Field experience with the use of Salenvac® and other measures as instruments in a Salmonella Control Program

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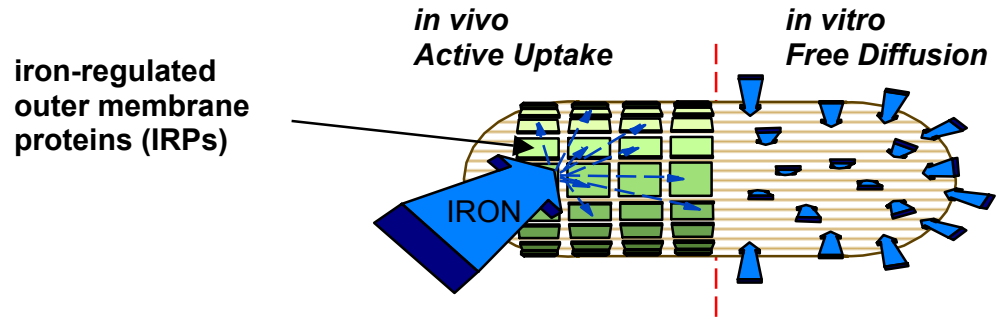
Introduction

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 of major importance as it became a 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. The contamination of the environment will stay a source for a continuous flow of new infections, because the knowledge and instruments to clear the environment completely from *S.e.* is not available.
In several European countries the poultry industry started using vaccines to protect their poultry and decrease the spread of *S.e.* from poultry to the food chain.
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.
This presentation will concentrate on the experience with Salenvac® as a major contribution to prevent the spread of *S.e.* 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 gutflora, in which the natural C.E.-mechanism can act as another hurdle.

Characteristics of Salenvac®

Salenvac® is an inactivated *Salmonella enteritidis*, phage type 4, vaccine. Aluminium hydroxide is used as adjuvant and it is therefor unlikely to cause harmful reactions, either in the vaccinated bird or in the operator, due to accidental self-injection.
The vaccine is produced under iron restriction. Research has shown that growing bacteria in “laboratory conditions” can result in antigenic differences from bacteria growing in their natural environment, e.g. the chicken’s intestine and other tissues. In the natural environment iron (Fe) is less available to the growing Salmonella-organism, because of iron binding to host proteins, normally present in intestines. In this environment Salmonella’s and also other Enterobacteriaceae, react with the formation of iron-transfer mechanisms on their surface. These Iron Regulating outer membrane Proteins (IRP’s) enable them to resorp iron actively. The bird also recognizes them as antigens and antibodies will be produced. If a vaccine is produced under the conditions of restricted iron availability, the growing organisms will form these IRP’s on their surfaces. The antibodies produced by the bird in response to such a vaccine are the same as those produced after a natural infection and will lead to a better protection.

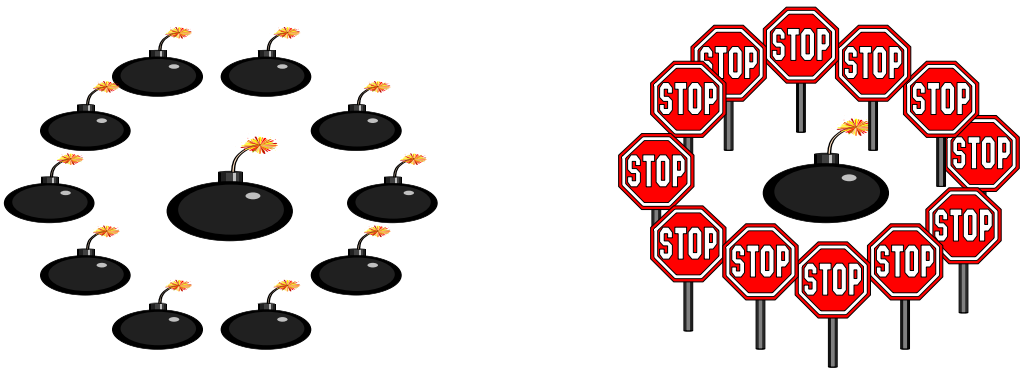
Figure 1. The effect of vaccine production under conditions of restricted iron availability (in vivo) as compared with an environment with unrestricted iron availability (in vitro).



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 the premises of poultry farms
- (Re) infection from the environment of the farm, originating from wild birds, mice, neighboring farms, slaughters 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 infection below the detection level of the monitoring program that is followed. A breeding flock will become infected between two moments of monitoring, often one month or even more separated. The corresponding hatching eggs will be transferred to the hatchery and will contaminate the hatchery and the equipment. Trolleys and other materials to transfer these hatching eggs are difficult the disinfect and are a risk for spreading the infection to other breeder farms, supplying the same hatchery, as these materials are used again and are not strictly assigned to an individual farm.
- Spread of infection in the hatchery, mainly the hatchers. At day old a few *S.e.*-organisms are sufficient to infect the chickens. The offspring of vaccinated birds is protected till 21 days of age by maternally derived antibodies that will limit the spread of the infection. (Anti-transmission effect of Salenvac®)
- Spread of infection in a flock where accidentally a few birds are not protected by vaccination. This can be due to not opening the last bottle for "just a few birds", or when birds are thought to be vaccinated but the bottle was already empty or when a flock is spiked with fresh, but unvaccinated males. The spiking with fresh males is commonly done at and age of the breeder flock of 40 weeks to improve the hatchability, that otherwise will decline fast after this age. (Anti-transmission effect of Salenvac®)

Figure 2. Anti-transmission effect of Salenvac®



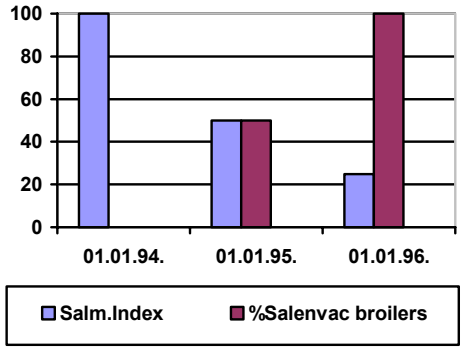
Experience with Salenvac®

United Kingdom

Salenvac® was first used under field conditions in the UK, where it was registered in 1995. Prior to the registration the product was thoroughly tested under an Animal Test Certificate protocol. Ca. 2 Mio breeder birds were vaccinated and placed on breeder farms that were known for their *S.e.*-infection in the previous flock. All flocks were monitored extensively by the national authorities and no *S.e.* infections were observed. Since it's launching a substantial part, over 75%, of all breeder flocks are being vaccinated as a standard preventive measure. *S.e.*-infections have been only observed in a few individual cases were not all birds, e.g. the males, were not vaccinated or an incorrect vaccination schedule was followed. The poultry industry has observed also other beneficial effects since the use of Salenvac®. From the field it is reported that *E.coli* problems in breeder flocks decreased in incidence as well as in magnitude. This probably is an explanation for the effect on the number of day-old chickens produced per hen housed. In a field test on 4 farms with each 2 houses, in total 76.200 birds were kept in two pens per house, separated by wire netting. In one pen the vaccinated birds were placed and in the other the unvaccinated birds. All farms were selected for this trial due to a *S.e.*-infection in the previous flock.

All flocks were monitored and stayed *S.e.*-negative till the end of production. The eggs of the vaccinated and unvaccinated birds were incubated and hatched separately in one hatchery. Till the age of 54 weeks the hatchery got more day-old chickens per hen housed out of the vaccinated group. The difference ranged from 1.91 till 2.15. After 54 weeks of age the eggs were not incubated separately anymore. A summary of this trial was published. (International Hatchery Practice 7/1995)

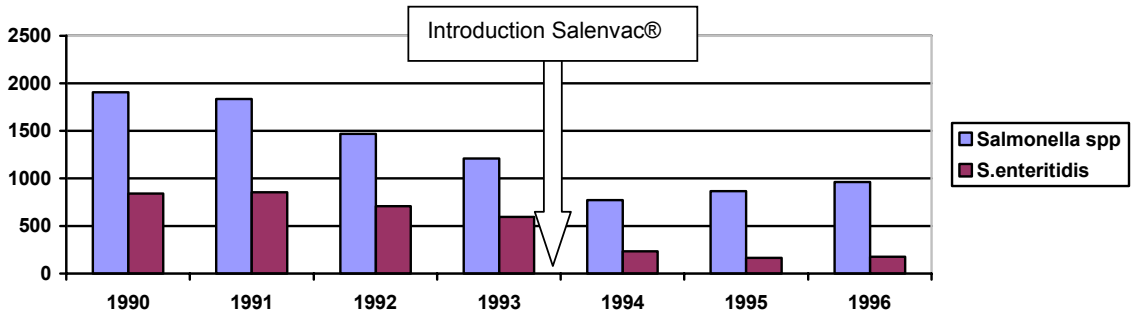
Figure 3. Salmonella Index vs. %broilers Salenvac®-origin



In the same period an integrator started a new Salmonella monitoring at slaughterhouses level. They started with a Salmonella Index for all Salmonella's, which they fixed as 100 at the start.
At that moment the *S.e.*-component in the index was already substantially below 50%.
The relation between this index and the percentage of broilers from vaccinated origin is shown in table 1.
These figures underline Salenvac®'s ability, when used at breeder level, to reduce the spread of *S.e.* to the broilers, even when the birds are samples at the slaughterhouse level.

The Ministry of Agriculture, Fisheries and Food publishes each year a report on "Salmonella in Livestock Production". In this report the effect of intensive Salenvac®-vaccination on the spread of Salmonella can be estimated.

Figure 4. Incidents of Salmonella spp and S.enteritidis between 1990 and 1996 in UK



These data show a gradual decrease in the incidents of Salmonella spp. between 1990 and 1993. This was probably due to the strict biosecurity rules that were implemented in the poultry industry. The effect it had on the incidence of *S.e.* however was only very limited. In fact the relative amount of *S.e.* as compared to all Salmonella's increased in this period from 44.2% to 49.3%. A sharp decline is the *S.e.*-figures was observed in 1994, the year the UK poultry industry started using Salenvac®, and afterwards. In this period the absolute figures decreased enormously as well as the relative amount of S.e., that dropped to 18.4%.
The layer industry started the use of Salenvac® in 1997 and over 3.2. Mio layers were vaccinated in this year. For 1998 the corresponding number has strongly increased, due to the advice from BEC and the pressure from retailers. Both want that the layer industry "uses all tools and does what it can" to reduce the spread of Salmonella.

Germany

Before it's registration in Germany, Salenvac® was tested in a field trial on 41 farms, that were all selected because the previous breeder flock was *S.e.*-positive. A very thorough Salmonella monitoring was carried out. In only one house out of three houses at the same site, *S.e.*-positive samples were obtained. The

isolation rate was extremely low compared to the previous flock. The age of the breeder flock at that time was 48 weeks. The other two houses stayed free of *S.e*. till the end of the production period.
Since it's registration in 1996, Salenvac® is being used as a standard preventive measure in ca. 90% of all broiler breeder flocks.

Belgium

Salenvac® was launched in March 1997. Since August 1997 ca. 85% of all broiler breeder flocks are vaccinated as a standard preventive measure. As a result the *S.e*.-incidence in broiler breeder flocks dropped dramatically. In 1996 6.3% of all broiler breeder flocks were identified as *S.e*.-positive. Till now no *S.e*.-infections were observed in Salenvac®-vaccinated flocks.
The layer industry started with the use of Salenvac® in heavy infected flocks, were the previous flocks and the premises of the farms were known for the *S.e.*-contamination. Due to this high infection level mortality figures doubled between 30 and 50 weeks. When these birds were autopsied almost all birds showed clinical signs of the *S.e*.-infection. Since the flocks were vaccinated, mortality decreased to the normal levels and no clinical signs of the infection were found.

The Netherlands

In a field-test in 1996 and under the coordination of the National Animal Health Service 1.1 Mio broiler breeders (53 flocks) were vaccinated preventively. All flocks were selected because they were identified as being "at risk". Flocks were identified as being at risk, when the previous flock on the same production or rearing site was *S.e*.-positive or when they originated from a grandparent flock, that became *S.e*.-positive in the period after the hatching eggs for the breeder flock concerned, were produced. Based on historical figures these breeder flocks had a chance of 17% to become *S.e.*-positive again. In these flocks no *S.e.*-infections were observed after they were vaccinated according to the advised vaccination schedule. This decrease from 17% to zero is a clear statistical significant improvement ($P < 0.01$). In the flocks that were not considered as being "at risk", the incidence of *S.e.* stayed at the same level (3.4%) as before, which confirms the continuous existence of the infection pressure in the Dutch poultry population. Two flocks were vaccinated after they were identified as *S.e*.-positive during the rearing period. In these flocks the vaccination was combined with a treatment with enrofloxacine and C.E.-flora. This treatment was not effective and both flocks stayed *S.e*.-positive.

Other countries

Besides the above mentioned countries Salenvac® is registered in Spain, Italy and Hungary. In all these countries the excellent results of Salenvac® as a major contribution to prevent the spread of *S.e*. are repeated.

Vaccination schedule

The vaccination schedule that is advised depends on when the risk of infection is expected.
Mostly the period of transfer from rearing farm to the production farm and the period directly afterwards is the most critical. During transfer the birds lack feed and water for one day, which will negatively effect the natural protection by a stable intestinal flora. Also the sub-optimal climate and the handling of the birds will increase the stress on the birds. The period directly after transfer is the period in which the birds reach maturity, which is a stress factor also. During the production breeding farms have regular direct contacts with the hatchery and direct or indirect contacts with other breeder farms, due to the transfer of hatching eggs.
A rearing flock can become infected when the premises are contaminated or when the corresponding grandparent flock is infected.
The advised vaccination schedule is given in table 1.

Table 1. Vaccination schedule for Salenvac®	
Risk during rearing	Day 1: 0.1 ml; week 4: 0.5 ml and ultimately 2 weeks prior to transfer: 0.5 ml
Risk during production	Week 10-12: 0.5 ml and ultimately 2 weeks prior to transfer: 0.5 ml
Remarks • Heavy breeders should be vaccinated intramuscularly in the breast • Light breeders and layer pullet should be vaccinated intramuscularly in the leg • Combination with other vaccinations/handling of the birds is possible • The birds can only develop a challengable immunity during the most important risk period (transfer to the production site and directly afterwards), if the last vaccination is given ultimately 2 weeks prior to transfer	

Positioning of Salenvac®

Salenvac® can be used in several options of a Salmonella Control Program:

1. Preventive vaccination of all breeder flocks during the rearing period
2. Preventive vaccination of all breeder flocks that are considered as being "at risk" during the rearing period
3. Vaccination of all *S.e.*-positive flocks (in rearing or production) eventually combined with an antibiotic and C.E.-treatment.

A maximal result will be obtained if all birds will be vaccinated preventively during rearing and if vaccination is combined with an integrated production, where the Salmonella Control Program has priority. Only if all "risk-factors" are known, it will be possible to obtain a maximal result by vaccinating only the "at risk" flocks. Vaccination of all *S.e.*-positive flocks, eventually combined with an antibiotic and C.E.-treatment, is not advised. The vaccination will not increase the success of the treatment (In The Netherlands estimated at 75-80%), but definitely increases the risk that intermittent shedders are introduced in the vaccinated flock. This is an undesirable situation, as it is practically impossible to identify these few birds in a flock. In most cases these flocks will be "false-negatives" and will frustrate the motivation of all concerned to co-operate with the Salmonella Control Program.

Experience with other measures to control spread of Salmonella

Since 1992 an effective treatment procedure to eliminate *S.e.* from infected flocks was developed by Dr. E. Goren from the PHC (Doorn) in The Netherlands. It comprises of 10 days medication with enrofloxacin (10-mg/kg bodyweight daily through the drinking water), followed by an administration of a C.E.-product the first and the third day after this treatment. When possible this treatment is combined with the transfer to a clean house. In The Netherlands this treatment is effective for broiler parent stock in 75-80% of all cases. In 1993 9 layer parent flocks were treated. Within less than 2 months all flocks were again (or still?) *S.e.*-positive. Since then this treatment has been restricted to the use in broiler parent stock.

As shown above the ability of certain antibiotics to eliminate Salmonella from the bird's body could be used as a beneficial instrument in a Salmonella Control Program. The disadvantage of this treatment however is that the complete intestinal flora will be eradicated, which will make the birds very susceptible for any (pathogenic?) micro-organism that will be the first to colonize the intestinal tract after the treatment was stopped. Therefor it is absolutely necessary to restore the intestinal flora, directly following the treatment. It is also possible that the use of antibiotics as a therapeutic for other bacterial diseases will affect the equilibrium in the intestinal flora. Mostly these treatments are not followed by a restoration of the intestinal flora and therefor may result in an unbalanced microflora, where pathogens (e.g. Salmonella's, Clostridia) may overgrow the beneficial (e.g. Lactobacilli) population and cause disease in the birds. Lactic acid is produced by these Lactobacilli and is used by other bacteria as a nutrient source to produce volatile fatty acids (VFAs) in the ceca, one of which is propionic acid. Lactic acid and VFAs are known as potent natural bactericidals, produced by the bird's intestinal flora that will depress the growth of Salmonella's and other pathogens. These natural defense mechanisms of the birds should be mobilized and not be destroyed. The effect of several antimicrobials that are used in feed as performance enhancers on the beneficial effect of lactic acid, mainly produced by the Lactobacilli was investigated by Free, Lindsay and Hedde (Zootecnica, Dec 1986)

Table 2. Antibiotic suppression of lactic acid production (mmol/L)

Treatment (Gastro-intestinal contents from 30- to 35-day-old broilers, incubated 5 hours at 42°C)	Small intestine	% reduction in small intestine
Start	4.21	------
Control (after 5 hours)	40.11	0
Flavomycin® (2 PPM)	36.16	9.9%
Lincomycin® (4 PPM)	22.16	44.8%
Bacitracin® (50 PPM)	8.16	79.7%
Virginiamycin® (15 PPM)	6.36	84.1%

These results indicate that antibiotic performance enhancers have differing effects on the level of lactic acid production in the birds' intestine and therefor have differing influences on the natural defense mechanism the birds have to depress the growth of pathogens.

The use of antimicrobials in feed in the EU is strictly regulated. The Fifth Amendment on the Regulation of Feed Additives states that of all products of which an anti-microbial activity is suspected, the effect on the Salmonella shedding-pattern should be investigated. For Flavomycin this study was done at ID-DLO, Lelystad, The Netherlands) the results are summarized in Table 3.

Table 3. Effect of Flavomycin® on Salmonella shedding (Bolder 1998)
Day 11 and 12: Challenge 10^8 cfu *S.enteritidis*, individual bacteriological sampling, Commercial broilers in housed individually in cages

	Number of broilers shedding S.e. at day of sampling		Mean S.e. cfu counts of shedders (log cfu/g faeces)	
	Control	Flavomycin®	Control	Flavomycin®
Week 2	20	21	4.75	4.51
Week 3	20	21	5.03	4.68
Week 4	20	19	3.32	2.82
Week 5	12	9	3.56	3.21
Week 6	14 p	4 q	3.43	2.89
Results within one week indicated with different letters differ statistically significant ($P < 0.05$)				

This study indicates 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 significantly the total "Salmonella load" of broiler flocks at the slaughter age, as well as it reduces the number of birds, that will be *S.e.*-shedding within such a flock. This reduction is of significant importance as it has its effects at the slaughterhouse, the stage in the production nearest to the consumers.
These results are confirmed by a monitoring done under field-conditions in the USA. In the Northern and Southern division of a broiler integrator (500.000 broilers/week, North Pacific region, both divisions 50 miles apart) each division were sampled in 1998 between February 15 and April 15, either by drag-sampling or by litter-sampling. At the slaughterhouse bacterial counts were done. The Northern division used only Flavomycin® (2ppm) as a performance enhancer in the feed. The Southern division used 50-PPM Bacitracin in the starter feed, 25-PPM Bacitracin in the grower feed and 10-PPM Virginiamycin in the finisher feed. The results are as follows in 6 paired flocks, labelled A-F

Figure 5. %-Samples Salmonella-positive Flavomycin-2 vs. BMD50/BMD25/Virg10

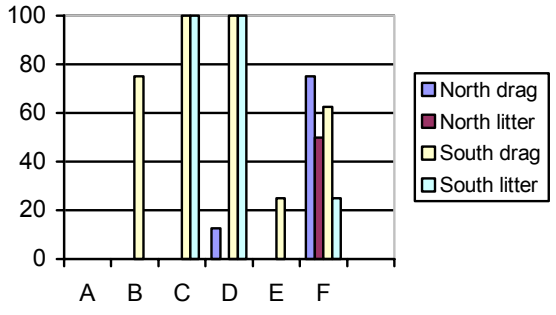


Table 4. Bacterial counts (cfu/g intestinal content)

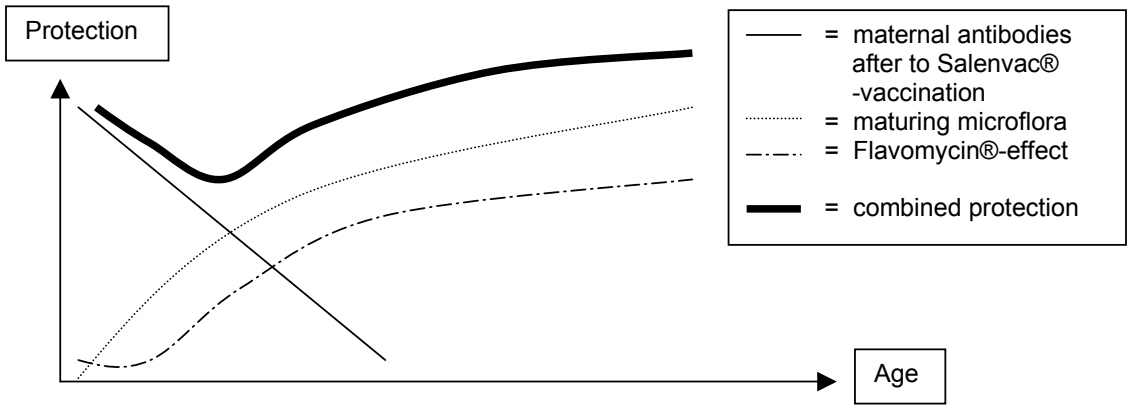
	Flavomycin-2	BMD50/BMD25/ Virg10
E.coli	229.000	506.500
Lactobacilli	16.115.000	3.685.000
Bifidobacteria	33.100.000	11.400.000
Cl.perfringens	590	10.130
%birds Salm.-positive	18%	50%

These results show clearly that Flavomycin® is an interesting extra instrument to decrease the spread of Salmonella during broiler production. Flavomycin® has a neutral effect on the equilibrium in the intestinal flora, e.g. Lactobacilli, and will result in an unaffected production of lactic acid. Other antibiotic performance enhancers can have a devastating effect on this equilibrium and on the production on lactic acid. They reduce the birds' natural defenses like the Competitive Exclusion mechanism and the bactericidal effect of lactic acid.
These are also signals that the use of certain antibiotic performance enhancers may (partly) responsible for the sometimes-unpredictable results of Competitive Exclusion.

Combination of Salenvac® and Flavomycin®

It is possible to combine the use of Salenvac® at breeder level with the use of Flavomycin® in broiler feed. This concept will combine the benefits of both. The offspring of vaccinated breeders will start uninfected and will be protected by maternally derived antibodies for ca. 21 days. An existing infection pressure on a broiler farm will be countered by the beneficial production of lactic acid that will not be depressed by the inclusion of Flavomycin® in the feed. This mechanism will act as a second barrier against infection during the period that the protection by the maternal antibodies is absent. It is known that due to the Competitive Exclusion of the normal intestinal flora the infectious dose necessary for an Salmonella infection decreases with increasing age. As Flavomycin® will not affect the equilibrium in the intestinal flora, the natural defense of the Competitive Exclusion mechanism will be the third barrier against Salmonella-infection of the broilers. In figure 6 an idea is given how these protective mechanisms can be used in combination and can provide a useful concept for further development.

Figure 6. Different protective mechanisms, that can be used in a Salmonella Control Program



Conclusions

- In all countries where it is licensed, Salenvac® has proven to be a major instrument to reduce the spread of *S.enteritidis* in poultry
  1. Vaccinated breeder birds are given protection against an infection
  2. The offspring of vaccinated breeders is protected till 21 days of age by maternally derived antibodies
  3. Salenvac® will be most effective in a Salmonella Control Program in which strict hygiene and monitoring are other important and effective components.
  4. From the field it is observed that Salenvac®-vaccinated breeder flocks perform better than unvaccinated flocks. These benefits are observed at breeder level, hatchery level and at broiler level. These benefits more than compensate the cost for vaccination.
- Competitive Exclusion can be a very helpful instrument to further reduce the spread of Salmonella. However it should be noted that
  1. The normal intestinal flora of every bird can act as a natural Competitive Exclusion defense. Certain antibiotic performance enhancers can negatively interfere with this natural mechanism as they may destroy the equilibrium that is responsible for the CE-effect.
  2. Flavomycin® has proven to have to not disturb this equilibrium.
  3. Laboratory and field studies show that inclusion of Flavomycin® in the broiler feed can be an interesting instrument to reduce the spread of Salmonella during broiler production.
  4. The treatment of S.e.-positive breeder flocks with a combination of a specified antibiotic and a C.E.-product should be restricted to incidental situations. As a preventive instrument vaccination of breeder flocks should be preferred.
- A combination of all possible preventive instruments should be used to reduce the risk of Salmonella-infection originating from the poultry.