of the IOBW or in addition. This was also seen in a review performed by Russell in 2012[410], which in itself is an oddity as the inside outside washer is a critical control point in most processing plants and a very efficient way to reduce surface and interior contamination prior to microbial to entrapment/attachment occurring [378,411].

3.5. Post Inside-Outside Washer/Pre-chill

Some post-IOBW treatments were explored in recent literature. Submersion in lactic acid reduced *Campylobacter* on carcasses by over 1 log_{10} CFU/carcass compared to water-immersed carcasses, while a lactic acid spray, and an electrolysed oxidising water spray and immersion had no significant effect[387]. A different study found that spray treatments of lactic acid could significantly reduce *Campylobacter* by up to 1.9 log_{10} CFU/g on carcass breast skin, but may worsen the appearance at high concentrations of lactic acid (8%)[412]. The use of a post-IOBW electrolysed NaCl solution spray (free chlorine of up to 18.4 ppm) in a commercial processing plant was found to have no significant effect on *Campylobacter* numbers on broiler carcasses[413].

3.6. Chill

The chilling stage of processing aims to reduce the carcass core temperature to <4 C within 4-8 h and can be achieved through immersion (in cold water), air or combination chilling[3]. The use of immersion chilling allows the use of sanitising agents to be added

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which primarily keeps the body of water bacteria free and aids in the reduction of enteropathogens.

Immersion chilling can significantly reduce *Salmonella* and *Campylobacter* prevalence relative to air chilling and combi in-line air chilling, of which neither had any significant effect on *Salmonella* or *Campylobacter* relative to untreated carcasses[414]. A different study found that there was no significant difference in *Salmonella* and *Campylobacter* prevalence between air-chilled and water immersion chilled carcasses processed in commercial conditions, but water chilled carcasses had a significantly lower average *Campylobacter* count[415]. A forced air chiller in a commercial plant has been shown to reduce *Campylobacter* counts by an average of 0.44 $\log_{10}$ CFU/carcass compared to carcasses sampled prior to chilling[406].

Chlorine based additives may be effective when added to immersion chill tanks. *Salmonella* prevalence was lowered in carcasses chilled in water containing 20 mg/L free chlorine in one study[416], and in another study, periodic addition of crushed ice and sodium hypochlorite was found to reduce carcass *Campylobacter* count relative to pre-chill measurements[417].Chlorine is susceptible to inactivation due to excess organic matter, but one study trialled the use of a proprietary chlorine stabiliser during immersion chilling and found that it significantly reduced the spread of *Campylobacter* and *Salmonella* from inoculated carcasses to uninoculated carcases relative to immersion chilling with chlorine only[418]. Calcium hydroxide was found to improve the efficacy of chlorine in chiller water containing organic matter in a similar manner, although it was a lab-scale study[419]. A different lab-scale study found that the use of pulsed-electric fields can reduce *Campylobacter* in chiller water[386].

Rapid freezing may help lower *Salmonella* and *Campylobacter* on carcasses, either as a chill or post-chill step. Freezing of carcasses immediately after IOBW reduced *Campylobacter* counts by an average of 1.44 $\log_{10}$ relative to untreated carcasses[406], and a post-chill immersion or spray of carcasses with dry ice was found to significantly reduce *Salmonella* spp. prevalence[420]. A prototype tunnel system for rapid surface freezing was trialled and shown to significantly reduce *Campylobacter* by at least 0.9 $\log_{10}$ CFU/g in post-chill carcasses, with the count remaining significantly lower after 7 d of storage[421].

The chilling methods reviewed above showed that there was a reduction in enteropathogens observed when processing aids were used during immersion chilling, or when very rapid chilling interventions were applied.

3.7. Post-chill

Some post-chill carcass interventions were explored (**Table 5**). Post-chill carcass treatments with peracetic acid, lactic acid, citric acid, sodium hypochlorite or SaniDate® (a commercial mixture of peracetic acid and hydrogen peroxide) were shown to reduce *Campylobacter* counts by a minimum of 1.26 $\log_{10}$ CFU/mL carcass rinsate[75]. For the reduction of *C.jejuni* in a post-chill treatment, peracetic acid was found to be more effective than sodium hypochlorite, and immersion treatments of these were generally more successful than spray treatments[422].

Peracetic acid added to a post-chill immersion tank was found to significantly reduce *Salmonella* and *Campylobacter* on carcasses relative to immersion in only water, while chlorine and lysozyme had no significant effect[423]. Peracetic acid, lactic acid, citric acid and sodium hypochlorite are all potentially effective immersion treatments for the reduction of *Salmonella* in post-chill carcasses[424]. Caprylic acid was also found to be a potentially effective processing aid against *C.jejuni* when trialled in lab-scale trial on post-chill carcasses[144].

Some studies also looked at post-chill portion treatment in commercial or simulated commercial conditions. One study trialled the use of acidic antimicrobials in a commercial equivalent spray-cabinet on chicken portions inoculated with *Salmonella*: on chicken thighs, lactic acid and buffered lactic acid significantly reduced *Salmonella* count relative to the water treated control in some temperature conditions, but on chicken breast no treatment was significantly different to the water-treated control[425]. A pilot plant study found that post-chill treatment of boneless breast and thigh meat with peracetic acid and

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cetylpyridinium chloride both significantly reduced *Campylobacter* and *Salmonella* counts in their resulting ground meat product compared to water treated samples, while no difference between water treatment and chlorine treatment was observed[426].

A study looked at the post-chill treatment of drumsticks with various combinations of disinfectants[427]and a further study looked at combinations of these disinfectants with ultrasonication[428]: it was found that a combination of trisodium phosphate (12% w/v) and capric acid sodium salt (5% w/v) was the most effective at reducing *C.jejuni*, but that overall there was no significant additive effect of using multiple disinfectants compared to just one. The use of ultrasonication in combination with disinfectants however did appear to significantly further reduce *Campylobacter* counts[428].

When broiler portions were treated in a post-chill decontamination tank at a pilot processing plant, *Salmonella* was reduced by 2.5-3.5 $\log_{10}$ CFU/mL and 1.5 $\log_{10}$ CFU/mL when treated with cetylpyridinium chloride (0.35-0.6%) or peracetic acid (0.07-0.1%) respectively, while *Campylobacter* was reduced by 4-5 and 1.5 $\log_{10}$ CFU/mL with these treatments. Chlorine and acidified sodium chlorite caused a minimal reduction in *Salmonella* and *Campylobacter* which was not significantly different to the water-treated control[171].

Treatment of portions before grinding can reduce *Salmonella* and *Campylobacter* in the ground product. Peracetic acid and cetylpyridinium chloride were both shown to reduce *Salmonella* on chicken frames when used as an immersion treatment, with a reduction remaining in the product when the frames were mechanically separated[429]. Treatment of chicken frames with peracetic acid, cetypyridinium chloride or lauric arginate also reduced *S.*Heidelberg and *C.jejuni* in the resulting minced product, while sodium hypochlorite was only effective against *C.jejuni*[430]. Treatment of boneless, skinless chicken thigs and legs with a commercial *Salmonella* bacteriophage additive (Salmonelex™) prior to grinding significantly reduced *Salmonella* in the ground product by 0.2-0.4 $\log_{10}$ CFU/cm^2 in a lab-scale study[431]. Ground chicken containing skin treated with peracetic acid had significantly lower *C.coli* counts, but there was no effect on *Salmonella,* relative to water or chlorine-treated skin immediately after treatment and throughout 9 days of storage[432].

Table 5: Post-chill carcass treatment trials and their findings on the effect on *Salmonella* and *Campylobacter* populations.

Type of processing aid	Treatment	Findings
Surface freezing	• Dry-ice spray or immersion[420] • Liquid nitrogen spray[421]	• Dry ice can reduce *Salmonella* and was not trialed for *Campylobacter* reduction. • Liquid nitrogen can reduce *Campylobacter* and was not trialed for *Salmonella* reduction.
Chlorine-based	• Sodium hypochlorite[75,422,424] • Chlorine, delivery method not specified[423]	• Chlorine based additives can reduce *Salmonella* and *Campylobacter* but were generally less effective than peracetic acid.
Organic acid based	• Peracetic acid[75,422-424] • Lactic acid[75,424] • Citric acid[75,424]	• Peracetic acid, lactic acid and citric acid can reduce *Salmonella* and *Campylobacter*
Miscellaneous/Combination treatments	• Peracetic acid with hydrogen peroxide[75] • Lysozyme[423]	• Peracetic acid with hydrogen peroxide can reduce *Salmonella* while lysozyme had no effect.

The above section described post-chill intervention strategies from literature, which tended to focus upon poultry carcasses and portions using very similar approaches to those seen in the chill process. Many of these trials were performed either as lab-based trials or small-scale processing plant trails. More data is required around commercial applications and the use of these processing aids over a standard production day.

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3.8. Lab-scale Meat Disinfection Studies

Some lab-based studies looked at chicken meat treatments that could be further tested in a processing environment. A range of chemical, physical, irradiation-based and biological treatments were found to reduce *Salmonella* or *Campylobacter* on chicken meat portions and are summarised in **Table 6**.

The use of non-chemical treatments can reduce waste and reduce the chance of product chemical contamination, improving safety. Therefore, some non-chemical approaches identified including hydrostatic pressure, UV irradiation and plasma are promising treatments that should be investigated further for practical use during processing.

Electrostatic spray may be a viable, more economic, alternative application method to immersion for *C.jejuni* reduction and was shown to be equivalent to immersion treatments with; peracetic acid, lactic acid, sodium hypochlorite, a mixture of citric and lactic acid and a mixture of peracetic acid and hydrogen peroxide. [433].

Table 6: Lab scale chicken meat trials of treatments found to be effective at lowering *Salmonella* and *Campylobacter* populations.

Treatment Category	Treatments effective against *Salmonella*	Treatments effective against *Campylobacter*
Chemical	• Peracetic acid[399,434-438] • Acidified sodium chlorite[398,399,434,437,439,440] • Electrolysed water[441-445] • Organic acids (including acetic acid, lactic acid, levulinic acid and succinic acid) [439,442,446-452] • Cetylpyridinium chloride[439,453] • Trisodium phosphate[398,399,437,454] • Essential oils[455,456] • Lauric arginate[442,453,457] • Sodium bisulfate[436] • Sodium hypochlorite[454] • Chlorine dioxide[399] • Organic acids with sodium dodecyl sulfate[450]	• Organic acids[458-464] • Essential oils[455,463,465-468] • Trisodium phosphate[454,458,469] • Sodium hypochlorite[454] • β-resorcylic acid[470] • Chitosan[471] • Pectin[471] • Sodium decanoate[458] • A proprietary low-pH additive (PoultrypHresh)[472] • Reducing water hardness[473]
Physical	• Wax coating[438] • Heat[399,434] • Steam[448] • Sonication[474] • High hydrostatic pressure[475-477] • Crust freezing[478] • Plasma[435,479]	• Crust freezing[480] • Sonication[458,481] • Steam treatment[464] • High hydrostatic pressure [459,482-484]
Irradiation	• UV-C [485-490] • X-ray [491]	• UV-C [489,492] • Gamma irradiation[493-495]
Biological	• Bacteriophage[453,496-500] • Bacteriophage and antimicrobials[453]	No studies

3.9. Equipment cleaning

Some *Salmonella* and *Campylobacter* species can form biofilms, which may help them persist on surfaces in processing plants[501,502]. This has lead to studies looking at disinfectants and other treatments that can be used to reduce *Salmonella* and *Campylobacter* on processing plant relevant surfaces.

When a range of surfaces in a commercial processing plant were sprayed with dry-ice, all surface swabs tested negative for *Salmonella*, while before treatment where the overall prevalence was 41%[420]. An anti-*Salmonella* bacteriophage cocktail was shown to

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significantly reduce *Salmonella* on worker's boots in a rendering-processing plant, with the most effective treatments being a combination of the phage and either sodium hypochlorite or scrubbing with a brush[503]. When a cleaning protocol focusing on continuous cleaning and sanitisation was implemented during a 30 d commercial operation, *Salmonella* carcass prevalence was 1%, compared to 6% when traditional daily cleaning was used for the same time period[504]. A glycerol monocaprate solution was found to significantly reduce *S.*Enteritidis on the surface of a plastic cutting boards spiked with meat juice containing *S.*Enteritidis, relative to the use of a washing-up-liquid[403]. Neutral electrolysed water, a quaternary ammonium and lactic acid were all show to be effective at reducing *S.*Typhimurium and *C.jejuni* on wooden and plastic cutting board surfaces[505].

Some lab-scale studies investigated antimicrobial treatments against *Salmonella* and *Campylobacter* on processing-relevant surfaces. One assessed the ability for combinations of disinfectants and steam to remove *S.*Typhimurium biofilms on stainless-steel coupons and found that the combination of antimicrobials with steam allowed for faster inactivation times compared to their use separately[506]. Steam or superheated steam alone can also reduce *S.*Typhimurium on stainless steel and PVC surfaces[507]. The use of UV-C irradiation to treat a range of processing-relevant surfaces including stainless steel and polyethylene has also been shown to be effective[492].

Bacteriophage cocktail treatment reduced *Salmonella* counts by at least 5.23 $\log_{10}$ on stainless steel coupons inoculated with *S.*Enteritidis and *S.*Typhimurium[496]. In a different study, immersion of inoculated stainless steel coupons in a solution of anti-*Salmonella* bacteriophage reduced counts by up to 1 $\log_{10}$[508]. Treatment of a range of surface coupons (stainless steel, glavanised metal, aluminium, plastic and pressure-treated wood) with a 1% solution of a N-Halamine (1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone) reduced *C.jejuni* and *S.*Typhimurium counts to below detection on all surfaces (~5 $\log_{10}$ reduction), which also remained below detection during 28 d of storage. A 0.1% solution was less effective: while counts were significantly reduced initially, they quickly regrew to control level by the end of storage[509].

A number of methods for reducing enteropathogens have been described in recent literature, and may be suitable for in-plant validation. In addition, many lab studies described treatments which can serve as a basis for future larger-scale trials.

4. Distribution

4.1. Packaging

Preliminary evidence on experimental active-packaging components shows they may be effective in controlling *Salmonella* and *Campylobacter*. Polylactide film containing cinnamon oil can reduce *S.*Typhimurium in inoculated meat during storage, with up to a ~3 $\log_{10}$ reduction relative to film without the oil[510,511], and further reductions can be achieved when this is combined with high-pressure processing[512]. Plastic films (either polylactide or linear low-density polyethylene) containing cinnamon essential oil and silver/copper nanoparticles have been found to reduce *C.jejuni* and *S.*Typhimurium on chicken meat during storage significantly compared to film without any additives[513,514].

A silk nanofiber film containing thyme essential oils reduced *S.*Typhimurium in chicken meat relative to alfoil-wrapped meat by nearly 6 $\log_{10}$ by day 7 of storage at 4°C[515]. ε-polylysine/chitosan nanofibers used as a packaging film reduced *S.*Typhiumurium and *S.*Enteritidis counts relative to chitosan nanofibers alone by up to 2.84 $\log_{10}$ in chicken meat after 14 d of storage at 4°C, however count did increase during storage[516]. Chicken breast packaged with a polyethyleneimine/polyacrylamide hydrogel was found to reduce *S.*Typhimurium during storage[517]. In one study, absorbent pads containing N-Halamine were found to significantly reduce *Salmonella* and *Campylobacter* when added to tray packs during storage relative to control pads[518].

The use of airtight packaging with an atmosphere tailored to reduce enteropathogen growth is another packaging-based intervention strategy, commonly referred to as modified atmosphere packaging (MAP). A range of MAP gas compositions were unable to

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reduce *Campylobacter* counts during storage relative to atmospheric packaging[519-523]. These included: N_2:CO_2 mixtures ranging in the ratio of 10:90 to 90:10, O_2:N_2 at 80:20, CO_2:O_2:N_2 at 40:30:30[519], 100% CO_2, O_2:CO_2:N_2 at 5:10:85, and 100% O_2[520]. A 99.5% CO_2 with 0.5% CO mixture did not significantly reduce counts relative to vacuum packaging[521]. *Campylobacter* "survived well" on chicken meat portions stored for 14 d in packaging containing 80% O_2 with 20% CO_2 or 70% N_2 with 30% CO_2, with no reduction in prevalence during storage for the former gas composition, and a reduction to 50% by d 14 in the latter (compared to air control with prevalence of 92%)[522]. Studies looking at the effect of MAP combined with irradiation also found that while irradiation before storage effectively reduced *Salmonella* and *Campylobacter* during storage, the combination with MAP had no further effect [521,523]. Relative to MAP without any additive, peracetic acid[524], *Bifidobacterium longum*[525] and seasoning mixtures[526] have been shown to reduce *C.jejuni* during storage when added to chicken meat packaged in MAP.

Fewer studies trialled MAP with *Salmonella*, and it is difficult to judge if the use of MAP can reduce *Salmonella* during storage. Packaging with 99.5% CO_2 and 0.5% CO did not reduce *S.*Typhimurium counts in chicken breast meat relative to vacuum packaging[523]. Chicken breast tested after 7 days showed that MAP containing 95% CO_2 and 5% O_2 reduced *Salmonella* counts by 0.4 $\log_{10}$ CFU/g, while pre storage treatment with a commercial bacteriophage (SalmoFresh™) reduced counts by 1 $\log_{10}$ CFU/g, with the combination of these treatments reducing counts by 1.2 $\log_{10}$ CFU/g[527]. The addition of evaporated ethyl pyruvate to packaging gas at 105 and 420 mg/L reduced *S.*Enteritidis on chicken leg meat by 1.51 and 2.43 $\log_{10}$ CFU/g respectively, relative to atmospheric gas packaging[528].The addition of a ClO_2 sachet to MAP containing 30% CO_2/70% N_2 led to significant reduction in *S.*Typhimurium on chicken breast relative to MAP alone[529]. In a similar experiment, the slow release of allyl-isothiocyanate within MAP (30% CO_2/70% N_2) also significantly reduced *S.*Typhimurium[530]. Storage of breast fillets in MAP (30% CO_2/70% N_2) with or without rosemary essential oil had no effect on *S.*Typhimurium counts[531].

Active packaging, provided it is shown to be safe, could be a useful tool for reducing enteropathogens during storage. While it may have unrelated beneficial effects, there was no strong evidence indicating that MAP can reduce *Campylobacter* or *Salmonella* during storage.

4.2. Lab-scale Shelf Life Studies

While treatments applied to meat right before packaging can lead to reductions in *Salmonella* and *Campylobacter* immediately (as summarised in Table 13 above), these reductions can also remain during storage and may even amplify, as seen in a number of lab-scale studies, summarised in **Table 7**. These results can provide a basis for further research in more practical conditions.

Table 7: Lab scale chicken meat treatments found to be effective at lowering *Salmonella* and *Campylobacter* populations during storage.

Treatment Category	Treatments effective against *Salmonella*	Treatments effective against *Campylobacter*
Chemical	• Lactic acid[447,448] • Acetic acid[447] • Citric acid[398,437] • A SCFA mixture[452] • Trisodium phosphate[398,437] • Acidified sodium chlorite[398,437] • Lauric arginate[457] • Essential oil additives[532] • Electrolysed water[443]	• Lactic acid[462,464,533,534] • Acetic acid[533] • Organic acids(including lactic acid and acetic acid)[462,464,533,534] • Propionic acid[461] • Malic acid[460] • Essential oil additives[471,535]
Physical	• Steam[448] • High pressure[476]	• Steam[464] • High pressure [483]

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	• Plasma[536]	• Crust freezing[480] • Plasma [536] • A combination of steam and lactic acid[464]
Irradiation	• UV-C [489]	• UV-C irradiation[489]
Biological	• Bacteriophage[497,499] • Probiotics[537]	No studies

Some coatings/marinades were also investigated for their ability to reduce *Salmonella* or *Campylobacter* during storage. Coatings of carboxymethyl cellulose[538] or chitosan and kappa-carrageenan[539,540] containing phytogenic extracts have been shown to reduce *Salmonella* and *Campylobacter* on chicken meat significantly during storage compared to untreated samples. A range of marinades were investigated for effect on *Campylobacter* and it was found that the addition of tartaric acid (6%) most effectively reduced *Campylobacter* over three days, and that in general the more acidic marinades were more effective against *Campylobacter*[541].

4.3. Freezing/storage Conditions

Some recent studies found that freezing chicken meat can reduce *Campylobacter* counts on various chicken portions during storage. Over 6 weeks of storage at -20°C, *Campylobacter* count dropped from 5.34 $\log_{10}$ CFU/g to 1.88 $\log_{10}$ CFU/g in chicken breast fillets[542]. When frozen for 14 d, carcass *Campylobacter* counts dropped from 1.74 to 0.63 $\log_{10}$[520]. Minced chicken meat stored at -22°C for 10 days was reduced in *Campylobacter* count by 2 log, while chicken skin stored at this temperature saw a 2 log reduction within 10-28 days[543]. In studies looking at chicken liver, freezing at -15°C to -25°C significantly reduced *Campylobacter* count by 2.5-4 $\log_{10}$ CFU/g[544,545].

4.4. Meat transport

One study looking at a meat transport intervention found that the use of ALIGAL™ Blue Ice (dry ice pellets containing ozone) in mock transport packaging reduced *C.jejuni* on contact surfaces by 3.9 $\log_{10}$ and on chicken breast by 1.3 $\log_{10}$ after 24 h of storage[546].

5. Discussion

The large number, 514, of relevant original research studies conducted in the last 10 years have identified a diversity of management and intervention strategies for the elimination or reduction of *Salmonella* and *Campylobacter* populations from poultry meat. While many of these studies were in laboratory or limited field trials, implementation in extensive trials or true commercial operations has proven problematic. More published statistically correct horizontal trial data using International Standards Organisation methods, or equivalent, for *Salmonella* and *Campylobacter* are required to fill this knowledge gap.

There are many studies looking at a single specific intervention with the production chain, e.g. feed additives, but limited studies showing how these interventions work alongside other interventions, e.g. vaccination, processing aids etc. There may be a synergistic effect that has not been explored in depth.

For entities considering using commercial anti-enteropathogen products and interventions it is highly advisable that an internal validation and fit for purpose trial is conducted by the individual entity as all *Salmonella* and *Campylobacter* serovars and biovars may react differently in the said entity's location.

Future research should start to focus on more non-chemical application within the processing plant and how synergistic through chain intervention may contribute to reducing the overall burden of enteropathogen load on the carcass.

6. Conclusions

A large number of intervention strategies spanning the chicken meat production chain were identified and summarised here, with many of these having the potential for

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commercial use. The management and application of intervention strategies is a costly exercise for the entity with the said cost passed onto the consumer. The consumer role in reducing the burden of enteric illness cannot be excluded. Proper food handling to avoid cross-contamination, cooking to ensure thermal death rates are achieved and organoleptic inspection prior to handling are a must to reduce and/or eliminate the risk.

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Appendix A: Literature search and screening methods

Information Sources

The following information sources were used

DATABASE / INFORMATION SOURCE	INTERFACE / URL
Science Citation Index – Expanded **Conference Proceedings Citation Index - Science**	Web of Science
Medline	Web of Science
CAB Abstracts	Web of Science
Agricola	USDA National Agricultural Library Citation Database: https://agricola.nal.usda.gov/
ANR-Index, ANR-Index Archive	Informit

Search Strategy

The search domain was limited to Title, Abstract and Keyword fields for articles published in the 10 years inclusive of January 2009 to November 2019. An example of the search strategy for the Web of Science was:

(broiler* OR chicken* OR gallus* OR poultry* OR "meat bird*") AND (control* OR reduc* OR hygien* OR risk* OR eliminat*) OR "sanitary dressing" OR "slaughter hygiene" OR "hygiene dressing") AND (salmonell* OR campy* OR enteropath*).

Full database records from searches (including Title, Abstract, Author, Year, Publication Title at a minimum) were downloaded, imported into Endnote X7 (Thomson Reuters 2013) and de-duplicated.

Citations were exported from Endnote in XML format and imported into Covidence (Veritas Health Innovation).

Selection Process

The screening process was managed by two reviewers using Covidence software. Duplicated studies were reviewed and removed in Title and abstract screening and Full text screening.

Title and abstract screening

Two reviewers independently assessed titles and abstracts against the eligibility criteria for progression to Full text screening.:

1. Meat chicken birds, carcasses or portions without undergoing any secondary processing;
2. Treated with an intervention
3. Included a control comparator group;
4. Measured *Salmonella* or *Campylobacter* levels
5. Described outcome measurement methods in detail or referred to a recognisable standard method (e.g. AS/NZ, BAM)

Abstracts where both reviewers scored [YES], or at least one reviewer scored [UNCLEAR] moved to full-text screening. Disagreements were resolved via discussion.

Full text screening

At the full text screening stage, the full texts of the studies were assessed against additional eligibility criteria:

1. Described the intervention method in detail sufficiently enough to implement the intervention
2. Used a randomised trial design.
3. Primary research
4. Was published in English (non-English publications were ineligible due to insufficient budget for translation)

Studies were excluded if the full text was unobtainable. Full texts where both reviewers scored [YES] were included in the review. Disagreements were resolved by discussion.

Studies that passed full text screening were included in this literature review.

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