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Methods and technologies to reduce or prevent salmonella infection using feed additives in poultry and swine production

written by

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INTRODUCTION

In food producing animals such as poultry, antibiotics that were used for growth promotion and improving feed efficiency have received increasing attention as a contributory factor in the international emergence of antibiotic-resistant bacteria (THITARAM et al., 2005).

In many countries, including the European Union, antibiotics have mostly been banned as animal growth promoters. Therefore natural methods have become a widespread interest in order to inhibit detrimental bacteria (VAN IMMERSEEL et al., 2006). This paper is a critical review of literature concerning the prevention or decrease of Salmonella caused diseases in poultry and pig using means alternative to antibiotics

1. About Salmonella in general

Salmonella belong to the family Enterobacteriaceae, which are Gram-negative bacteria, consisting of medium sized rods (0.4-0.6 x 2-3 µm). The genus Salmonella consists of a single species called Salmonella enterica, which has been divided into over 2500 serotypes. These serotypes are based on the *Kauffmann-White* Scheme, according to the O (somatic), H (flagellar) and occasionally capsular (Vi) antigens. Some classifications divide the genus into 7 subgroups, where subgroup I contains the most significant animal pathogens. The full name for example is Salmonella enterica subsp. enterica serovar Typhimurium. A simplified nomenclature is often preferred, with the named serotypes of Salmonella regarded as "species", for example S. Typhimurium.

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The reservoir for salmonellae is the intestinal tract of warm- and even cold-blooded animals. The majority of infected animals become carriers and subclinically excretors. In the environment Salmonellae can survive for 9 months or more in moist soil, water, faecal particles and animal feeds, especially in blood, meat-and-bone and fish meals (QUINN et al., 1999).

1.1. Salmonellosis

Salmonellosis occurs worldwide and in many animal species. The frequency of the disease has increased with the intensification of livestock production. The more common Salmonella species are as follows: In cattle the S. Typhimurium, S. Dublin and S. Newport; In the sheep and goats the S. Typhimurium, S. Dublin, S. Anatum and S. montevideo; In horses the S. Typhimurium, S. Anatum, S. Newport, S. Enteritidis and the serovar IIIa (KAHN et al., 2005).

The focus of the present paper will be on Salmonella infecting pigs and poultry which will be dealt with in detail in the following chapters.

1.2. Transmission

Infection occurs usually by the faecal-oral route of viable salmonellae. However infection via mucos membranes of the conjunctiva or upper respiratory tract is suspected (QUINN et al., 1999). The outcome of the disease depends upon the colonization resistance of the host animal, the infectious dose and the given species of Salmonella (DWIGHT et al., 1999). Salmonellae are frequently facultative intracellular parasites. Host macrophages take up the invasive strains, which are then spread via the lymphatic system, bloodstream, or both (CARTER et al., 1995). In recent years, the incidence of human salmonellosis has increased. Transmission to humans occurs via contaminated drinking water, milk, meat, eggs and foods such as fast food mixes that use contaminated ingredients (KAHN et al., 2005) as can be seen in figure 1.

Figure 1. Salmonella transmission to humans via food products.

The role of wildlife as a carrier of Salmonella species and the transmission of the bacteria to farm animals or directly to humans via game hunted for human consumption has become a matter of increasing concern. Hedgehogs, wild birds, white-tailed deer, wild boars and rabbits have been highlighted in several studies as important Salmonella carriers. A very recent study in Portugal reported that 22% of the wild boars (*Sus scrofa*) and 48% of the wild rabbit (Oryctolagus cuniculus) presented Salmonella spp. in their faeces. In Northern Portugal the predominant Salmonella serovars were S. Typhimurium, S. Rissen, S. Enteritidis and S. Havana (VIEIRA-PINTO et al., 2011). Hence, wildlife may represent a potential role as important faecal spreaders of this zoonotic agent. Therefore, attention should be reinforced on effective measures to keep wildlife separate from farm animals and on precautions during game meat preparation.

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1.3. Clinical Signs

Salmonellosis is characterized by one or more of three major syndromes: septicaemia, acute enteritis and chronic enteritis. Young piglets usually develop the septicaemic form. Chronic enteritis may develop in growing pigs. It may also cause abortion (KAHN et al., 2005). The asymptomatic carrier animal is common and a serious problem in all host species. In humans there are also three principle forms: enteric fevers, septicaemia, and gastroenteritis (CARTER et al., 1995).

1.4. Pathogenesis

The pathogenesis of Salmonella can be divided in two distinct phases, which are the intestinal and the systemic phase of the infection. Both are regulated by genes of a different Salmonella pathogenicity island (SPI). Figure 2 shows that Salmonella pathogenicity island I (SPI-1) function is required for the initial stages of salmonellosis. Firstly, SPI-1 controls the entry of Salmonella in non-phagocytic cells by triggering invasion and the penetration of the gastrointestinal epithelium. Furthermore, SPI-1 function is required for the onset of diarrhoeal symptoms during localized gastrointestinal infections. The function of SPI-2 is required for later stages of the infection, i.e. systemic spread and the colonization of host organs. The role of SPI-2 for survival and replication in host phagocytes appears to be essential for this phase of pathogenesis (VAN IMMERSEEL, et al., 2002b).

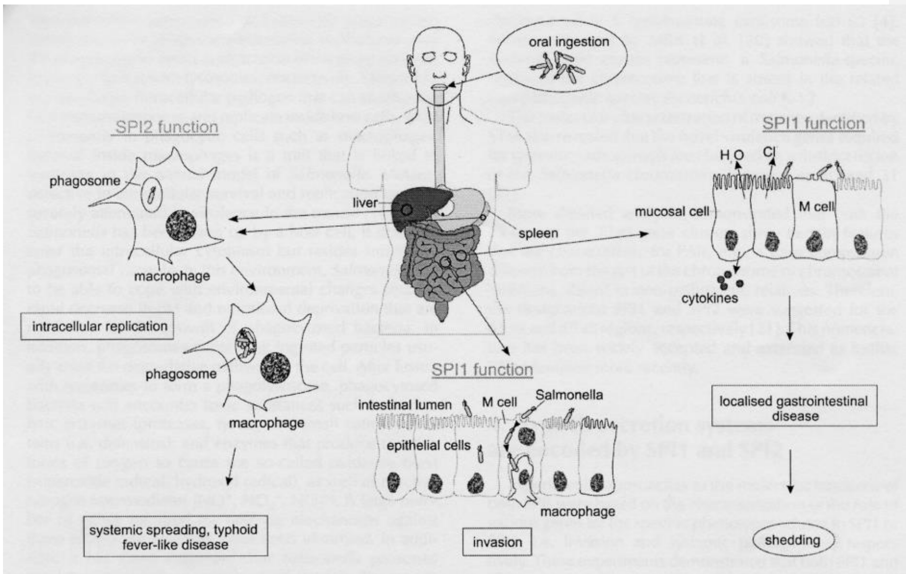

Figure 2. Schematic representation of host-pathogen interactions during pathogenesis of Salmonella infections.

To initiate enteric disease, Salmonella spp. have to colonize the ileum or colon. The indigenous normal anaerobic flora produces volatile fatty acids like butyric acid, which usually inhibit the growth of Salmonellae. The normal flora also usually blocks access to attachment sites required by the Salmonella species. However, factors such as antibiotic therapy, diet and water deprivation may disrupt the normal intestinal flora and therefore increases the host’s susceptibility to infection. Other predisposing factors are reduced peristalsis, transportation and overcrowding stress. Invasive strains that produce septicaemia, are able to escape destruction by the host and to multiply within the macrophages of liver and spleen as well as in the vessels’ lumen. The invasive ability of some serovars can be increased by the presence of special genes carried on a plasmid, for example S. Typhimurium carries O-repeat units of lipopolysaccharide, which masks the bacterial cell surface and thus prevents destruction within the bloodstream by the host’s complement system (QUINN et al., 1999). This can be solved by feed additive non-antibiotics chemicals. (INDUSTRIAL PATENT).

Disruption on a farm can possibly be severe and economic losses high, which emphasizes the difficulty and crucial need to minimize pathogen intake into the food chain. Salmonella control schemes have been put in place in most EU countries in recent years such as directive EU-92/117 and subsequent amendments, by the Council of the European community.

2. Special Poultry Features

The consumption of chicken eggs and meat is the leading cause of human foodborne infections. The primary cause of pandemic salmonellosis was previously Salmonella Typhimurium. Since the mid seventieths this serovariant has been replaced by Salmonella Enteritidis and since 1990 the latter has become the primary cause of human salmonellosis worldwide (GUARD-PETTER, 2001). Interestingly, the increase in S. Enteritidis isolates coincided with a decrease in S. Gallinarum in poultry. It has been proposed that eradication of S. Gallinarum resulted in loss of flock immunity against the O9-antigen, enabling S. Enteritidis to spread (BÄUMLER et al., 2000). In Hungary the predominant species in broiler houses is Salmonella Infantis, from which multi-drug resistant strains have been increasingly detected (NÓGRÁDY et al., 2008)

Horizontal transmission is as important as vertical transmission in Salmonella infections in poultry. The so-called "invasive serotypes" are serovars known to pass from the intestine into the tissues of poultry. These constitute the greatest risk, as they are transmitted vertically in the poultry population, when follicles in the ovary are infected or the developing eggs become infected in the oviduct. Invasive serovars are for example Enteritidis, Typhimurium, Bertha, Thompson, Infantis and Hadar. Horizontal transmission can increase because Poultry can become carriers and asymptomatically excrete Salmonella intermittently, as is the case for S. Enteritidis, or re-excretion is induced by stress conditions.

For non-invasive serotypes horizontal transmission is of major importance, as only eggshell contamination can lead to vertical transmission (VAN IMMERSEEL et al., 2002).

2.1 Background to Salmonella colonization

2.1.1Caecal invasion

Young chickens are very susceptible to infection by Salmonella enterica ser. Enteritidis because their lymphoid organs are not yet fully developed. Also if infection occurs at a young age, there is a greater risk to evolve into a carrier state. Experimental peroral infection showed that Salmonella bacteria, after attachment to the intestinal mucosa, cross the intestinal epithelium. Within few hours after the infection, they replicate in the lamina propria. The bacteria may proceed further to deeper tissues to disseminate via the bloodstream, invading organs such as liver and spleen, within a little more than one day post-inoculation. Non-specific inflammatory response, mainly macrophages and granulocytes, within the caecal lamina propria mediate the clearance of bacteria, reducing the possible number of Salmonella entering the blood stream. Chemotaxis attracts T-lymphocytes, which in turn, contribute to an antigen specific B-cell response. Evaluation of the leukocyte infiltration in the caecal lamina propria concludes that structural maturation of gut associated lymphoid tissues, GALT, is antigen driven (FALUS, 2004).

The results open the possibility to accelerate future immune responses against pathogenic strains, by priming the non-antigen-specific immunity of GALT, using non-pathogenic bacterial strains (VAN IMMERSEEL et al., 2002).

The invasion mechanism of Salmonella into intestinal epithelial cells involves the key regulatory protein **HilA**, which activates genes located on the Salmonella pathogenicity **Island I, SPI-1**. The latter assembles a three secretion system which allows injection of bacterial proteins into the cytosol, thus allowing intracellular multiplication within epithelial cells of the caeca. HilA also regulates genes on SPI-4, which upon activation contribute to uptake by macrophages and subsequent survival within the macrophages. Experiments using a HilA deficient mutant strain of S. Enteritidis illustrated a strong reduction in caecal colonization and feacal shedding. Although inactivating HilA regulatory protein did not prevent colonization of internal organs completely, suggesting the existance of additional mechanisms for invasion in Salmonella (BOHEZ et al., 2006).

2.1.2. Invasion of the Reproductive Tract

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S. Enteritidis has become the primary cause of human foodborne infection, in part because it has the unique ability to contaminate eggs without causing clinical illness in the birds infected (GUARD-PETTER, 2001).–

The pathogenesis of egg contamination is still not completely understood. Studies are difficult and time-consuming, because of the low incidence of egg contamination in an infected flock. Therefore the intermittent production of contaminated eggs by infected hens and because of the possibility of different mechanisms being involved (DE BRUCK et al., 2004a).

Vertical transmission either may occur during the development of the egg via infected reproductive tissues, or when the egg passes through the cloaca resulting in shell surface contamination. Under experimental conditions, several Salmonella serotypes can infect the chicken ovary, nevertheless in natural infections S. Enteritidis is the most frequent serotype found in table eggs. Several studies confirmed that the most infected site within the egg is the shell's inner side, which contains the shell membranes. This indicates that egg shell contamination mostly takes place inside the upper reproductive tract, most importantly within the isthmus and uterus. Cloacal contamination is less important, since experiments have shown that positive cultures of contaminated eggs were detectet even after intestinal carriage of Salmonella had ceased (DE BRUCK et al., 2003).

Three separate assays have reported that the ratio of colonization in the oviduct of laying hens is always higher in the isthmus than in the magnum. *S. Enteritidis* is therefore suggested to have adapted best to the isthmus segment of the chicken oviduct. These assays have confirmed that S. Enteritidis bacteria are detected intracellularly within the tubular gland cells, and few or none are observed attached to the surface epithelium. The intracellular proliferation in the oviduct during long periods can explain the clustered and intermittent production of infected eggs, since the Salmonella bacteria appear to wait for an undefined stimulus to come out of the cells, and colonize the forming egg (DE BRUCK et al., 2004c).

2.1.3. The Role of fimbriae in invasion

Surface structures, such as fimbriae, play a vital role in S. Enteritidis pathogenesis. Research has revealed that S.Enteritidis harbours at least four morphologically distinct fimbriae, which are encoded within a serotype associated plasmid (SAP) of 58 kb in size. These four Salmonella Enteritidis fimbriae are denoted SEF14, SEF17, SEF18 and SEF21 respectively. The first mentioned SEF14 has already previously been implicated with persistent infection in chicken. The SEF17 structure generates an aggregated phenotype and binds fibronectin. SEF18 genes are colocated with the genes encoding SEF14. SEF21, also referred to as the Type 1 fimbrial structure, binds laminin and promotes mannose sensitive haemagglutination (WOODWARD et al., 1996). The type 1 fimbriae can be seen in figure 3 as the thin projections sticking out from the surface of the cell. Some of the fimbriae have broken off, indicating they are quite brittle.

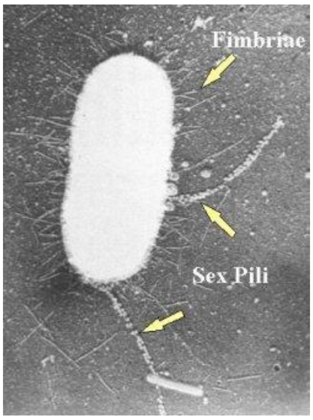

Figure 3. Transmission electron microscope picture showing bacterium with type 1 fimbriae

Experiments have shown that after natural infection of laying hens, S. Enteritidis can be found in the tubular gland cells of the oviduct. Detection can be as early as 24hours after an intravenous infection within the tubular gland cells in the magnum and isthmus of adult laying hens. The receptor of the binding has been localized inside the tubular gland cells of the isthmus. About one third of Salmonella Enteritidis isolates show adhesion to isthmal secretions. These adhesions proved to be mannose sensitive, which concludes the type of fimbriae predominantly involved in such adhesions are the Type 1 fimbriae (SEF21). Although the majority of Salmonella isolates are believed to be able to express type 1 fimbriae, optimal culturing is necessary for their expression. This suggests that most Salmonella isolates would in theory be able to attach to the isthmal secretions. During egg development, isthmus secretions generate the fibres of the shell membranes. S. Enteritidis bacteria localized in the isthmal glandular cells can easily be transported along with the secretory products of the cells, due to their affinity for such glandular cell secretions. The Salmonella contaminated secretory products coalesce within the duct to form a fibre, that is extruded from the opening of the gland into the lumen. The bacteria within the inner shell membrane are more or less protected from the antimicrobial factors in the egg white.

Overall the exposure of the bacteria to the immunological system of the hen is reduced to a minimum, because of the efficient transport of the bacteria from an intracellular location within the reproductive tract to the egg membranes at the moment of its formation. Also, vertical transmission of S. Enteritidis usually does not affect fertility rates or hatching percentage, since the embryo does not get infected until late during incubation or even until pipping, because of the bacteria’s favourable position in the shell membranes (DE BRUCK et al, 2003).

A fimbriate mutants (**fimD** mutant) have been used in separate studies, which supports earlier findings. Compared to the parent strain, infection with the **fimD** mutant leads to a reduction in egg shell contamination. Inoculation of laying hens with the **fimD** mutant, results in distinctly prolonged bacteraemia, which in turn has the effect in heavier and more frequently contaminated internal organs. Despite a heavier infection of the ovaries by the **fimD** mutant, shell membrane contamination remains low, because of the absence of type 1 fimbriae. Thus the mutant strain cannot adhere to the isthmal secretions and subsequently is not carried along the developing shell membrane fibres (DE BRUCK et al., 2004a).

Many virulence factors are involved that enable Salmonella Enteritidis to contaminate chicken eggs. Other factors that have been suggested are high molecular weight lipopolysaccharides and a capacity for growth to high densities. Analysis of fresh eggs have shown that S. Enteritidis can be associated not only with egg shells and their membranes, but also with yolk and egg white (DE BRUCK et al.,2004a).

A systemic infection with S. Enteritidis in laying hens can also lead to the colonization of the ovary as well as the oviduct. Reproductive organs can be infected independently from each other, at the same time or in consequential order. If present in the ovaries, the bacteria have shown to be able to interact with the cellular components of the preovulatory follicle, in particular the granulosa cell layer. From here, the Salmonella may penetrate the perivitelline layer and multiply within the interior yolk contents, or can be found on the intact egg yolk membrane. Contamination of the albumen is believed to occur during the passage of the egg through the oviduct. According to different authors and reports, the egg compartment that is most frequently contaminated varys, appearing to be highest in the shell membrane and lowest in the albumen (DE BRUCK et al. 2004b).

2.2 Surface contamination of eggs

A wide range of Salmonella serovars has been recovered from eggshells. Surface contamination can be the result of either infection of the lower reproductive tract or feacal contamination. In a healthy hen, faecal contamination is unlikely during oviposition, because of the evertion of the vagina beyond the alimentary tract and the stretching of the cloacal lining. Faecal contamination is most likely to occur in the environment after oviposition, therefore the hygiene in the chicken house and during egg handling and processing is critical.

Penetration of the eggshell by S. Enteritids, S. Typhimurium and other serovars has repeatedly been described under experimental conditions exclusively, and not in practice (DE BRUCK et al. 2004b).

3. Strategies to control Salmonella infection in laying hens (domestic fowl)

In 1992, The Council of the European Community issued a directive (EU-92/117 and subsequent amendments) requiring member countries to monitor for zoonotic agents, to control Salmonella in parent and layer flocks.

The ambition of the EU is to reduce the infection pressure of specified zoonotic agents, such as Salmonella, at all levels of the animal production chain. This can be done by a combination of pre-harvest, harvest, and post-harvest measures (VAN IMMERSEEL et al., 2002). The present paper focuses on feed additives, which constitutes an important group of pre-harvest measures.

3.1 Irradiation

Irradiation of poultry feed at predetermined doses can result in complete destruction of salmonellae, as well as other pathogens such as Enterobacteriaceae, moulds, fungi and insects. Effective doses range from 10 to 40 kGy. However, research indicates loss in potency of certain nutrients, such as all fat-soluble vitamins. There is also an increases in the peroxidation of fats, which could be controlled by the inclusion of antioxidants in the diet (LEESON – MARCOTTE, 1993). Other possible destructive effects on major nutrient components include depressed absorption of amino acids and particularly of fat.

This treatment may also have beneficial effects on nutritional value. Irradiation appears to also effectively improve growth of chicks fed on oat, rye or barley diets. The improvement of the nutritive value is due to irradiation-induced depolymerization of pentosans and β-glucans (CAMPBELL et al., 1986).

The irradiation of poultry feed for control of Salmonella is also approved by the FDA, food and drug administration, (21 CFR § 579.40) (Code of federal regulations, 2011).

3.2 Traditional feed additives

The purpose is replacement of banned antibiotics using prebiotics, probiotics and synbiotics, as well as other feed additives such as organic acids which will be focused on later. The aim is to stabilize the intestinal microflora, to decrease the colonization of intestine with pathogens and to improve the animal's health status (FEKETE, 2005).

3.2.1 Prebiotics

A prebiotic was originally defined in 1995 as a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (GIBSON and ROBERFROID, 1995). A more recent definition stated that "A prebiotic is a selectively fermented ingredient that allows specific changes, both in composition and/or activity in the gastrointestinal microbiota that confers benefits upon host wellbeing and health" (GIBSON et al., 2004).

Besides indigestible carbohydrates, there are other substances thought to have a positive effect on the intestinal flora, for example organic acids, however they will be treated separately.

The mechanism of action of prebiotics can be either direct or indirect. An example of direct effect is by increasing the osmotic value in the intestinal lumen, or by direct binding of the pathogens. Substances such as MOS that work via such a direct effect may strictly speaking not be classified as a prebiotic, because there is no involvement of the microflora. However they will be included here because in addition to direct binding they may also function as a substrate for indigenous microflora. Figure 4 explains the mechanism of direct binding of Salmonella by MOS. As previously explained, Salmonella adhere to the mucosal surface of eukaryotic cells with type 1 (F1) fimbriae, which attach to the mannose residues of glycoproteins present on the surface, a prerequisite for the colonization of the host. Prebiotics with indigestible mannose residues may bind the type 1 (F1) fimbriae and therefore block the adhesion of bacteria to the epithelial cells (VAN IMMERSEEL et al., 2002).

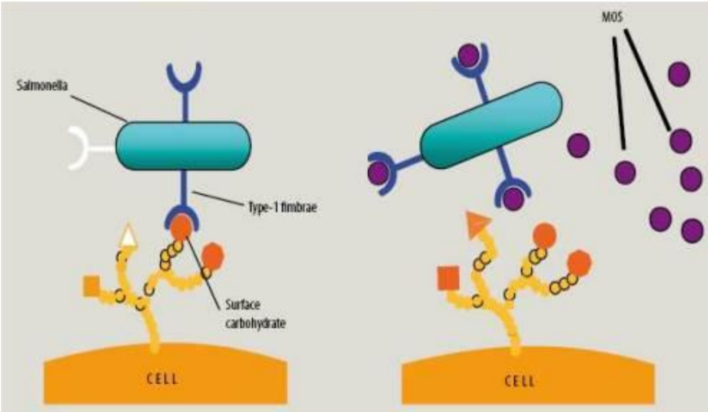

Figure 4. Direct binding of Salmonella by prebiotic - Mannose sensitive agglutination.

In the more traditional sense, prebiotics' mode of action is an indirect effect via influencing the local microflora. The positive influence on the microflora may follow the metabolism of the prebiotic by the intestinal flora, resulting in the production of metabolites such as short-chain fatty acids, lactate, polyamines and bactericins. Prebiotics may represent a substrate for the growth of normal endogenous intestinal flora, thus inhibiting the colonization with pathogenic bacteria by competitive exclusion. This growth promoting effect has mainly been demonstrated in vitro, in vivo demonstrations are lacking.

Another possible mechanism of action of prebiotics is by modifying the metabolic activity of normal intestinal flora (VAN IMMERSEEL et al. 2002).

3.2.1.1 Classes of Prebiotics

Prebiotics are either of natural or synthetic origin, and they can be divided into groups based on their molecular length: mono-, di-, oligo-, and polysaccharides.

The most important monosaccharides are hexoses (glucose, fructose, galactose, mannose) and pentoses (ribose, xylose, arabinose). The most commonly feed additive used from this group is mannose. Galactose is available mostly under the disaccharide form of lactose.

The most important disaccharides are sucrose, lactose and maltose, as well as their isomerization products. Lactose, lactulose and lactosucrose reportedly have protective effects in chicken on Salmonella colonization (VAN IMMERSEEL et al. 2002). However, lactose feeding in broilers changes the consistency of ceacal contents and can lead to mild scouring. Data also suggests that provision of lactose in the drinking water during the last 5 to 11 days of growout prior to slaughter will not be useful in an integrated Salmonella control program under commercial conditions (BARNHART et al. 1999).

Oligosaccharides are usually defined as glycosides that contain some hexose or pentose units. Natural forms can be used, but mostly they are obtained through enzymatic synthesis or hydrolysis (VAN IMMERSEEL et al., 2002). Preferred are the non-digestible oligosaccharides, NDO, which can be metabolized by bacteria but are resistant to intestinal digestive enzymes due to the configuration of their osidic bonds (ASAHARA et al., 2001). Oligosaccharides and related carbohydrates are neither degraded nor hydrolyzed in the upper intestinal tract of animals and, hence, reach the cecum intact (THITARAM et al. 2005). NDO's with prebiotic effect include lactulose, fructo-oligosaccharides, FOS, galacto-oligosaccharides, GOS, soybean oligo-saccharides and xylooligosaccharides. Transgalacosylated oligosaccharides, TOS, are produced by converting lactose using β-galactosidase (ASAHARA et al., 2001). FOS are the oligosaccharides most extensively studied in chickens with respect to their prebiotic effect and their activity against Salmonella. When the animals received competitive exclusion flora in addition to FOS, the reduction of colonization of the intestine by Salmonella was even more pronounced. FOS have been shown to promote growth of normal endogenous intestinal flora in vitro, such as that of Enterococcus faecium, Lactobacillus lactis and Pediococcus species. GOS can be produced industrially, but have so far not been tested in poultry. Mannan-oligosaccharides, MOS, or mannose-based carbohydrates occur naturally in many products such as yeast cell walls. There is a commercial product available for poultry, which contains yeast cell wall fragments derived from Saccharomyces cerevisiae (**Ffigure 4**). Day-old chicks that are fed with MOS as part of the diet show reduced caecal Salmonella colonization. Also hens fed dietary MOS protects chicks from colonization (VAN IMMERSEEL et al., 2002) as explained in figure 4. In mice that were experimentally infected with Salmonella Typhimurium showed increased resistance when provided with dietary NDO's such as oligofructose and inulin. The findings are consistent with enhanced immune functions in response to changes in the composition and metabolic characteristics of bacteria resident in the intestinal tract (BUDDINGTON et al., 2002). A newly developed compound derived by fermentation is isomaltooligosaccharide, IMO. Broiler chickens fed with 1% IMO diet show a significant reduction in the level of experimentally inoculated S. Typhimurium present in the caeca. The effect of IMO is to enrich cecal bifidobacterial populations and thus reducing Salmonella colonization levels. However a 2% or 4% IMO diet made no difference, which suggests that 1% IMO is the optimum level. However the 1% IMO diet resulted in significant reduction in bodyweight of the birds, for which the precise mechanism remains unknown. Undetermined factors such as stress, temperature, animal health, and others may influence the efficacy of IMO on broiler chicken performance (THITARAM et al., 2005).

The most commonly used polysaccharide prebiotic for chickens is guar gum, which is produced from the seeds of the guar bean, Cyamopsis tetragonolobus. By selectively cleaving the mannan backbone-chain of the guar gum, a mixture of galactomannans is obtained, called partially hydrolysed guar gum, PHGG. Feeding 0.0025% PHGG to hens not only reduces the S. Enteritidis colonization but also decreases its presence on eggshell surfaces, egg white and yolk (VAN IMMERSEEL et al., 2002).

3.2.2 Probiotics

The term probiotic is derived from Greek and means "for life". Probiotics are generally defined as live microorganisms that improve animal health or well-being by modifying the intestinal microflora. Adequate amounts can help form the proper bacterial balance and improve gut health, or may even prevent or cure some diseases. Thus, probiotics can promote livestock growth and production (WANG and ZHOU, 2007).

Indigenous gastrointestinal micro-flora provides a good degree of resistance to colonization by exogenous potentially pathogenic micro-organisms.

Any antibiotic treatment disrupts the normal intestinal bacterial flora and lowers the

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resistance to oral infection with Salmonella (ASAHARA et al., 2001).

A very crucial event in the history of development of probiotics was proving the concept of competitive exclusion: Nurmi and Rantal demonstrated in 1973 that newly hatched chickens could be protected against colonization by Salmonella Enteritidis by dosing a suspension of gut contents derived from healthy adult chickens (VAN IMMERSEEL et al., 2002).

Manipulation of the intestinal microflora thus created conditions that are unfavourable for Salmonella colonization. The proper mechanism of competitive exclusion is multifactorial, such as competition for nutrients and receptor sites (MEAD and BARROW, 1990).

The concept of competitive exclusion and the use of complex dietary carbohydrates has opened a new, promising approach to the control of Salmonella in poultry (THITARAM et al., 2005).

In practice treatment may be used in two ways: firstly for newly hatched chicks that are dosed via the first drinking water or by spray-inoculation in the hatchery. This is good prophylaxis because commercially reared poultry are slow to develop an intestinal microflora that would help to prevent them becoming carriers of host non-specific salmonellas, thus treatment would enhance the rate at which such a flora becomes established. The second method is applicable to older birds that are known Salmonella carriers. Since a protective effect in chicks usually begins within one hour of treatment. attention has been given to the ability of protective bacteria to adhere to the caecal wall. This method has been successfully combined with the additions of organic acids to the feed to maximize protection.

Undefined treatment material such as cultured caecal content is normally used to treat birds of the same species as the donor. However, material from chicken can also be used to protect turkeys and vica versa. Protection of duckling with chicken caecal cultures is less successful.

Protection with competitive exclusion treatment has been demonstrated with at least 10 Salmonella serotypes, including both invasive and non-invasive strains, although treatment is only partially effective against the host specific S. Gallinarum which causes systemic infection and mortality in chicks (MEAD and BARROW, 1990)

3.2.2.1 Lactobacilli as a probiotic

Many research efforts have shown that Lactobacilli has growth-inhibiting effect against S. Enteritidis, and are able to reduce the attachment of S. Pullorum and Typhimurium to chicken intestinal epithelial cells (VAN IMMERSEEL et al., 2002). When dosing one-day-old chicks with a Lactobacillus salivarius strain together with S. Enteritidis directly into the proventriculus, the Salmonella bacteria can be completely removed from the birds after 21 days. The great capability of L. salivarius to reduce S. Enteritidis colonization in vivo, together with its ability to colonize the gastrointestinal tract of chicken after a single inclusion in the feed mixture, highlights it as a suitable strain for widespread use in the avian industry in order to minimize Salmonella colonization (PASCUAL et al., 1999).

Little is known about the normal microflora of genital organs in poultry, however the predominant resident organism present in the cloaca and vagina of laying hens are lactobacilli. Over fourty strains have been isolated, of which the most prevalent species were Lactobacillus acidophilus and L. salivarius and few L. fermentum. All three species can be found in cloacal contents, and only L. acidophilus and L. salivarius in vaginal mucus.

Experiments involving in vitro inhibition assays have demonstrated that all lactobacilli species have an inhibitory effect on the growth of Salmonella Enteritidis, with no difference between lactobacilli derived from the cloaca or those from the vagina. The mechanisms by which the lactobacilli inhibit the growth of other bacteria are varied. Suggested are, the production of hydrogen peroxide, production of organic acids such as lactic and acetic acids to decrease pH, and production of specific proteins such as bacteriocins.

The aim is to inhibit ascending infection of genital organs by Salmonella Enteritidis, which is able to colonize and proliferate in the cloaca, and then ascend into the vagina, where colonization can result in an increased production of Salmonella-contaminated eggs.

Using Lactobacilli as a probiotic is therefore a promising control measure against Salmonella. However further research is needed for administration of such probiotic, such as artificial implantation into cloaca or vagina (MIYAMOTO et al., 2000).

Commercial feed mixtures exist which include a strain of Lactobacillus salivarius, a good way to supply it on the farm, however the strain may show sensitivity to storage temperatures (VAN IMMERSEEL et al., 2002;).

Lactobacillus reuteri should receive special attention, because it produces and secretes an intermediary metabolite, reuterin, which has antimicrobial activity against Salmonella and other enteric pathogens. In contrast to other probiotic bacterial cultures, inoculation in ovo does not affect hatchability, and decreases Salmonella colonization after hatching. Additionally it reduces mortality due to in-hatcher exposure to Salmonella.

Lactobacillus plantarum possesses mannose sensitive receptors, a rare phenomenon in gram-positive bacteria, and thus can compete for the same adhesion sites in the intestine as the gram-negatice pathogens (LORENZONI, 2011).

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3.2.2.2 Other Probiotics

Bifidobacteria are mentioned under synbiotics.

Enterococcus faecium can inhibit the growth of Salmonella Pullorum, Gallinarum, Typhimurium and Enteritidis in vitro. The antibacterial action is thought to be the combined effect of lactic acid and bacteriocin.

Non-pathogenic yeast, such as Saccharomyces boulardii, can be used as living oral biotherapeutic agent (**Ffigure 5**). Day-old-chicks show a well reduced intestinal colonization of S Typhimurium when given a dose of 100 g/kg feed (VAN IMMERSEEL et al. 2002).

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Figure 5. Probiotic for poultry containing Saccharomyces cerevisiae.

3.2.3 Synbiotics

A synbiotic is, in its simplest definition, a combination of probiotics and prebiotics. Such a combination should improve the survival of the probiotic organism, since its specific substrate is directly available for fermentation. Examples of synbiotics are bifidobacteria and FOS, or lactobacilli and lactitol. (VAN IMMERSEEL et al., 2002).

In rats, dietary Calcium Phosphate (CaP_i) leads to significantly greater numbers of ileal and fecal lactobacilli, which in turn decreases the severity of both colonization and translocation of Salmonella. Dietary CaP_i has a trophic effect on the intestinal surfactants, such as bile acids and fatty acids. The mechanism by which CaP_i favours growth of the microflora is by reducing the cytotoxicity and the concentration of bile acids and fatty acids of ileal contents and fecal water, as well as changing the composition of ileal bile acids in a less cell-damaging direction. The increased number of lactobacilli then exert their antagonistic actions: competition for nutrients and adhesion sites, production of antimicrobial compounds such as organic acids and hydrogen peroxide.

The growth promoting activity of dietary CaP_i for the endogenous lactobacilli is probably also relevant for the functionality of other probiotic strains used in foods (BOVEE-OUDENHOVEN et al., 1999).

Bifidobacteria are another example for probiotics. Studies have shown that Salmonella colonization of the gut can be decreased when the bifidobacterial population is increased. This can be done by either administering bifidobacteria as a probiotic strain or by addition of certain types of oligosaccharides that stimulate proliferation of these bacteria in the gut (VAN IMMERSEEL et al.;, 2006, VAN DIJK, 2012).

In mice, strains such as Bifidobacterium breve (strain Yakult) and B. pseudocatenulatum showed anti-infectious activity against S. Typhimurium. Explosive intestinal growth and subsequent extra-intestinal translocation of orally infected S. Typhimurium were inhibited by B. breve colonization. This anti-Salmonella activity was strengthened by synbiotic administration of prebiotic transgalactosylated oligosaccharides, TOS. The anti-infectious mechanism is due to both the increase in the concentration of organic acids and the lowered pH in the intestine. Bifidobacterium strains such as B. bifidum and B. catenulatum conferred no activity, even when they reached high population levels. These results indicate that certain bifidobacteria together with prebiotics may be used for prophylaxis against intestinal pathogens (ASAHARA et al. 2001).

Another study in rats confirmed that a stimulation of intestinal lactobacilli and bifidobacteria lead to an inhibition of Salmonella Enteritidis colonization, by studying the faecal excretion of this pathogen. However, simultaneously the translocation of salmonella was observed, by analysis of urinary nitric oxide metabolites over time and by classical organ cultures. The latter showed that feed supplement containing the prebiotics lactulose and fructo-oligosaccharides, FOS, significantly enhanced translocation of Slamonella. Thus, stimulation of endogenous lactobacilli and bifidobacteria is no guarantee of improved host defence against intestinal infection, furthermore, FOS and lactulose impair the resistance of rats to intestinal salmonella infection (BOVEE-OUDENHOVEN et al. 2003).

3.3.Feed additives to reduce Salmonellae: Organic Acids

Fermentation acids have been used by man as a method of food and feed preservation for over 6000 years, largely based on their antimicrobial activity outside the intestinal tract. In the late 1960s the use of acidic compounds to control Salmonella first appeared, and mainly focused on decontamination of carcass meal.

Recently their value as feed or drinking water additives have been reported, such as enhancing digestibility and diet palatability, as well as pathogen control. Few really convincing studies have been made, however overall the use of fermentation acids in pig and poultry appear to improve feed conversion efficiency (FCE) and growth (VAN IMMERSEEL et al. 2006).

3.3.1 Bacterial metabolism of organic acids

In most of the literature organic acids are divided into short-, medium- and long-chain fatty acids, depending on the number of carbon atoms (≤C5, C6 to C12, ≥C12 respectively). Bacteria such as Salmonella or Escherichia coli can use organic acids as both carbon and energy sources. Long- and medium-chain fatty acids are transported across the bacterial cell membrane by carrier mechanisms, involving both outer (**fadL**) and inner (**fadD**) membrane proteins. Short-chain as well as some medium-chain fatty acids diffuse freely across the membrane if only in the undissociated form. Once inside the bacteria, cell degradation occurs through the β-oxidation pathway, yielding multiple acetyl-CoA molecules. Degradation of long-chain fatty acids having an odd number of carbon atoms also yields propionyl-CoA as an end product. Acetyl-CoA is also generated by butyric acid breakdown or by acetate conversion, which is then used by the bacteria in the citric acid cycle (TCA-cycle) for energy production.

Long- and medium-chain fatty acids can also be used for incorporation in the membrane as phospholipids (VAN IMMERSEEL et al., 2006).

3.3.2 Antimicrobial activity of organic acids

Fermentative environments are typically acidic and bacteria capable of utilizing fatty acids are anaerobic. The simplest fermentation is conversion of sugar to lactate by lactobacilli, streptococci, lactococci and enterococci. However when sugars are scarce, all of these bacteria are able to switch to a fermentation that produces acetate, formate, butyrate or ethanol, so ATP production can be enhanced.

Fermentation acids are inhibitory when the pH is low, but some bacteria show more resistance than others.

There are two theories, the traditional “uncoupling model” and the newer “anion model”. The first model compares organic acids with synthetic uncouplers, able to pass across cell membrane and remain associated with it, then dissociate in the more alkaline interior and acidify the cell cytoplasm. This is done by shuttling protons in a cyclic manner wich would dissipate the pH gradient across the cell membrane. However this theory does not take into account that organic acid anions are charged and not lipid permeable and it does not explain the difference in sensitivity of some bacteria.

The anion model of organic acid toxicity explains why bacteria differ in their sensitivity to organic acids. Many fermentative bacteria are able to let their intracellular pH decline when the extracellular pH becomes highly acidic. Thus the bacterium has a much smaller pH gradient across the cell membrane and is protected from anion accumulation. If the pH gradient should remain high, then it causes a logarithmic accumulation of the fermentation acid anions (JONES, 2012). The organic acid toxicity can be represented by MIC, minimal inhibitory concentration. For example the MIC of acetic acid is 250 times lower for Bacillus subtilis than for lactobacilli. In the case of *E. coli* the MIC values for acetic, butyric, lactic and caprylic acid are less than 4g/l, but the same bacterium is approximately 10 times more resistant to malic acid, tartaric acid and citric acid (HSIAO and SIEBERT, 1999).

The anion model however does not provide information on the antibacterial effect of one acid versus another, for example acetate versus lactate: Experiments with the K-12 strain of E.coli showed that when intracellular acetate concentration increases, there is a nearly equal molar increase in intracellular potassium. Thus osmotic stress is the toxic effect. In a different experiment using Clostridium sporogenes, intracellular lactate ion accumulation caused a secondary effect of intracellular glutamate loss.

Thus, in addition to the two models, the factors which affect the antimicrobial activity of organic acids are such as chain length, side chain composition, pKa values (acid dissociation values) and hydrophobicity (VAN IMMERSEEL et al. 2006).

3.3.3 Short-chain fatty acids

The short-chain defines the length of the aliphatic tails of this subgroup of fatty acids, which is less than 6 Carbon atoms (<C6), such as formic acid, acetic acid, propionic acid and butyric acid. Currently, short-chain FA are far more commonly used than medium-chain FA in the poultry industry to combat Salmonella (VAN IMMERSEEL et al. 2004a).

To assess the effect of these acids on the virulence of Salmonella, several experiments used various concentrations of each fatty acid to supplement growth media of epithelial or ceacal cells. Then S. Typhimurium or S. Enteritidis was preincubated in forementioned medias, and any change in Salmonella's invasiveness into the epithelial cells was observed and compared.

Khan & Katamay evaluated in 1969 the efficacy of 32 different acid preparations to decontaminate bone meal, and concluded that low-molecular-weight volatile fatty acids were the most promising. Their results were prophetic and these acids are nowadays added to feed, drinking water, and other matrices. Poultry feed is considered the major source for Salmonella introduction to the farm, thus the original concept of incorporating acids into feed was thought to decontaminate the feed itself and prevent Salmonella uptake by the chickens. This is true in case of adding formic and proprionic acid, which has been proven in several studies such as those conducted in 1988 by Humphrey & Lanning, Hinton & Linton, and in 1995 by Iba & Berchieri.

The antibacterial activity of organic acids is dependent on temperature and moisture, and since the water content of poultry feed is generally low, the action of acids is not always optimal. Hence the in-feed effects are not necessarily the major reason for protection.

When acid treated feed is eaten, it is both warmed and moistened and the activity of the short-chain FA should increase. In 1997 Thompson & Hinton fed laying hens with supplemented formic and propionic acids and assessed pH changes in the digestive tract. Results show that the pH values of the crop, gizzard, jejunum, caecum and colon were not altered relative to control animals, however formic and propionic acid concentration in the crop and gizzard were significantly increased. Concomitantly, lactic acid concentration in the crop decreased significantly, suggesting that lactobacilli were either inhibited or killed.

In the 1980s and 1990s many studies examined the effects of supplemental acids on

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Salmonella colonization of chicken tissues. The results were largely dependant on the infection protocol of each study. Low dosage and a short time between infection and sampling usually showed ineffective results. The most striking proof of the efficacy of formic and propionic acids as feed additive were given in three independent studies, which added the acid-supplement from the day of hatch, as compared to acid-treated feed given at a later age (16 or 32 days). If fed acid supplement from day one, Salmonella infection can be dramatically decreased, from as high as 30-60% down to 3-0%. However preventing initial colonization of Salmonella is most important. Once an infection is established, it is very difficult to counteract using acid-treated feed.

Studies with both layers and broilers have confirmed that mixtures of 0.5-1% formic acid and propionic acid are effective in reducing Salmonella colonization, including Salmonella Kedougou, S. Pullorum and S. Gallinarum (VAN IMMERSEEL et al. 2006).

Butyric acid exposure has shown to directly decrease invasion of intestinal epithelial cells by both Salmonella serovars S. Enteritidis and S. Typhimurium. This effect occurs on a genetic level, whereby butyrate specifically down-regulates up to 17 genes localized on the Salmonella pathogenicity island 1, SPI-1. These included the SPI-1 regulatory genes **hilD** and **invF** (GANTOIS et al., 2006).

Small-chain FA have also been used as drinking water sanitizers. Lactic acid, formic acid, and even to some extent acetic acid added to drinking water (DZANIS, 2013), can decrease crop contamination and the incidence of Salmonella in pre-chill carcass rinses (BYRD et al. 2001). However drinking water acidification is not significantly effective when chickens are moulted or highly stressed (HOLT, 2003).

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3.3.3.1 Microencapsulation of short-chain fatty acids

The caecum is the main fermentation site, therefore the concentrations of small-chain FA are already higher here than in other intestinal segments. In an adult chicken acetic acid is the predominant short-chain FA in the caeca, with concentrations ranging between 70 and 90 µmol/g caecal content, butyric acid concentration ranges between 10 and 40 µmol/g, and the propionic acid concentration is even less. If small-chain fatty acid production in the caeca could be altered by changes in feed composition, then in theory Salmonella colonization of the caeca could thus be influenced (VAN IMMERSEEL et al. 2006).

Recent experiments have focused on attempting to transport the organic acids further down in the gastrointestinal tract by micro-encapsulation, which should prevent absorption of the acids in the upper tract, and ensure a slow release further down in the gastrointestinal tract. Encapsulation is done in mineral carriers as film-coated microbeads for acetic, formic and propionic acid, and as spray-cooled microcapsules for butyric acid. The acid supplements ranged from 0.15-0.27%. Five groups of 20 chicken were given feed containing one of the abovementioned acid supplements or no supplement at all for the control group. After artificial inoculation with S. Enteritidis day 5 post-hatch, each group was examined on the degree of colonization in caeca, liver and spleen 3 days post-infection. The best result was achieved with butyric acid impregnated microbeads in feed, which showed a significant decrease of colonization by S. Enteritidis in the caeca, but not in the liver and spleen. Colonization of internal organs was the same as in the control group for propionic acid coated microbeads, and even increased in case of formic acid microbeads. The highest degree of Salmonella colonization of internal organs occurred with acetic acid microbeads. Thus the type of acid supplement may even enhance the virulence of Salmonella (VAN IMMERSEEL et al. 2004).

Another study focused on butyric acid as a feed additive, and compared the ability of powder form and coated form to reduce Salmonalla colonization of ceca and internal organs, using the same infection protocol. Caecal colonization at slaughter age was equal for both groups, however the group of broilers receiving coated butyric acid had a significantly lower number of broilers shedding Salmonella bacteria. This study concluded that butyric acid decreases ceacal colonization shortly after infection, decreases fecal shedding and consequential environmental contamination. However, a complete elimination of Salmonella-infected broilers can only be achieved with a combined approach using both hygienic measures and different protection measures (VAN IMMERSEEL et al. 2005).

3.3.4 Medium-chain fatty acids

Medium-chain are C6 to C12, such as caproic acid, caprylic acid, capric and lauric acid. Data indicates that these have the greatest antibacterial activity against Salmonella, but large-scale studies are lacking (VAN IMMERSEEL, 2006).

In vitro, free medium chain triglycerides have been shown to be more bactericidal to numerous gram-negative and gram-positive bacteria than the short-chain FA (NAKAI and SIEBERT, 2003).

All medium-chain FA have growth inhibiting effects on S. Enteritidis in vitro, with caproic acid being the most potent. In chicks fed additions of 3 g/kg feed there was a significant decrease in the level of colonization of ceca and internal organs by S. Enteritidis. The mechanism of action is on the genetic level, where all medium-chain FA have the ability to decrease the expression of **hilA**. This gene is a regulator of the Salmonella pathogenicity island I and is directly involved in the invasion of intestinal epithelial cells. In addition the expression of the SipC gene is also impaired, a protein that promotes internalization of the pathogen when injected into the eukaryotic cell. In comparison, the short-chain FA propionic and butyric acid decrease Salmonella invasion t2- to ten-10fold respectively. Medium-chain FA seem to decrease invasion at least to the same extent as butyric acid but at lower concentrations, therefore their antibacterial activity appears higher (VAN IMMERSEEL et al., 2004a). This appears to be the firswot report demonstrating possible use of medium-chain FA in controlling Salmonella in poultry.

3.4 Phytogenic feed additives

Phytogenic feed additives, also called phytobiotics or botanicals, are substances derived from plants and comprise a wide range of substances. They are classified according to botanical origin, processing and composition. Additives include herbs, which are non-woody flowering plants known to have medicinal properties; spices, which are herbs with intensive smell or taste improving palatability and therefore increase feed intake; essential oils, which are aromatic oily liquids derived from plant materials such as flowers, leaves, fruits and roots; and oleoresins, which are extracts derived by non-aqueous solvents from plant material.

The mode of action of phytogenic feed additives covers a wide range and some of it is still incompletely understood. Gut function may be improved by direct stimulation of the digestive enzymes or pharmacologic actions such as relaxant and spasmolytic effects. Aside from antimicrobial activity, they potentially provide antioxidative effects that have been attributed to the phenolic terpenes in the essential oils. Most beneficial effects claimed from using phytogenic feed additives are based on experience from the field of human medicine (JACELA et al. 2010).

Traditional Chinese medicine uses natural medicinal products originating from fungi and herbs and have been used as feed additives for farm animals in china for centuries. They have many medicinal properties such as antimicrobial activity, immune enhancement and stress reduction and are rumored to prevent and cure many animal diseases (WANG and ZHOU, 2007).

In one trial the growth performance of 720 broilers was examined, comparing dietary Chinese herbal medicine, CHM, as an alternative to the antibiotic virginiamycin. The CHM dietary treatments produced increased body weight gain at 7 to 21days of age, but not at 21 to 28 days of age. However, the CHM groups had a higher feed intake and a higher feed conversion ratio between 21 and 28 days. Further studies are needed to elucidate the underlying mechanisms (GUO et al., 2004).

3.5 Bacteriocins, Antimicrobial Peptides and Bacteriophages

Bacteriocins, antimicrobial peptides and bacteriophages have recently attracted attention as potential substitutes for antimicrobial compounds. Regulatory issues and the high cost of producing such alternative agents are factors which might prevent application of these agents in the near future (JOERGER, 2003)

3.5.1 Bacteriocins

Bacteriocins are proteinaceous compounds of bacterial origin that are lethal to bacteria other than the producing strain. It is assumed that many of the bacteria in the intestinal tract produce bacteriocins as a means to competitive advantage, for example Fusobacterium mortiferum isolated from chicken ceca.

Regulatory approval for use in certain foods has currently only been given to nisin, which is produced by certain strains of Lactococcus lactis subsp. lactis. The bacteriocin nisin actually has GRAS (generally recognized as safe) status (21 CFR 184.1538). Nisin's use for poultry products has been studied extensively. Although gram-negative bacteria such as Salmonella are considerably less sensitive to nisin than are many of the gram-positive bacteria, additions of chelating agents such as EDTA and detergents such as Tween 80 have been used to enhance the activity of nisin against gram-negative bacteria (JOERGER, 2003).

Lactobacillus reuteri produces reuterin, a metabolic product that is secreted during anaerobic metabolism of glycerol. Reuterin has broad-spectrum antibiotic activity, decreasing both Salmonella and E. coli intestinal colonization in chicks and poults when given in ovo (FULTON et al. 2002).

Other purified or partially purified bacteriocins could be used for the reduction or elimination of certain pathogens including Salmonella. For example avain Escherichia coli strain genetically engineered to produce the bacteriocin microcin-24 has shown to lower intestinal Salmonella Typhimurium counts in chickens, when administered continuously in the water supply. Similarly, the bacteriocin-producing Enterococcus faecium strain J96 exhibits some protective effect on chicks infected with S. Pullorum.

The issue of resistance has to be considered. Although the mechanism of action is not known for all bacteriocins, most of the low molecular weight bacteriocins appear to interact with the bacterial membrane. Resistance is therefore usually the result of changes in the membrane of bacteria.

A more cost-effective approach might be the administration of bacteriocin-producing bacteria rather than the bacteriocins themselves. However, before such an approach will be feasible significant progress in developing suitable producer strains will have to be made.

Investments in research and development can be expected to be high (JOERGER, 2003).

3.5.2 Antimicrobial Peptides

In general, antimicrobial peptides are small molecules with a molecular mass of 1 to 5kDa. The production of small antimicrobial peptides is not confined to bacteria, but appears to occur in all organisms studied so far. Their structure usually contains elements to facilitate the interaction with negatively charged membranes, resembling a similar mode of action as small bacteriocins. In this respect, the development of resistance to the eukaryotic peptides might therefore also require changes to the membrane.

The application of antimicrobial peptides from sources other than bacteria to poultry has not yet been explored to a significant extent. There has been some evidence that chickens have the ability to provide such antimicrobial peptides, and research has already uncovered some of the gene sequences that potentially code for them. Three peptides have been purified from chicken leukocytes and also from turkey heterophil granules. In a subsequent study their antimicrobial activity was demonstrated against S. Typhimurium and S. Enteritidis as well as many other pathogens.

As with bacteriocins, the proteinaceous nature of antimicrobial peptides makes them vulnerable to proteolytic enzymes. This is of little concern for peptides produced by the immune system or epithelium where bacterial targets are in close range, in contrast to interventions involvine injection or ingestion of such peptides. Here their administration might have to include encapsulation methods or chemical modification, which would add to the costs of antimicrobial peptide treatment.

For large-scale production chemical synthesis appears too costly currently, therefore biological production with microorganisms, tissue cultures or in transgenic animals will have to be attempted. Peptide-containing transgenic plant material could be added to animal feed.

Extensive research will be required to identify peptides that influence intestinal microbiotica in the same way as currently known for antibiotics (JOERGER, 2003).

3.5.3 Bacteriophages

Bacteriophages, or phages, are viruses which infect and multiply in bacteria. Viral replication usually causes lysis of the host bacterium. They were discovered separately by F.W. TWORT andAND F. D'HÉRELLE in the early 1900s. Early historical failures for their in vivo use was attributed to not understanding the specificity of phage-host interaction at that time (PELCZAR et al. 1993). The initial euphoria about phage as therapeutic agents dissipated with the onset of antibiotic era. Recently, bacteriophages have received renewed attention. Evidence from several trials indicates that phage therapy can be very effective under certain circumstances. Bacteriophages are generally very stable entities and survive storage relatively well.

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There is no known phage that is lytic for all Salmonella serovars. A particular Salmonella phage will only lyse a small part of the pecturm of Salmonella serovars and even will not be lytic for all members of one particular serovar. This degree of host specificity necessitates the use of phage mixtures for prophylaxis of bacterial infections (JOERGER, 2003).

In an effort to exploit target specificity, a trial was conducted using so-called tailspike proteins of the bacteriophage Podoviridae P22, which recognizes the lipopolysaccharides of Salmonella Typhimurium. A formulated form allows protection against proteases. When administered orally to chickens, P22 phage tailspike protein significantly reduces Salmonella colonization in the gut and its further penetration into internal organs (WASEH et al. 2010).

The chief obstacles for phage application are the narrow host range, phage resistance, and phage-mediated transfer of genetic material to bacterial hosts. The same technical and financial challenges are present as faced by most other large-scale operations involving microorganisms. One significant difference of phage production could be safety concerns regarding the bacterial host, since it is mostly pathogenic, relatively costly safety measures to protect plant workers and the surrounding community would have to be implemented.

Arguably, bacteriophages are the most promising agents that could complement or replace antibiotics, but their use on the farm or for food safety applications is uncertain. (JOERGER, 2003).

3.6 Vaccination

Live vaccines confer better protection than killed vaccines, because the former stimulate both cellular and humoral responses, while the latter stimulate antibody production only (QUINN et al., 1994). Currently, two types of Salmonella vaccines are commercially available: the bacterins and the live vaccines (GANTOIS et al., 2006a).

It is essential that live, attenuated vaccine strains cannot revert to virulence. Modern genetic techniques are used to construct stable, genetically defined, attenuated bacterial strains suitable for widespread use. Several genes have been identified that when mutated result in attenuation. Salmonellae can be attenuated by auxotrophic mutations such as **galE**, **aroA**,or **purA**. The **aroA** mutants are dependent on aromatic compounds for growth in vitro, and the limited availability of one or more of these compounds in vivo is responsible for attenuation. Salmonella **aroA** mutants have now been well characterized and have been shown to be excellent live vaccines against salmonellosis in several animal species (DOUGAN et al., 1988)

Protection during the first days of their life is especially important, because of the lack of normal microflora in the intestine. This fact in turn allows easy colonization by live attenuated Salmonella strains.

Inoculation of newly hatched chicks with Salmonella Enteritidis **aroA** mutant induces a rapid onset of resistance to intestinal colonization by other Salmonella strains. Vaccinated animals have a much lower number of challenge bacteria in their organs and caecal contents the first days post-challenge. The mechanism of this early colonization-inhibition was previously unclear, because most parts of the newly hatched chick's immune system do not mature until about one week post-hatch, such as B- and T-cell responsiveness and phagocytic activity of macrophages. Analysis of the kinetics of immune cell infiltration in the caecal wall has shown that heterophils play a much more important role than the previously mentioned immune cells. Data implys that the rapid onset of colonization-inhibition is because immune cells had already colonized the caecal wall at the time of challenge (VAN IMMERSEEL et al., 2002a).

3.6.1 Immunisation with Type 1 fimbriae

Vaccination of laying hens might be the most effective way to reduce egg contamination by S. Enteritidis. The basis for the development of new vaccines should be understanding the S. Enteritidis-specific factors involved in the egg contamination process as well as the host immune responses. Type 1 fimbriae have been shown to play a role in the intestinal stage of infection, in colonizing the reproductive organs, and even in binding to the secretions of the oviduct constituting the forming egg.

Subcutaneous or intraocular immunization with type 1 fimbriae elicits an IgG, IgM and IgA response. Maternal Igs can be transferred to the egg white mainly through glandular cells in the magnum of the chicken oviduct. The vaccine has no significant effect on the numbers of eggs laid, and demonstrated a reduction in the colonization of the reproductive organs and a reduction in the number of contaminated eggs. However immunization did not have any influence on colonization of liver and spleen.

Protection against an oral challenge of S. Enteritidis is much higher than against an intravenous challenge. Anti type-1-fimbriae antibodies act only on fimbriated bacteria, thus non-fimbriated Salmonella bacteria have an additional advantage especially during the acute phase of infection, because non-fimbriated Salmonella can stay blood borne much longer than the fimbriated ones and can disseminate better to the internal organs. Hence eggs from vaccinated hens cannot be guaranteed to be Salmonella free.

Type 1 fimbriae could therefore be supplemented to existing vaccines or constitute a component of a subunit vaccine (DE BRUCK et al., 2005).

3.6.2 Live vaccine strains of TAD Salmonella vac®

EU regulations require compulsory vaccination against S. Typhimurium and S. Enteritidis for poultry meat producers. In 2003 Britain launched its first live, oral vaccine against Salmonella Typhimurium in poultry by Lohmann Animal Health. Following 10years of research TAD Salmonella vac T is designed for the broiler-breeder sector where vaccination is a requirement. Three doses added to the birds's drinking water make it ideal for flock vaccination via drinking water, which saves labour costs and the stressful task of individual injection of thousands of birds (VETBUZZ, 2003).

Figure 6. Live oral Salmonella Typhimurium Vaccine for poultry

In 2006 a separate study was carried out to assess the effect of oral vaccination with both TAD Salmonella vac E, and TAD Salmonella vac T; against S. Enteritidis and S. Typhimurium respectively (see Figure 6.) Active immunization with these live vaccines protects the birds from mortality and significantly reduces faecal excretion by Salmonella Enteritidis and Salmonella Typhimurium. Immunisation with both vaccine strains shows a remarkable reduction in reproductive tract colonization and internal egg contamination. These live vaccines should be considered as a valuable tool to combat Salmonella in poultry. These results underline the high value of routine vaccination practices (GANTOIS et al., 2006a).

Vaccination of poultry is becoming one of the most important control measures, because of the cost and impracticability of improvements in hygiene and the increasing antibiotic resistance of bacteria (ZHANG-BARBER et al., 1999).

4. Turkey feed additives to reduce Salmonella infection

Turkey flocks can be colonized by many Salmonella species. Different phage types have different degrees of colonization and duration of shedding (HAFEZ and STADLER, 1997). On the American continent S. Arizonae is the most important serovar (KAHN et al., 2005). In Poland a recent epidemic spread of new multi-drug resistant strains related to Salmonella Kentucky was observed in non-diseased turkeys and is posing a serious public health risk (WASYL–HOSZOWSKY, 2011). Other common serovars are S. Heidelberg, S. Muenster and S. Worthington, three examples out of 29 Salmonella serotypes isolated from turkey caeca and the production environment (NAYAK et al., 2004). In August S. Heidelberg in ground turkey was claimed to be responsible for one death and 76 illnesses in people across 26 states (HENDRICK, 2011).

4.1 Turkey prebiotics

Addition of lactose to the feed starting with one-day-old poults can decrease Salmonella Senftenberg cecal colonization. If challenged at 3 days of age, dietary provision of 5% lactose significantly decreases S. Senftenberg growth in the caecal contents at 10 and 30 days of age. (CORRIER et al. 1991). Treatment with 2.5% lactose has no effect on Salmonella Typhimurium colonization in the crop, after challenge occurred at 3 weeks of age (JOHANNSEN et al. 2004). This negative result is mainly because samples were taken at time points less than 24hours postchallenge, therefore this trial only proves that dietary lactose has no effect on short-term crop colonization, however this does not confirm or deny a change in colonization of the internal organs.

4.2 Turkey probiotics

A series of experiments was conducted to evaluate the ability of Lactobacillus based commercial probiotic culture (FloraMax, IVS-Wynco LLC, Springdale) to reduce Salmonella enterica serovar Heidelberg. Poults have been found to be more susceptible to S. Heidelberg colonization, however probiotic treatments 1hour postchallenge resulted in significant reductions in the concentrations of Salmonella within the ceaca. For evaluation, Caecal tonsils and caeca were collected 24 and 72 hours posttreatment (MENCONI et al. 2011).

The efficacy of a 9-bacteria probiotic culture, consisting of 7 Enterobacteriaceae and 2 lactic acid bacteria and two lactic acid bacteria isolates alone were evalutated. Both had the ability to significantly reduce recoverable environmental Salmonella in commercial turkey flocks 2 weeks prior to processing; the 9-bacteria culture being the slightly more effective one (VINCENTE et al., 2004).

In 2007 VINCENTE et al. repeated the experiment with the following probiotic cultures: P1 consisted of 5 strains of Escherichia coli, Kluyvera ascorbata, Klebsiella travesanii, Lactobacillus casei and Lactobacillus cellobiosus. The second culture, P2, consisted of L. casei and L. cellobiosus. Two weeks after treatment, a significant reduction of environmental salmonella was observed within each treatment. One of the six turkey houses receiving a high dose of P1 culture alone experienced a 90% reduction in Salmonella-positive swabs (VINCENTE et al. 2007).

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4.3 Turkey synbiotics

A combination of intracloacal inoculation with anaerobic cultures of caecal microflora (probiotic) and addition of lactose (prebiotic) to the feed results in a protection against colonization by Salmonella Sentenberg equal to or higher than either of the two treatments administered separately (CORRIER et al., 1991). Addition of Lactobacillus acidophilus and lactose to the drinking water has no effect on crop colonization by Salmonella Typhimurium (JOHANNSEN et al., 2004).

4.4 Turkey organic acids

Vincente et al. combined each of the two probiotic cultures from the 2004 experiment mentioned above with commercial organic acid called performax. Reduction in Salmonella recovery was even higher by using these combinations, compared to using the probiotic cultures alone; even reaching 100% reduction in the case of the 9-bacteria probiotic culture combined with organic acid. This study suggests a synergistic effect of selected probiotics in combination with organic acids (VINCENTE et al., 2004).

In 2007 a series of trials was performed using aforementioned cultures P1 and P2 (see figure 7) alone or in combination with organic acids. Trials included administration of the cultures prior to organic acid treatment and vica versa, each confirming a significant reduction in Salmonella-positive swab samples. This data confirms an apparent additive effect of organic acid and prebiotics when administered via drinking water to turkeys preslaughter (VINCENTE et al. 2007).

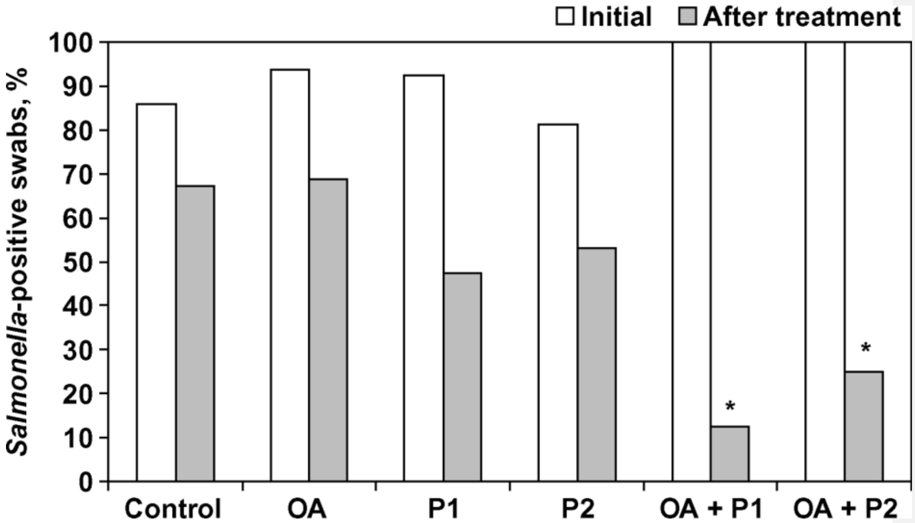

Figure 7. Administration of probiotic cultures (P1 and P2) or organic acid (OA) in the drinking water alone or in combination. *= significant differences (P>0.05) within a treatment within sampling times.

5. Duck feed additives to reduce Salmonella infection

Duck meat is a traditional Chinese delicacy and is becoming increasingly more popular. Very little is known about the effect of feeding additives to meat ducks other than antibiotic growth promoters. The investigative focus so far has been on body weight, feed conversion ratio, FCR, and carcass characteristics (WANG and ZHOU, 2007).

Ducklings can be infected with Salmonella Enteritidis directly or by contact with infected birds. Under production conditions Salmonella is shed from infected ducklings and can be transmitted horizontally to flock mates. Shedding most likely takes place prior to seven days postinfection (FULTON et al., 2002).

Wang and Zhou conducted a study to compare the effect of three types of feed additives on the performance of pekin meat ducks. The supplements consisted of Chinese herbs, probiotics and prebiotics. At the end of the second week, the ducks on probiotics had the highest body weight, however overall the FCER was lowest compared to the other two groups. At the end of week 7, the Chinese herb group had the highest body weight, but the difference did not reach statistical significance. The FCER was highest in the Chinese herb and prebiotic group. Mortality was lowest in the Chinese herbs group, equally low when fed antibiotics (0%). In all groups the carcass characteristics were similar, therefore it can be concluded that these supplementations have no negative affect on meat duck performance (WANG and ZHOU, 2007).

5.1 Duck prebiotics

Mannose-oligosaccharides, MOS, were used in this study and showed neither positive nor negative effect on meat duck performance. Possibly other factors influenced the ducks' response to MOS or inefficient quantities were fed, however duck health or productivity were not compromised. Optimum quantity of these additives and their effects on meat ducks merit further investigation (WANG and ZHOU, 2007)

5.2 Duck probiotics

Mixed cultures of Lactobacillus acidophilus and Bacillus subtilis are superior in terms of weight gain and FCR to antibiotics and can be proposed as a replacer for antibiotics. Bacillus coagulans has also proven beneficial effects on the mean body weight (WANG and ZHOU, 2007).

Lactobacillus reuteri decreases Salmonella intestinal colonization by competitive exclusion and by secreting the bacteriocin reuterin (FULTON et al., 2002).

5.3 Oral antibodies

Studies have shown that in ducklings probiotics can act synergistically with oral antibodies. Previously it has been proven that chicken egg-derived antibodies, when given orally, are effective in the prevention of clinical disease and infection with Salmonella in piglets and calves. In an experiment with 200 Pekin ducklings, when given chicken-egg-derived anti-S. Enteritidis antibody orally, prevents Salmonella infection if the antibody is given at least five days before infection (FULTON et al., 2002).

5.4 Duck phytogenic feed additives

Chinese herbs improve metabolism and clearly promote growth performance and reproduction. Most importantly this practice is economically feasible because these herbs are very inexpensive and their sources are abundant. Chinese herbs can be considered a substitute for antibiotics (WANG and ZHOU, 2007).

6. Swine feed additives to combat Salmonella

6.1 Generally about Swine Salmonellosis

While many other Salmonella species may cause disease, the more common ones in pigs are S. serovar Typhimurium and S. serovar Choleraesuis. Outbreaks of septicaemic salmonellosis in pigs are rare and usually can be traced to a purchased, infected pig (KAHN, 2005) In the past few years an increase in the number of clinical cases of salmonellosis associated with S. Typhimurium was noted in animals and humans following the consumption of animal products. Since the disease may occur at the end of the fattening period, from a public health point of view it is critical to better understand the survival of the bacteria in faeces and organs following infection (COTÉ et al., 2004).

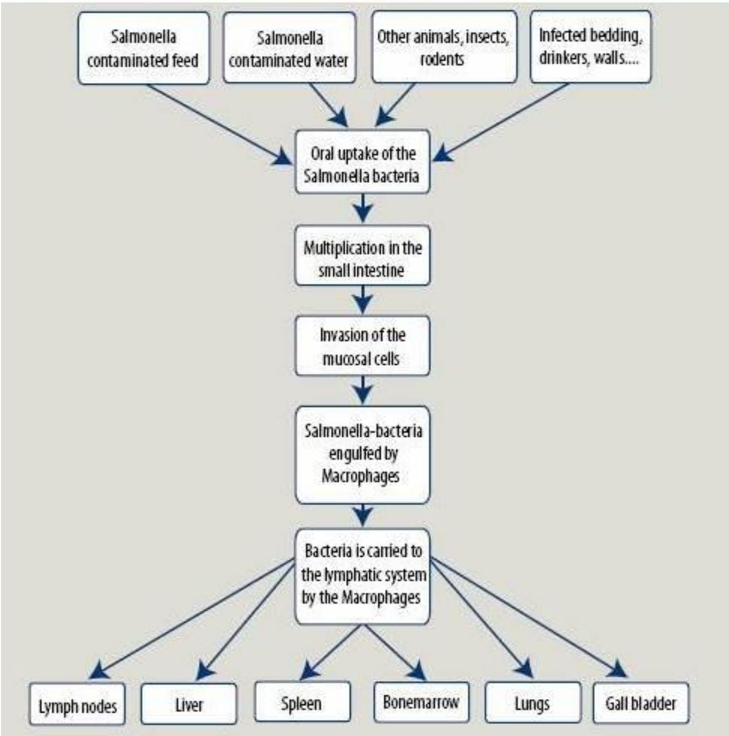

Figure 8. Salmonella infection in pigs, source and route of infection.

While most animals colonized by this bacterium will remain healthy carriers, clinical signs associated with salmonellosis in pigs are yellowish diarrhea with fever, prostration, and/or mortality (COTE et al., 2004). Figure 8 summarizes the source and route of infection. The pathogenesis can be characterized by three phases: first, colonization of intestines; second, invasion of enterocytes; and third, bacterial dissemination to lymph nodes and organs. Several organs, including the tonsils, serve as important sites for persistence and dissemination of Salmonella (SZABÓ et al., 2008) Infectious diarrhea of neonatal animals is one of the most common and economically devastating conditions encountered in the animal agriculture industry (GUSILS et al., 2002). To minimize exposure the purchase of feeder pigs should be from salmonellae-free herds and the use of the "all-in/all-out" policy should be implemented in finishing units (KAHN, 2005). Studies show there is a strong correlation between the number of live animals that carry Salmonella species in their faeces and the number of contaminated carcasses in the end of the slaughter line. Transport to the slaughterhouse and especially holding in pens at the slaughterhouse provide many opportunities for cross contamination and thus increase the prevalence of Salmonella-positive pigs (MIKKELSEN et al., 2004). With the rise in antibiotic resistance and the subsequent removal of antibiotics from pig feed there is a need to identify alternatives which can reduce incidence of Salmonella in pig herds. In the case of the pig, the problem is exacerbated by the lack of epithelial cell lines, their inadequacy in situations where feed constituents can induce a plethora of effects on the gastrointestinal tract, and the difficulties in maintaining mucosal integrity in organ culture models (NAUGHTON et al. 2001)

6.2. Effects of physical properties of feed

There is a strong evidence that the physical characteristics of the feed influence the susceptibility of pigs to Salmonella. A study was conducted to study the effect of grinding (fine and coarse) and feed processing (pelleted and non-pelleted) on microbial populations and survival of S. Typhimurium in the gastrointestinal tract of pigs. It was concluded that there is a strong effect of diet on stomach parameters, whereas the effect was less in other parts of the gastrointestinal tract. The best results were demonstrated in pigs fed coarse non-pelleted diet as it decreases the survival of Salmonella during passage through the stomach. Coarsely ground meal increases stomach concentrations of organic acids and undissociated lactic acid, which is in strong correlation to the death rate of S. Typhimurium. Pigs fed coarse diets also showed increased concentration of butyric acid in the caecum and colon compared with pigs fed the fine diets.

However, feeding a coarse and/or nonpelleted feed to pigs results in poor growth performance and consequently is an economic disadvantage for the pig producer (MIKKELSEN et al. 2004).

<u>6.3 Swine prebiotics</u>

It has been proposed that NDOs can be utilized preferentially by lactobacilli and bifidobacterial species, which leads to the production of lactic acid and an increase in short-chain FA production. Commercially available FOS and GOS were used to study their effects in the jejunum and ileum intestinal segments. It is generally accepted that the ileum is the main site of invasion in pigs. In this study, GOS had no effect on the association of S. Typhimurium in the culture model. FOS feed inclusion slightly reduced Salmonella numbers in the organ cultures, but the finding was not significant. Microbial fermentation of NDO is limited in the small intestine, therefore this result is more likely due to a direct action of FOS on the gut, such as direct inhibition of bacterial binding sites (NAUGHTON et al., 2001).

Oligofructose and inulin are other examples that have been used as substrates for the pig. Unfortunately, research trials that show consistent beneficial effects in pigs are limited (JACELA et al. 2010).

<u>6.4 Swine probiotics</u>

The stressful physiological and environmental conditions experienced in particular by young pigs promote the proliferation of pathogens in the digestive tract. Probiotics such as lactic acid-producing bacteria, Bacillus species and yeast have been resported to improve microbial balance in the gastrointestinal tract through bacterial antagonisms, competitive exclusion and immune stimulation (ZIMMERMANN et al., 2001).

In the specific case of swine livestock probiotic foods should be administered immediately before the weaning and at the beginning of the breeding stage.

Out of a hundred strains of lactic acid bacteria that were isolated from the gastrointestinal tract of pigs only six strains were determined to have potential probiotic use, showing inhibition against S. Enteritidis, S. Choleraesuis and S. Typhimurium. These six bacteria were identified as four strains of Enterococcus faecium (7c, 10c, 14c, 19c) and two strains of Lacobacillus acidophilus (4c, 13c). These selected strains fulfill the conditions of probiotic bacteria, such as resistance to pH 3.0 and bile salts, and therefore they could be selected for elaborating pig probiotic feed (GUSILS et al., 2002).

A study conducted in 2008 tested the effect of a probiotic strain of Enterococcus faecium given via feed supplement to 89 weaning piglets on day 14 postpartum. After challenge with Salmonella Typhimurium both the probiotic and the control group showed equally mild clinical signs of salmonellosis. The piglets fed E. faecium demonstrated significantly greater fecal excretion and colonization of Salmonella in organs, thus the probiotic-treated animals were less able to defend themselves against a Salmonella infection. However, the elevated intestinal bacterial load, and the increased levels of bacteria in tissues and lymphoid organs also resulted in earlier and greater humoral immune response, suggesting the treatment leads to an enhanced course of infection.

In contrast to these results, in vitro studies have demonstrated that this E. faecium probiotic strain decreases the rate of invasion of a porcine intestinal epithelial cell line by S. Typhimurium (SZABÓ et al.~~,~~ 2009).

A five-strain probiotic combination fed to weaned pigs via milk for 30 days significantly improved both the clinical and microbiological outcome of a Salmonella Typhimurim infection, orally challenged one week after treatment begin. The probiotic mixture consisted of two strains of Lacctobaciullus murinus and one strain each of Lactobacillus salivarius subsp. salivarius, Lactobacillus pentosus and Pediococcus pentosaceous. Probiotic treated animals showed reduced incidence, severity, and duration of diarrhea, including significantly reduced mean fecal numbers of Salmonella. In addition, these animals also gained weight at a greater rate than the control pigs. This study demonstrates the validity of using commensal lactic acid bacterial strains in the prevention of gastrointestinal infection (CASEY et al.~~,~~ 2007).

The variation in responses suggests several possibilities. The fact that some studies improve pig performance whereas others do not, indicates the influence of environment and production practices which may differ from one setting to another. Also the number of viable organisms in each dose of probiotic can possibly be insuffient for intestinal establishment. Another factor might be that the microorganisms included in the probiotic product were not isolated from pigs but from other animal species (JACELA et al.~~,~~ 2010).

6.5. Swine organic acids

Medium chain triglycerides have been shown to be good alternatives for nutritional antibiotics in piglets, due to the high antibacterial activity of the medium chain FA (VAN IMMERSEEL et al.~~,~~ 2004a)

Combined feed additives containing 5% triacylglycerol, coconut and butter oil medium-chain FA, and two lipolytic enzymes to piglet diet resulted in a strong in vitro and in vivo suppressive and stabilizing effect on the pig proximal gut flora. Previous experiments confirmed the enzymatic release of medium-chain FA from specific triacylglycerols and their antibacterial effects. A minimum concentration of 0.35 g FA per 100 g in the medium was necessary to obtain a significant suppression of luminal flora (DIERICK et al., 2002).

6.6 Swine phytogenic feed additives

Regarding feed intake studies have shown inconclusive results especially in the case of essential oils, presenting both increase and decrease as well as no change in feed intake. Limited evidence exists in pigs to support improvement in gut function. Plants high in terpenes with high anti-oxidative properties include rosemary, oregano and thyme. However, whether they can be added in amounts sufficient to replace the effects of antioxidants commonly used in pig diets, such as ethoxyquin and butylated hydroxytoluene, remains to be seen. . Two of the most common phytogenic substances evaluated in swine include the spices oregano and thyme because of their good antimicrobial effect. Oregano contains the monoterpene carvacrol and thyme contains thymol, both of which have demonstrated high efficacy in vitro against several pathogens found in the intestinal tract. This suggests phytogenic feed additives may be suitable replacement for in-feed antibiotics, however numerous in vivo studies have showed inconsistent responses in pig health improvement and growth performance. Hence, more evidence is needed to confirm the apparent beneficial effects before these products are added to swine diets on a regular basis (JACELA et al. 2010).

Two different industrial products obtained from garlic, Allium sativum, which are referred to as PTS and PTS-O, have been tested in a number of in vitro experiments on the gastrointestinal microbiota of pigs. Both products have significant antimicrobial activity against total aerobic and anaerobic bacterial groups including a bactericidal effect against Salmonella Typhimurium. Results showed that PTS-O had a significantly stronger antimicrobial effect than PTS. In vivo trials are now necessary to study the potential use of these garlic derived products as alternatives to antibiotics in pig feeds (RUIZ et al., 2009).

6.7 Swine Vaccination

In pigs, Salmonella monitoring is based on meat juice serology. Immunization with conventional Vaccines cannot be used, as it interferes with current control programs relying on serology as a means of herd classification. A new negative-marker has been developed, SalmoporcΔompD, which allows the differentiation of infected from vaccinated animals (DIVA)-. The protective efficacy of two peroral immunizations using the DIVA live vaccine was proven using a challenge with a multiresistant S. Typhimurium isolate. Both clinical symptoms and colonization of lymph nodes and intestinal tract were significantly reduced.

Since genetically modified organisms are involved, it is necessary to license these vaccines at the European Agency for the Evaluation of Medicinal Products. Relatively high costs and experimental difficulties are involved in bacterial DIVA live vaccine construction. They have been used in good success for the elimination of viral infections such as Aujeszky's disease in pigs, however DIVA vaccines against food-borne pathogens are described only in their experimental stage so far (SELKE et al., 2007).

CONCLUSION

All these feed approaches deserve further investigation in order to develop an integrated control strategy which should guarantee an acceptable level of protection from Salmonella. The list of feed additives is not meant to be complete, and there is no doubt that there are more aspects to the nutritional strategies, such as physical and chemical decontamination of feed and drinking water. Any of these additives may slightly or considerably add to the cost of feed production, thus each is faced by thorough cost – benefit analysis, which may hinder future research or application.

The use of feed additives to combat Salmonella is definitely a good alternative to antibiotics. Most of these tools undoubtedly can reduce the colonization and excretion of Salmonella, however probably none can eliminate this pathogen completely in a field situation, especially when used on their own. By no means does the application of feed additives replace good, effective hygienic and health management of farms, transportation and slaughter houses.

In my opinion, Salmonella control should start early in newborn piglets and post-hatch poultry, due to the susceptibility of young animals for Salmonella at that age. Both stimulation of the host immune system and reduction of virulence of the bacteria should be combined to decrease colonization and shedding of the animal. The first can be achieved by feeding pre- and probiotics, as well as vaccination with live mutant strains resulting in protective non-specific immune responses. The latter can be achieved by the use of feeding organic acids, namely microbeads of the short chain fatty acid butyric acid for broilers, and medium-chain fatty acids for swine.

Phytogenic feed additives deserve much more attention, because of their widespread mode of action and their negligible harmful effect. Especially herbal traditional chinese medicine should be increasingly incorportated into western countries. Research ought to be devoted into the results of over 2000 years worth of tradition and its practical application to improve health management of modern-day farming.

SUMMARY

Salmonellosis is generally accepted to be one of the most important zoonoses transmitted by meat and eggs in the developed world. Whilst the majority of salmonellosis infections arise from consumption of poultry products, the pork industry is not blameless. Salmonellosis is caused by a Gram-negative, facultative anaerobic intracellular bacteria. There are over 2500 different serotypes of Salmonella and the epidemiology is complex. The problem of increasing microbial resistance to antibiotics resulting from years of overuse and the resulting ban of antibiotics in animal production have led to increased interest in alternatives to antibiotics in animal production.

A large spectrum of feed additives have been tested for their suppressive effect on Salmonella shedding and carriage, mostly in broilers.

These include the classical probiotics, such as cultures of the Lactobacillus species, for which the beneficial effects are well documented, especially in poultry. Results of growth performance trials in pigs with prebiotics, like lactose, and probiotics have been inconsistent and require further investigation. Synbiotics, which consist of combinations such as Lactobacilli and FOS, have received merit in many studies.

Organic acids have been increasingly introduced, short-chain fatty acids being mostly used in the poultry industry, where dietary propionic acid or microencapsulated butyric acid are the most effective. Swine industry uses medium-chain fatty acids, caproic acid being the most potent.

Phytogenics such as oregano and thyme in the swine industry have been introduced, but consistency in a significant increase in growth performance is still lacking. Trials with dietary Chinese herbal medicine have been very positive in the case of ducks.

Regulatory approval for new experimental approaches such as the bacteriocin nisin, antimicrobial peptides and bacteriophages is slow.

Several vaccinations are already compulsory, nevertheless, development of new vaccines is getting increasingly more attention, for example immunization of laying hens with type 1 fimbriae.

Multiple combinations of feed additives appear promising, for example for turkey a combination of organic acids with mixed probiotic cultures have shown most impressive results.

Research will continue into the additive and adverse effects of all these promising alternatives to antibiotics.

ÖSSZEFOGLALÁS

Vanessa, Erbslöh: Takarmányadalékok révén a Salmonella-fertőzést csökkentő és megelőző módszerek és technológiák a baromfi- és sertésiparban

Salmonellosis az általános fölfogás szerint egyike a legfontosabb hús és tojás által átvitt zoonósisnak a fejlett országokban. Bár a szalmonellás fertőzések többsége baromfi-termékek fogyasztása révén keletkezik, a sertéstermékek sem mentesek. A salmonellózist. A szalmonellózist *Gram* negatív, fakultatív anaerob, intracelluláris baktériumok okozzák. Több, mint 2500 különböző Salmonella szerotípus létezik, s a betegség járványtana is összetett. Az egyre növekvő arányú antibiotikum rezisztencia sok év túlzott és indokolatlan használatából ered és összefüggésben áll az antibiotikumoknak mint hozamfokozóknak a teljes betiltásával.
Több takarmányadalékot próbáltak ki – főként a pecsenyecsirke vonatkozásában - vajon van-e korlátozó hatásuk a szalmonellák ürítésére és hordozására. Így kipróbáltak klasszikus probiotikumokat mint tejsavbaktériumok kultúráit, amelyek esetében, főleg baromfi vonatkozásában jók bizonyított a pozitív hatás. A prebiotikumokkal (pl. laktózzal) végzett sertéskísérletek nem egyértelműek és további vizsgálatokat igényelnek. Számos pozitív eredménnyel zárult kutatást végeztek a szimbiotikumokkal, amelyek tejsavbaktériumok és a FOS kombinációjával. A baromfiiparba egyre inkább bevezetik a szerves savakat (főként rövid szénláncú zsírsavakat), s a takarmányba kevert propionsavat és a mikrokapszulázott vajsavat.
A sertéshús-előállításban inkább közepes hosszúságú zsírsavakat, nevezetesen a kapronsavat találták a leghatékonyabbnak. Az oregánóból és a kakukkfűből készült növényi kivonatokat is alkalmazzák a sertéshizlalás során, de a súlygyarapodás következetes javulása nem mindig következik be. Kacsákon pozitív hatásúnak találtak a takarmányba kevert kínai gyógynövény-keveréket.
A kísérleti szinten bevált új típusú szalmonella-ellenes szerek (bakteriocin, nizin, antimikrobák peptidek és bakteriofágok) engedélyezése hosszadalmas folyamat.
Több vakcinázási típust is vezettek be, a vakcinafejlesztés nem zárult le, így például a tojótyúkoknak az I-es típusú fimbria elleni vakcinája.
A takarmánykiegészítők többszörös kombinációja tűnik biztatónak, így például pulykának szerves savakból és vegyes probiotikus kultúrából álló keverék adta a legmeggyőzőbb eredményt.
Az antibiotikumokat kiváltó ígéretes takarmányadalékok esetleges káros mellékhatásait további vizsgálatoknak kell kizárnia.

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Vanessa Erbslöh

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Budapest,September 2013

…………………………………

Prof. Dr. Sándor György Fekete

DVM, DSc, Head of the Unit for
Laboratory Animal Science

ABBREVIATION KEY

μm = micrometer
ser. = serovariant
S. Enteritidis = Salmonella enterica serovar Enteritidis
E. coli = Escherichia coli
subsp. = subspecies
GALT = gut associated lymphoid tissue
SPI = Salmonella pathogenicity island
C4, C6, C10, C12 = respective number of carbon atoms within fatty acid chain
FCE= feed conversion efficiency
kb = kilo base pairs = 1000 base pairs (1 base pair = two nucleotides on opposite complementary DNA or RNA strands that are connected via hydrogen bonds)
SAP = serotype associated plasmid
SEF = Salmonella Enteritidis Fimbriae
ATP = Adenosine triphosphate
E. coli = Escherichia coli
MIC = minimal inhibitory concentration
NDO = non-digestible oligosaccharides
FOS = fructo-oligosaccharides
GOS = galacto-oligosaccharides
MOS = mannan-oligosaccharides
PHGG = partially hydrolysed guar gum
TCA-cycle = tricarboxylic acid cycle = citric acid cycle = Krebs cycle
TOS = transgalactosylated oligosaccharides
FA = fatty acids
CaP_i = calcium phosphate
kGy = kilo Gray, unit of absorbed ionizing radiation
IgG = Immunoglobulin G
IgA = Immunoglobulin A
Igs = Immunoglobulins
CFR = code of federal regulations
kDa = kilo Daltons, unit for molecular mass
DIVA = Differentiation of Infected from Vaccinated Animals
i.e. = id est (Latin for “that is”)

Significant refers to statistically significant ($P < 0.05$). The P value is “the probability of obtaining a test statistic at least as extreme as the one that was actually observed.” When the p- value is less than the significance level, which is often 0.05, then the null hypothesis is rejected, and the result is said to be statistically significant. The null hypothesis corresponds to there being no relationship between two measured phenomena or that a potential treatment has no effect (http://en.wikipedia.org/wiki/P-value. Downloaded 17.12.2011).

pKa = acid dissociation constant Ka, in logarithmic measure

$$pK_\mathrm{a} = -\log_{10} K_\mathrm{a}$$

Where the acid dissociation constant Ka is: $$K_\mathrm{a} = \frac{[\mathrm{A}^-][\mathrm{H}^+]}{[\mathrm{HA}]}$$

HA is a generic acid that dissociates by splitting into A−, known as the conjugate base of the acid, and the hydrogen ion, H+, which, in the case of aqueous solutions, exists as a solvated hydronium ion. The equilibrium can be written as $HA \rightleftharpoons A^- + H^+$, (http://en.wikipedia.org/wiki/Acid_dissociation_constant. Downloaded 31.juli.2011)

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Figure 1: Salmonella transmission to humans
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Figure 2: Schematic representation of host-pathogen interactions during pathogenesis of Salmonella infections.
Source: (VAN IMMERSEEL, et al., 2002b) page 14.
Figure 3: Transmission electron microscope picture
Source: http://www.ncl.ac.uk/dental/oralbiol/oralenv/tutorials/fimbriae.htm
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Figure 4: Direct binding of Salmonella by prebiotic
Source: http://www.pigprogress.net/public/image/Figure2.jpg
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Figure 5: Probiotic for poultry
Source: http://in.all.biz/img/in/catalog/33809.jpeg
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Figure 7: Administration of probiotic cultures (P1 and P2) or organic acid (OA) in the drinking water alone or in combination.
Source: (VINCENTE et al., 2007) page 474.
Figure 8: Salmonella infection in pigs, source and route of infection
Source: http://www.pigprogress.net/background/nutritional-approaches-to-controlling-salmonella-5919.html
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