# The spread of Salmonella in animal production
# An ongoing reduction is possible

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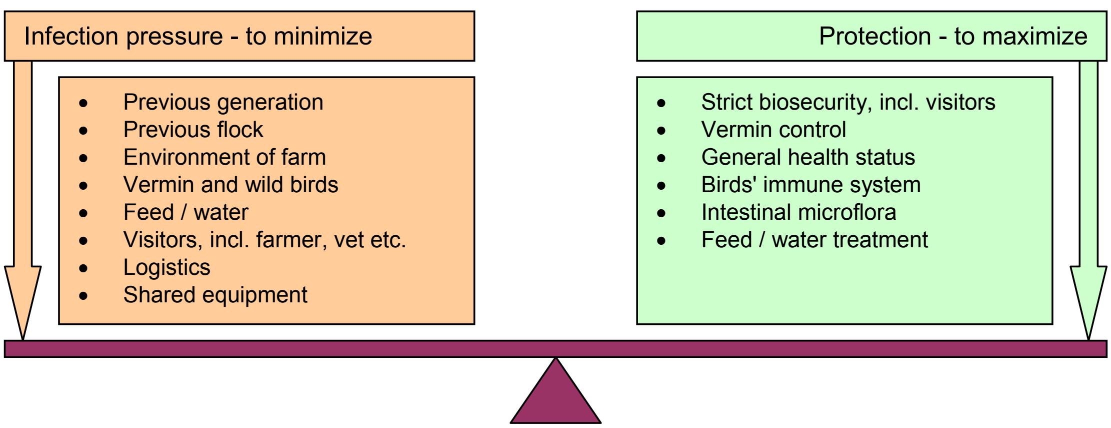
## Introduction

The human health authorities and the poultry industry worldwide recognize the implications of Salmonella infections in humans and poultry. During the 1980s one particular type of Salmonella, *Salmonella enteritidis (S.e.)* became the predominant source for human salmonellosis originating from poultry products. Meanwhile this serotype has contaminated the environment and the poultry industry to a level that an eradication program is unrealistic. This contamination of the environment will stay a source for a continuous flow of new infections.
Since 1994 Salenvac® is used as a major tool in Salmonella control programs. The success of such programs does not solely depend on the use of vaccines. The more “hurdles”, that will decrease the spread of Salmonella, are incorporated, the more success may be expected.
Simultaneous implementation of a adequate monitoring program, in case of observed contamination followed by corrective measures, and adequate biosecurity is essential. Another important, sometimes neglected phenomenon is the birds’ own natural intestinal microflora that can provide protection against Salmonella infections. In general an effective Salmonella control program needs to minimize infection pressure and to maximize protection. (figure 1)

Figure 1. Salmonella infection: a balance between infection pressure and protection



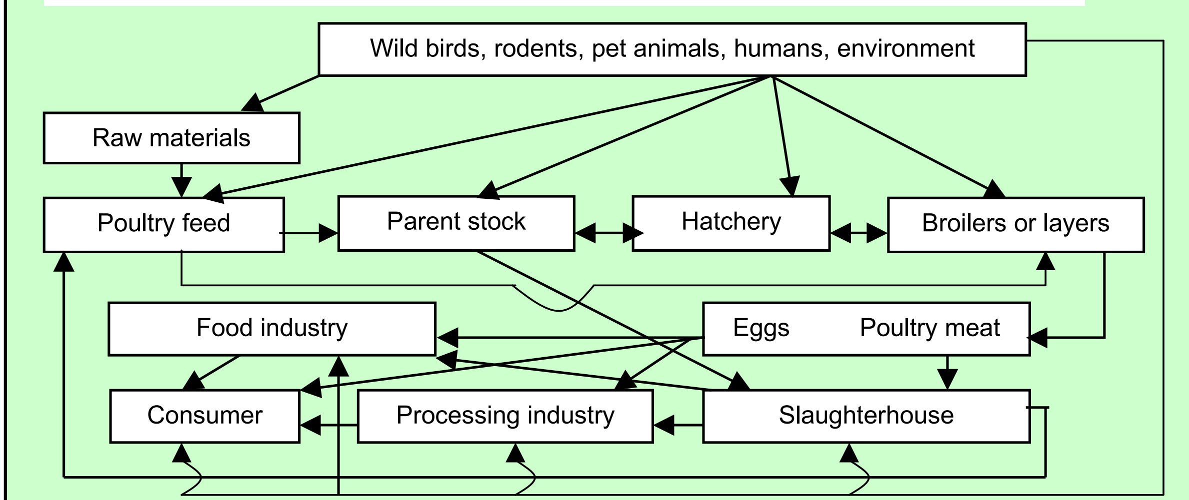

## The basics for a Salmonella Control program

Reduction of spread of salmonella can only be achieved by a coordinated sequence of measures. In general terms these can be described as follows:

1. Start with proven Salmonella-negative origin. This is the barrier between the different generations. An accurate monitoring program will provide the essential information in the Salmonella-status in the previous generations.
2. An effective biosecurity management will act as the barrier between individual farms and their environment and the logistic activities that link them together.
3. Maximalization of each individual animals’ own protective mechanisms that enable them to withstand an environmental infection pressure. This can be achieved by a specific activation of immune system and the optimalization of the protection that can be derived from a timely developed and balanced intestinal microflora. These two phenomena can be interpreted as a barrier around each individual animal.

The above mentioned sequence does not imply any ranking. However it should be realized that due to the practical situation ~~(e.g. detection~~ limits in monitoring programs, impossibility to eliminate all rodents, individual variability in immunological response and / or in the development of a balanced intestinal microflora, non of the above mentioned measures will be 100% effective. The possibilities for Salmonella to spread in animal production are to complex, as shown for poultry in figure 2.

Figure 2. Infection routes of Salmonella in poultry production




Ref.: varied after Int. Poultry Production, Vol. 4, No.2, 1996

It can be concluded from this figure that the contamination of the environment will stay a source for a continuous flow of new infections. There for the success of Salmonella Control Programs does not solely depend on the use of a single measure. The more “hurdles”, that will decrease the spread of Salmonella, are incorporated, the more success may be expected.
This presentation will concentrate on the maximalization of each individual animals’ own protective mechanisms that enable them to withstand an environmental infection pressure. It will summarize the experience with vaccination as a major contribution to prevent the spread of Salmonella in poultry, but will also mention the experience with an in-feed approach that was developed recently. This approach is based on the stimulation of the development of a harmonious intestinal flora, in which the natural C.E.-mechanism can act as another hurdle that can provide protection against Salmonella infections.

## Optimalization of the animals’ own protective mechanisms

As mentioned the animals’ own natural protective mechanisms can be separated in two classes:

1. The immune system that enables them after an adequate stimulation to develop a specific protection against a defined pathogen. To maximize this protection the following is essential:
   - A well functioning immune system, not negatively influenced by immunosuppression factors like stress, diseases or nutritional deficiencies.
   - An accurate stimulation, preferably by a safe, non-disease causing antigen.
   - An accurate vaccination schedule (timing and sequencing of vaccinations)
   - A proper vaccination (the correct volume at the correct place in all animals)

   When the correct choice of vaccines (mainly the inactivated vaccines) is made it is possible to transfer immunity via matermal antibodies to the offspring that will last for the first weeks of life.
2. The intestinal microflora can also be activated as a natural protective mechanism against Salmonella colonisation in broilers. This phenomenon, Competitive Exclusion, was first described by Nurmi and Rantala (1973). The basic mode(s) of action are still not fully understood. Most probably it is a combination of:
   - Production of chemical compounds by certain, non-pathogenic bacteria-populations, that have a negative influence on the growth-potential of the unwanted (pathogenic) populations. This was described by Barnes et al. (1980) and Dawson (1997). Recently van der Wielen et al (2000) described that Volatile Fatty Acids (VFA’s) in non-dissociated form were responsible for an observed reduction in *Enterobacteriaceae* in the ceca of broilers,
   - Competition for nutrients, that may also be used by the unwanted species
   - Competition for adhesion-sites, that may also be used by the unwanted species
   - Immunomodulation

## Benefits of vaccination

The EC-zoonosis-directive (EC/92/117) mentions both *Salmonella enteritidis* and *Salmonella typhimurium (S.t.)* as predominant sources for human Salmonellosis. Experience with the use of live and inactivated Salmonella vaccines is available. This paper will concentrate on the experience with an inactivated *S.e.* vaccine (Salenvac®) and a live *Salmonella gallinarum* vaccine (Nobilis SG 9R®). In 2000 an inactivated vaccine (Salenvac T®) containing both *S.e.* and *S.t.* is registered in UK. A broader protection against other serovars of the D-group and B and D-group of the Kauffman-White scheme (figure 3) is proven for the inactivated vaccines respectively and the live vaccine.

Figure 3. Kaufmann - White Classification for some Salmonella serovars

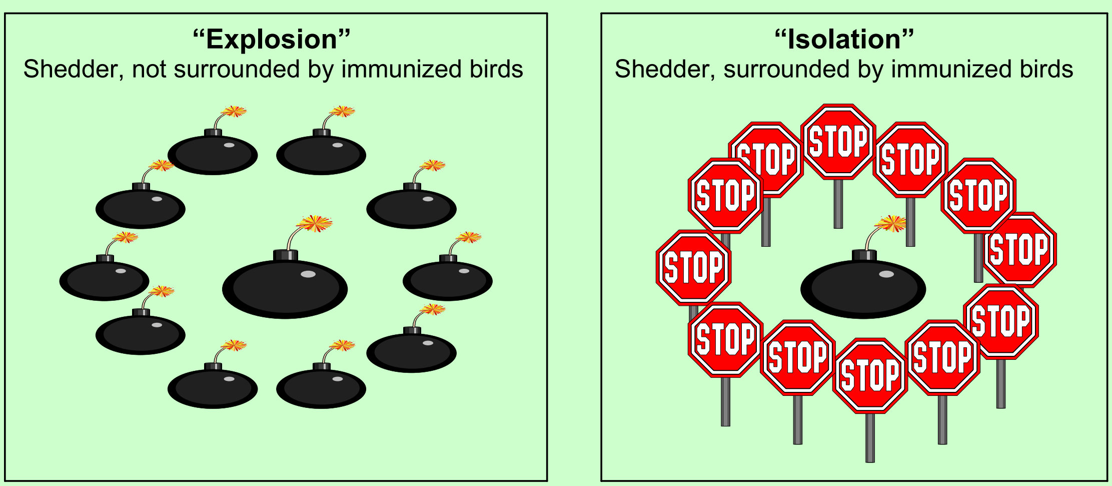
| Group | Serovar | Somatic (O) antigens | Flagellar (H) antigens | |
|---|---|---|---|---|
| | | | Phase 1 | Phase 2 |
| B | *S.typhimurium* | 1, 4, 5, 12 | I | 1, 2 |
| B. | *S.paratyphi B java* | 1, 4, 5, 12 | b | 1, 2 |
| D | *S.enteritidis.* | 1, 9, 12 | g, m | -- |
| D | *S.gallinarum/pullorum* | 1, 9, 12 | Non mobile | Non mobile |

The safety profile of these vaccines is excellent. No vaccination reaction in the vaccinated bird or after accidental self-injection is observed. Additionally these vaccines have the advantage that they do not bear any antibiotic resistances towards antibiotics used in human therapy as might be for some live S.e. vaccines

When birds are vaccinated against Salmonella, the vaccination gives protection against:

- (Re-)infection by rodents and other vermin, that are commonly found and can not be eradicated on poultry farms
- (Re-)infection from the environment of the farm, originating from wild birds, mice, neighboring farms, slaughterhouses etc.
- Infection by contaminated feed, which normally will be a small chance, but the risk for spreading the infection is enormous, when it occurs
- Spread of an undetected infection. A breeding flock will become infected between two moments of monitoring. The infected hatching eggs will be transferred to the hatchery and will contaminate the hatchery and the equipment, thus creating a risk for infection of other breeder farms, supplying the same hatchery.
- Spread of infection in the hatchery, mainly in the hatchers. The offspring of vaccinated birds is protected during the first weeks by maternal antibodies that will limit the spread of the infection. (Figure 4. Anti-transmission effect of Salenvac®)

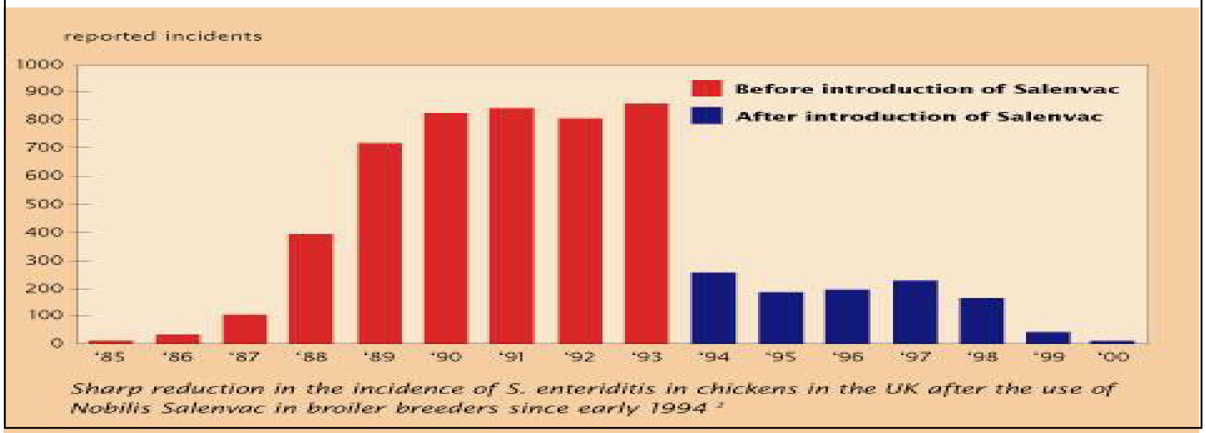
Figure 4. Anti-transmission effect of Salenvac®




## Experience with vaccination in broiler breeders

On behalf of the UK Ministry of Agriculture, Fisheries and Food the Veterinary Laboratories Agency publishes each year a report on "Salmonella in Livestock Production". A sharp decline in the *S.e.*-figures was observed since 1994, the year the UK poultry industry started using Salenvac, and afterwards. (Figure 5.)

Figure 5. Reported Salmonella incidents in UK (ref. Salmonella in Livestock Production 2000, MAFF)

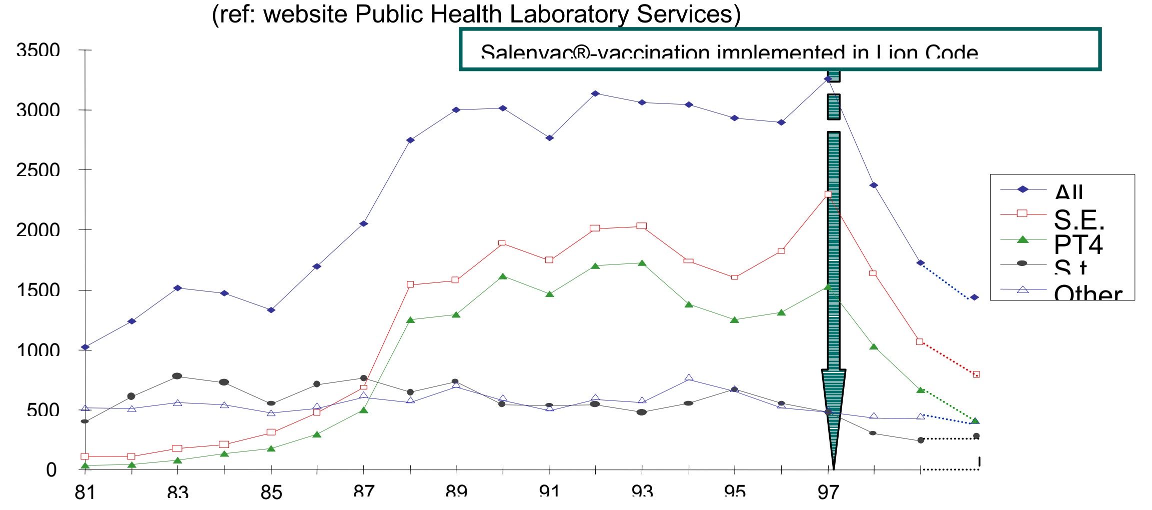



The poultry industry has observed several beneficial side effects since the use of Salenvac. A positive effect of vaccination is also observed on hatchery economics. At 54 weeks of age Salenvac vaccinated groups produced already 1,5 – 2 day old chickens more then the non-vaccinated controls, housed on the same farm. (International Hatchery Practice, 7, 1995)
In a pre-registration field-test in The Netherlands in 1996 under the coordination of the National Animal Health Service 1.1 Mio broiler breeders (53 flocks) were vaccinated with Salenvac. This extensive fieldtrial is published by Feberwee et al. in Avian Diseases in 2000. All vaccinated flocks were identified as being "at risk" and had a historical chance of 17% to become *S.e.*-positive again. In the vaccinated flocks no *S.e.*-infections were observed ($P < 0.01$). In the control flocks, not considered as being "at risk", the incidence of *S.e.* stayed at the same level as before, which confirms the continuous existence of the infection pressure in the Dutch poultry population. This excellent trackrecord is repeated in all other countries, where it is registered.

## Experience with vaccination in layers

A large scale sampling and testing of free range eggs that could be traced to a vaccination or a non-vaccination origin was performed by the Public Health Laboratory Service (UK). 44.700 eggs of vaccinated origin were all tested negative for Salmonella. On the contrary out of the 42.642 tested eggs of non-vaccinated origin 39 were positive.. After the general implementation by the British Egg Industry Council of vaccination in the Code of Practice for Lion Quality Eggs (Lion Code) a steady decrease in the amount of human Salmonellosis in England and Wales is observed in the data of the official monitoring. This program represents the vast majority of the UK table egg production. The data are summarized in figure 6. The authorities indicated vaccination as the major rationale behind the observed decline in the number of cases of human Salmonellosis.

Figure 6. Trends in human Salmonellosis in England and Wales (ref: website Public Health Laboratory Services)



In several countries the vaccine Nobilis SG9R® is available. This live vaccine is based on the so called "Rough" strain of *Salmonella gallinarum*. Vaccination with this Salmonella serovar results in a good cross-immunity to *Salmonella enteritidis* as both belong to the D-group in the Kaufman-White scheme. This rough strain can easily be differentiated from field strain as these all are "rough" strains. Another advantage is that *Salmonella gallinarum* is specific for poultry and is 100% safe for humans.
Between 1995 and 1998 2.3 Mio " at risk" layers were vaccinated using the live vaccine Nobilis SG9R in a field study in The Netherlands. Only 1.25 % of the vaccinated flocks became S.e.-positive (1 flock at at 72 weeks of age), whereas in the un-vaccinated, not " at risk" population 11.5 % of all flocks became Salm.-positive in the same period. This difference was clearly significant (P<0.05).
All these results confirm the efficacy of the mentioned vaccines.

### Experience with Flavomycin® to reduce the spread of Salmonella

In the European Union a new registration procedure, known as the Fifth Amendment of the Feed Additive Directive (70/524/EEC) requires, among other data, the submission of information on the possible effect of feed additives on the excretion of food-borne pathogenic bacteria like Salmonella and Campylobacter. For Flavophospholipol (Flavomycin®, a performance enhancing antibiotic from Intervet) this study was done at ID-Lelystad, The Netherlands. The results of this study are published by N.M. Bolder et al. in Poultry Science (1999).
In this study Flavomycin® reduced significantly (P<0.05) the level of Salmonella-shedding at slaughter age. Less broilers in the Flavomycin®-group were Salmonella-positive after the initial oral infection at day 12 and 13 and mean fecal Salmonella cfu counts were significantly (P<0.05) lower in the Flavomycin®-group then those in the control group. The results are summarized in figure 7 and 8.

Figure 7. *S.enteritidis*-shedding, group average

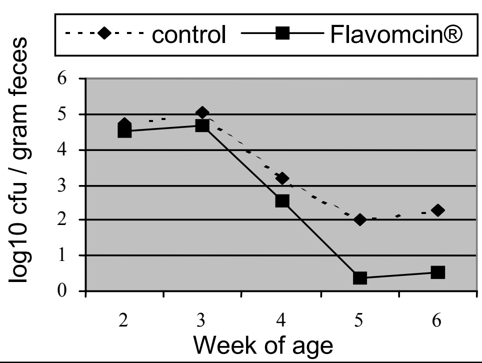
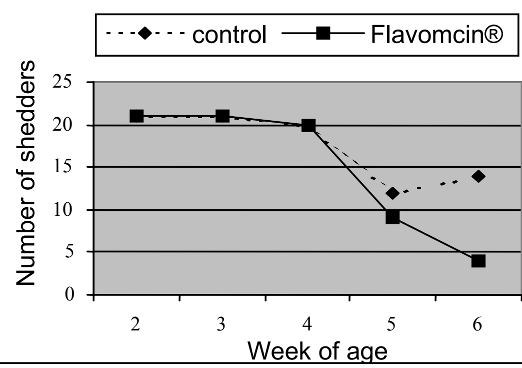



Figure 8. Number of broilers shedding *Salmonella enteritidis*




These results confirm earlier studies in broilers. In the Bolder study an effect to the same extent was found on Clostridium perfringens shedding, confirming the publications of Fukata et al (1991) and Kling et al (1995). The level and incidence of Campylobacter was not affected. Studies in pigs and calves show identical results on the level of Salmonella-shedding. (Dealy and Mueller, 1976 and 1977)
Under USA-field conditions broiler rations containing Flavomycin® were compared to rations containing other antimicrobial growth promoters. Intestinal samples were collected at the slaughterhouse and these were analysed for the numbers of Salmonella. A part of this work is published by Schleifer et al. (1999) and Oostenbach (2000) The results are summarized in figure 9.

Figure 9. Percentage of Salmonella-positive samples at slaughterhouse-level under USA-field Conditions

| | Positive control | Flavomycin®-group |
|---|---|---|
| Integrator A | 50 % | 18 % |
| Integrator B | 45 % | 21 % |
| Integrator C | 11 % | 5 % |
| Integrator D | 81 % | 15 % |

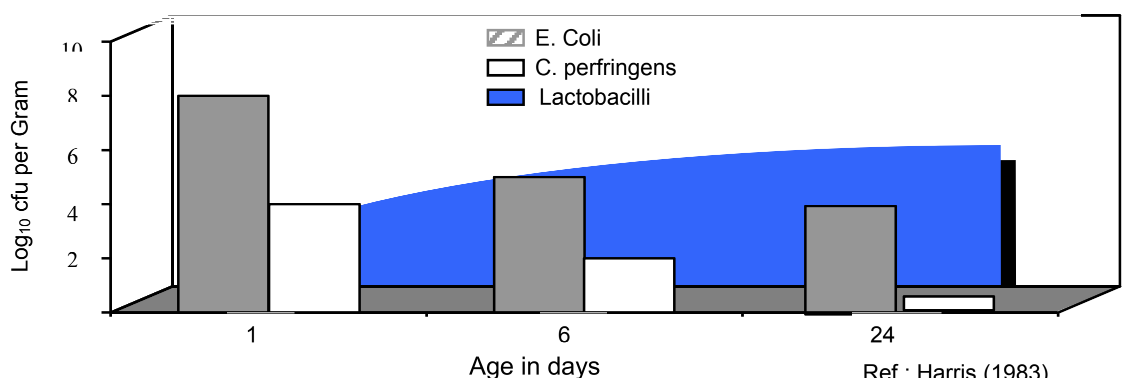
These data cover all major production regions in USA. The only difference between the positive control and the Flavomycin®-group is the used antimicrobial growth promoter.

These results indicate that Flavomycin® could be a very useful tool in a HACCP-program for the slaughterhouse and in a Salmonella Control Program for the complete production chain, as it reduces substantially and significantly the total "Salmonella load" of broiler flocks at the slaughter age, as well as the number of shedding birds. This reduction is of major importance as it has its effects at the slaughterhouse, the stage in the production nearest to the consumers.

## The stimulating effect of Flavomycin® on the intestinal microflora

Another aspect of the use of Flavomycin® is that it supports the intestinal microflora. One of the observed benefits is the beneficial effect it has in litter quality. This dryer litter results in improved climate conditions, decreased infection pressure, cleaner animals and less down grading at the processing level. The most probable mode of action is the protective role of the natural, balanced intestinal microflora.
This important Food Safety benefit of Flavomycin originates from it's stimulation of the production of organic acids by the natural occuring intestinal microflora. Free, Lindsay and Hedde published on the stimulation effect of Flavomycin as compared to other antimicrobial growth promotores. Lactic Acid Producing Bacteria (LAPBs) are an important natural barrier against unwanted pathogenic bacteria as they are among the first bacteria to colonize the intestinal tract of young chickens, thereby limiting the growth potential of pathogens. Figure 10 describes the development of the intestinal microflora of young chickens.

Figure 10. Development intestinal microflora in the chickens' ileum




This underlines the importance of a quick and complete colonization of the intestinal tract by Lactobacilli. This mode of action enables Flavomycin to reduce the growth of organisms against which it has no direct antimicrobial effect (MIC-value). This effect of Flavomycin is not only published for Salmonella as cited above but also for other pathogens, e.g. Clostridium perfringens. Besides this Competive Exclusion -effect of LAPB's the production of lactic acid itself is another important part of the mode of action. Lactic acid is not only known for it's direct bactericidal effect in the first part of the intestinal tract, but also is used as an essential nutrient by other bacteria like Bifidobacterium. This group of bacteria produce Volatile Fatty Acids (VFA's). VFA's do have an even more pronounced bactericidal efect, mainly targeted at the last part of the intestinal tract, as described by van der Wielen et al. (2000).
In addition to this indirect effect on pathogens, e.g. *Cl. Perfringens*, less therapeutic treatments will be necessary. This has the beneficial effect for Food Safety that there is a decreased risk on residues. Experience in all countries that have banned or substantially reduced the use of antimicrobial performance enhancers tells us that this has been closely related to an increase in the amount of therapeutics, often used against *Cl. Perfringens*..

## Alternatives for antimicrobial performance enhancers

The research for alternatives for mainly concentrates on the application of (mixes of) organic acids and herbal extracts. Till now none of these products are registered as growth promoter. A substantial part does not even have a validated registration dossier as feed additive, they just are allowed (not explicitely forbidden) to be used in feed. To stay on the market is will come absolutely necessary that these products will have a validated registration dossier, consisting of data on efficacy, safety, standardized content of identified active compounds, mode of action per active compound and methods to analyze active compounds, metabolites and potential for residues. A statement that these products are of natural origin cannot be interpreted as a guarantee for safety as such.
At this moment it is difficult to predict the likelihood that products with this background will be complete alternatives for the classical antimicrobial growth promoters. The long term effects are still unknown and it is very probable that their field experience is positively influenced by the previous use of AMGPs.

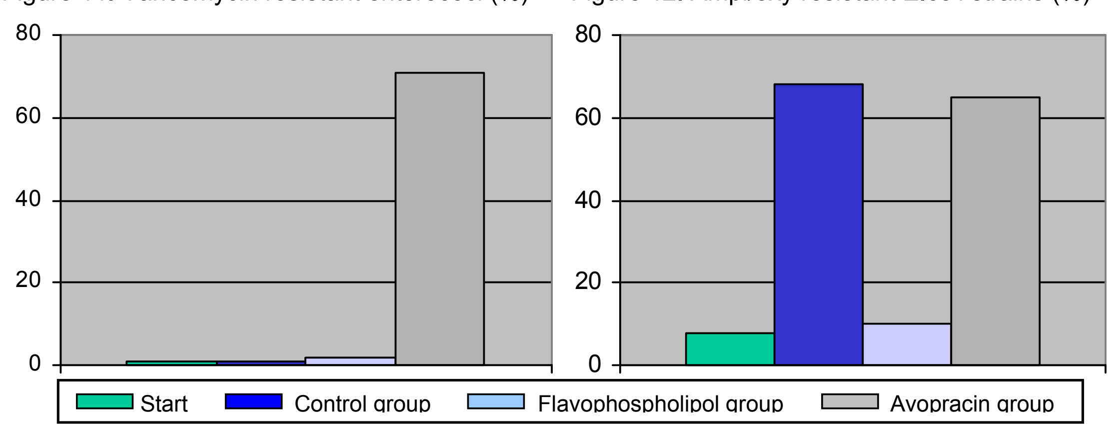
Field observations in several countries that stopped to use AMGPs give indications for the effect of a sudden change. In some countries the elements Zinc and Copper are used (again) for their bactericidal effect, however these dosages exceed the EC-limits and there for cannot be accepted as alternatives.
Another group that is often mentioned among the potential alternatives for AMGPs consists of (mixes of) organic acids. Several of these products have a registration as preservatives in food and feed industry. It is known that pathogens can develop tolerance against organic acids. Usage of these products in primary production can induce this acid tolerance that will have a negative effect on their efficacy as preservatives. This is a negative effect for Food Safety. Recently this phenomenon for several pathogens, among which E.coli, has been published by Bower and Daeschler (1999) and Cheung, Bartelt and Knorr (1999), who described situations that lead to food poisoning.
The development of acid tolerance in primary production due to a selection pressure induced by the use of (mixes of) organic acids is unacceptable from a Food Safety point of view (Brul and Coote, 1999). In fact the identical rationales as that were used in 1999 to ban all AMGPs that were related in any sense to therapeutically used antibiotics.

### Characteristics of Flavomycin

Flavomycin® (Flavophospholipo), belonging to the antibiotic-class of the phosphoglycolipids, is licensed as a digestive enhancing antibiotic by the regulatory authorities in the EU, the USA and most countries world-wide. Due to it's limited efficacy against human bacteria and it's poor pharmaceutical properties it is not related to any antibiotic currently in use or under development for the treatment of human or animal diseases. Therefor it was not affected by the decision of the EC to ban the digestive enhancing antibiotics are related in any way to antibiotics used for human or animal therapy.
The very limited direct antibacterial activity is restricted to the gram-positives. The enzyme glycosyltransferase, that plays an essential role in the synthesis of the cell wall of this group, can not distinguish between Flavophospolipol and the natural compound. This results in an instable cell wall, leading to the death of this cell. In gram-negatives glycosyltransferase plays no role, due to the different cell wall structure. However when a plasmid bridge (pylus) between to bacteria is formed it's biosynthesis is disrupted in a similar way, leading to the death of the donor-cell. This plasmid bridge plays an essential role during the transfer of genetic information on antibiotic-resistance from one bacterium to another.
The concern on antibiotic resistance is that the plasmids carrying this antibiotic resistance may be transferred by this plasmid bridge from non pathogenic intestinal bacteria in animals to human pathogens. Flavophospholipol interferes in the biosynthesis of this pylus and therefor prevents this genetic transfer and actually reduces the number of resistance carrying bacteria. This reducing effect on plasmid-bound antibiotic resistance has already been described since the early 70's (Watanabe T. et al. (1971), Lebek G. (1972). George B.A. and Fagerberg D.J. (1984) and Dealy and Mueller (1976 and 1977)). Of more recent date are the publication of Riedl et al. (2000 2x) and van den Bogaard (2002) The results of a field study in pigs of the latter are summarized in figure 11 and 12.

Figure 11. Vancomycin resistant enterococci (%)

Figure 12. Ampi/oxy resistant E.coli strains (%)

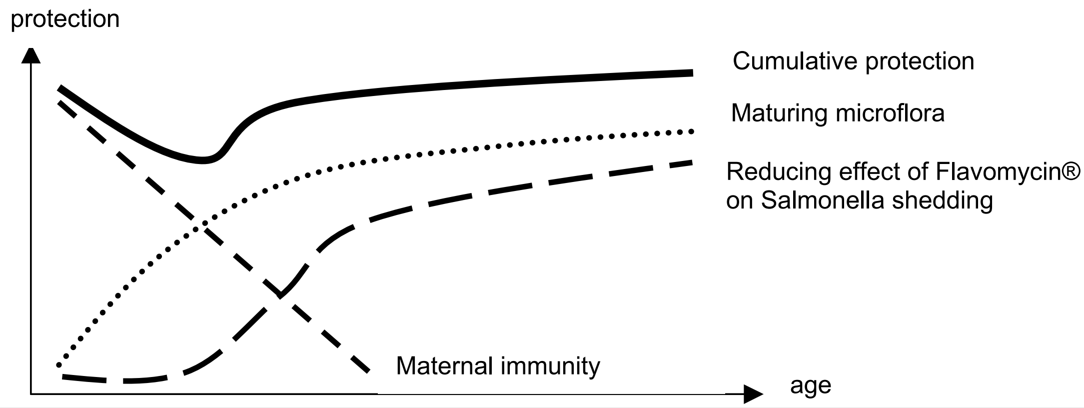



These results indicate clearly that Flavophospholipol reduces significantly vancomycin resistance in enterococci (VRE) and antibiotic resistance in E.coli and confirm the cited, older in vitro and in vivo studies.

**The synergetic effect of the general use of Salmonella vaccines in broiler breeders combined with Flavomycin® in broiler diets.**

As mentioned before a successful approach is a salmonella control program in which as much as possible measures have been implemented. In that situation synergetic benefits can be achieved as the weaknesses of one measure can be overcome by another measure. This will be the case when vaccination in breeders that realizes maternal immunity In their offspring is followed by the use of Flavomycin in broiler feed.
The offspring of vaccinated breeders will start uninfected and will be protected by maternally derived antibodies during the first weeks. Especially spread of just a few Salmonella's in a hatchery will lead to an explosion in the day old chickens as at this age the infectious dose is extremely low, e.g. only one S.*enteriitdis* bacteria is sufficient for an infection. An existing infection pressure on a broiler farm will be countered by the inclusion of Flavomycin® in the feed. It is also known that due to the Competitive Exclusion of the normal intestinal flora the infectious dose necessary for a Salmonella infection increases with age. As Flavomycin® will not affect the equilibrium in the intestinal flora, the natural defense of the Competitive Exclusion mechanism will be an additional barrier against Salmonella-infection of the broilers.
Figure 13 provides the concept how these protective mechanisms can be used in combination and can provide a useful concept for further development. (Oostenbach, 1998)

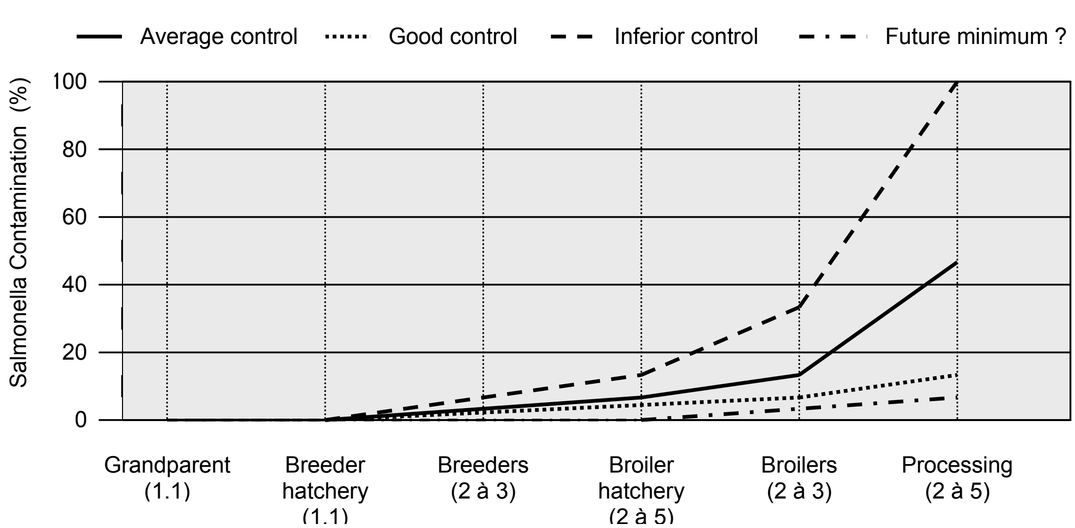
Figure 13. Effect of different protective mechanisms, to be used in a Salmonella Control Program




**The relation between the level of Salmonella contamination at different levels in the production chain**

In general it can be stated that the level of salmonella infection at a certain level in the production chain is not only related to the level in the previous stage, but also by the possibilities for this infection to transferred to the next stage and spread between individual farms at that next level. This relation is visualized in figure 14. The numbers between brackets are personal estimations for this multiplication of the infection from one level to the next.

Figure 14. Relation Salmonella infection between the levels of the poultry production chain



This clearly indicates that the levels broiler hatchery, broiler production and processing can add substantially to the infection level before the products reach the retail level. This is mainly influenced by high animal numbers per level, the intensive contacts between levels and between the individual farms that all can lead to an increased cross contamination. For the broiler level this is even amplified by the phenomenon that due to their young age they are very sensitive to even the lowest infection pressure.

## Conclusions

The best efficacy in a salmonella control Program may be expected by a simultaneous implementation of monitoring, biosecurity, vaccination and the correct choice in feed additives. Several of these measures have been discussed above and can be summarized as follows:

- Vaccination results in an immune response and antibodies are produced that protect against an infection pressure.
    - The chance that vaccinated birds will become infected is minimized.
    - The amount of spread of infections at the own level of the production chain is minimized.
    - In case of the inactivated vaccine Salenvac, the vaccinated breeders will transfer maternal antibodies to their offspring via the yolk. This will protect the offspring during the first weeks of life when they are most vulnerable.

  Thereby also minimizing the risk of spreading infections to the next generation and the following levels of the production chain.
- Inclusion of Flavomycin® in broiler diets is an efficacious instrument to reduce Salmonella at the processing level.
    - The percentage of broilers shedding Salmonella is reduced.
    - The mean fecal Salmonella counts level of the broilers still shedding is reduced.

  Both effects are very important for Food Safety as they affect the level of Salmonella contamination in primary [production closest to the consumers.The impact thereof is underlined by the experience that all benefits in earlier stages of the production may be lost, due to the vulnerability of broilers to Salmonella-infection.
- Combination of the use of Salmonella vaccines at breeder level and the inclusion of Flavomycin® in broiler diets is a synergetic instrument in a Salmonella Control Program.
- It is of utmost importance to realize that the efficacy of a Salmonella Control Program does not solely depend on the preventive use of vaccines and/or the use of the mentioned feed additive. Simultaneous implementation of an adequate monitoring program, in case of observed contamination followed by corrective measures, and adequate biosecurity is essential.
- Another aspect of today's Food Safety concerns is the level of antibiotic resistant organisms in animal production. Flavomycin® is capable of reducing this level.

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