Multi-hurdle feed additive approach prevents Salmonella

A multi-hurdle feed additive concept to control Salmonella in poultry looks promising. Combinations of organic acids, MCFA, butyrate and mannobiose appear to significantly reduce caecal *S. enteritidis*. The principle is based on combining additives that control multiplication, block attachment, and prevent invasion of Salmonella in the intestinal tract of broilers.

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The European Union has set clear targets to combat *Salmonella spp*. in poultry, and EU regulations have been implemented in the egg and broiler production chain, or will be implemented from 31 December 2011 onwards (*Table 1*). Surveys by the European Food Safety Authority (EFSA) indicate that Salmonella prevalence varies significantly between countries. It will be a challenge to meet the EU demands to combat Salmonella, with much effort required from the poultry industry.

Infection in broilers

The aetiology of birds becoming Salmonella-positive has been studied extensively. Birds may be contaminated at hatch, after ingestion of contaminated feed or water, or become infected via other environmental routes. Once ingested, Salmonella may colonise the gastrointestinal tract, and invasive Salmonella types such as *enteritidis, hadar, infantis, typhimurium, virchow, java*, and *gallinarum* may invade the body and migrate to organs. Salmonella can spread from infected birds to the rest of the flock via the excreta.

Although strict biosecurity measures can significantly lower the risk of contamination, it is not realistic that this will lead to its complete eradication. Additional measures, such as the use of feed additives, will help to prevent feed contamination, or to prevent infected birds from contaminating the rest of the flock.

Controlling multiplication with fatty acids

The antimicrobial properties of organic acids can be utilised in feed additive concepts for the control of Salmonella. Their mode of action can be explained by the decrease of environmental pH and by the ability of these acids to pass the cell membrane where they dissociate and disturb the metabolism of the microbe. Acid treatment of feed with organic acids, such as formic and propionic acid, is applied to reduce the risk of Salmonella entering the gastrointestinal tract via feed or the feeding system. Formic acid, in particular, has potent antibacterial properties to control Salmonella. The acids in the feed will also have a bacteriostatic effect on growth of Salmonella in the crop of the bird, but their effect on colonisation in the caeca seems to be inconsistent. Medium-chain fatty acids (MCFA, C6 to C12) are also effective in inhibiting growth of Salmonella. Supplementation of broiler feed with MCFA reduced caecal colonisation of Salmonella which, besides growth inhibition, can be partly explained by down-regulation of virulence genes. From a mode of action point of view, organic acids and MCFA could be complementary for the control of Salmonella in the gastrointestinal tract.

Blocking attachment of Salmonella

Attachment of Salmonella to intestinal epithelium is an important step in the proliferation, colonisation and systemic persistence of Salmonella in the animal. Inhibition of adhesion to intestinal epithelium is therefore effective as one element in the "multi-hurdle" approach. The mechanism of this adhesion process is now largely understood. An important adhesion mechanism is the mannose-

dependent or mannose-sensitive adhesion through Type 1 fimbriae. The attachment of Salmonella to enterocytes can be studied *in vitro*. Nutreco recently used an in vitro model in which the adhesion inhibition of *Salmonella spp*. to a monolayer of a defined pig jejunal enterocyte cell line, denoted as IPEC-J2 cells, was studied. The aim of this study was to estimate the adhesion-inhibiting effects of some natural compounds containing mannose, mannobiose, galactomannans and manno-proteins, against a blanco culture medium control. The results of this *in vitro* test confirmed that the mannose-dependent adhesion mechanism of *S. enteritidis* can be inhibited substantially (50-80%) by natural mannose-containing feed substances (*Figure 1*). Mannose and mannobiose effectively inhibited adhesion *in vitro*, whereas the commercially available galactomannan preparation and yeast cell wall sources were less or not effective. *In vivo*, however, mannobiose will have a higher efficacy than mannose. Mannose is absorbed by the small intestinal tract and a relatively low proportion of the ingested mannose will reach the caeca. In contrast, β-1,4 mannobiose is an indigestible disaccharide that will act as a Salmonella blocker throughout the intestinal tract, including the caeca (*Figure 2*).

Prevent invasion with butyrate and MCFA

Butyrate and MCFA are able to down-regulate the pathogenicity of Salmonella, thereby reducing Salmonella colonisation of caeca and internal organs. It was demonstrated by others that Salmonella exposed to butyrate and MCFA *in vitro* had lower expression of the virulence genes *hilA* and *fimA* that determine the capability of Salmonella to become invasive. Birds challenged with Salmonella and fed feeds supplemented with butyrate and MCFA indeed had lower caecal and organ Salmonella counts than control animals, which significantly reduced the risk of transmission of Salmonella from infected to non-infected birds. Coated butyrate proved to be more effective than the non-coated form, probably because of higher butyrate concentrations in the caecal contents.

Table 1 - EU regulations implemented in the egg and broiler production chain, or implemented from the 31 December 2011

Egg chain	Legislation	Reduction target
Eggs	EC 2073/2005	Salmonella-free consumption eggs by 01-01-2009
Laying hens	EC 1168/2006	Max 1% positive flocks by 31 Dec 2011 Annual minimum percentage reduction of positive flocks *S. enteritidis & S. typhimurium* dependent of prevalence
Broiler chain	**Legislation**	**Reduction target**
Broiler meat	EC 2073/2005	Salmonella: Absence in 25 g in poultry meat by 01-01-2011
Broilers	EC 646/2007	Maximum 1% positive flocks by 31 Dec 2011 *S. enteritidis & S. typhimurium*
Poultry breeding flocks	EC 1003/2005	Maximum 1% positive flocks by 31 Dec 2009 *S. enteritidis, hadar, infantis, typhimurium & virchow*

Figure 1 – Percent of *S. enteritidis* adhesion inhibition vs. blanco culture medium in an IPEC-J2 cell culture. The test substances were D (+) mannose (reference), mannobiose of plant origin (MB), a botanical source of galactomannans (GM) and two yeast based manno-protein products (YCW-1 & 2). (Source: WUR Livestock Research, Lelystad, the Netherlands, 2010)

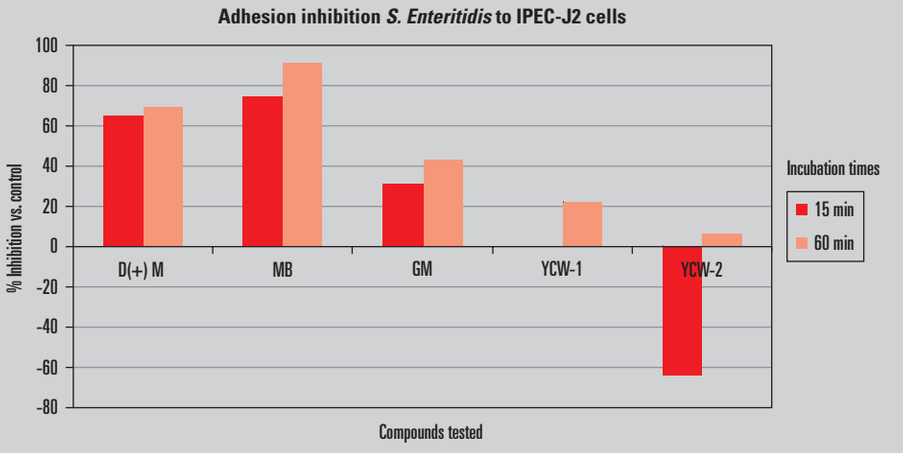


Figure 2 - Faecal *S. enteritidis* counts from 3 groups of 20 chicks fed either the control diet or supplemented diets containing 0.1% D-mannose or 0.1% β1-4 mannobiose. Chicks were inoculated orally with $2x10^7$ cfu SE/ml and shedding was monitored up to 19 days post infection. (Source: Agunos et al, 2007)

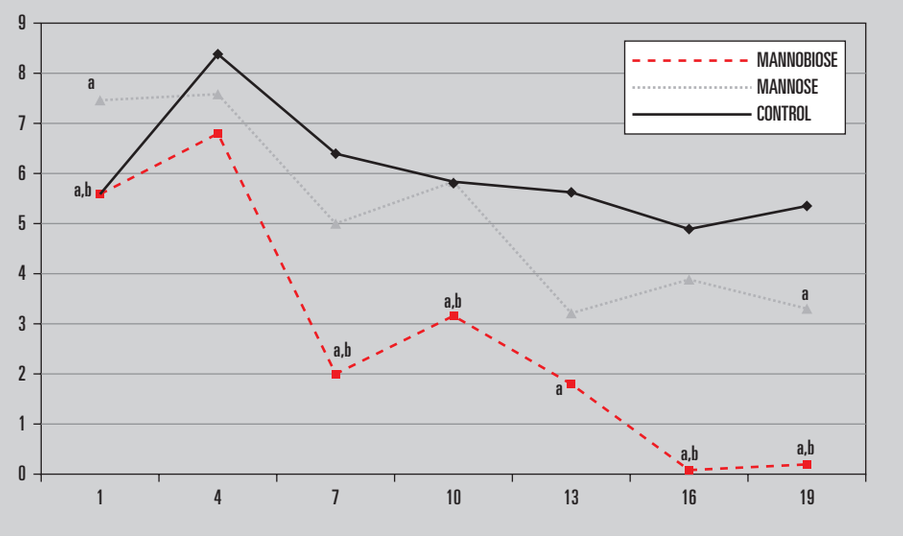


Figure 3 - Salmonella status of broilers fed diets with Mannobiose (0.1%), a commercial galactomannan premix (0.1%), with or without coated butyrate (0.1%) at 35 days of age. Birds were infected at day 7 and 8 with 10^3 *Salmonella enteriditis*. (Source: Nutreco PRRC, Corujo, 2009)

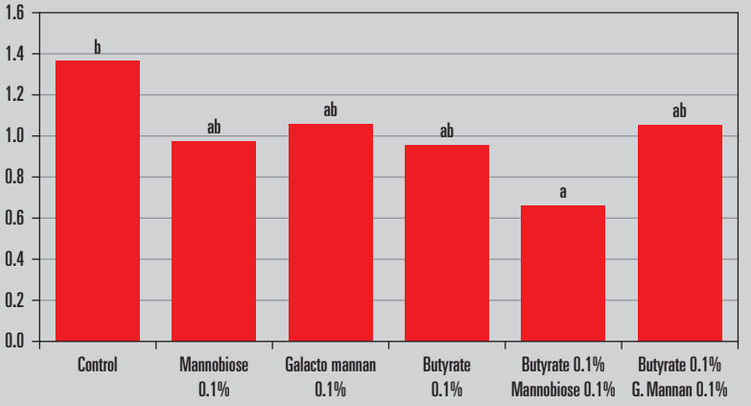
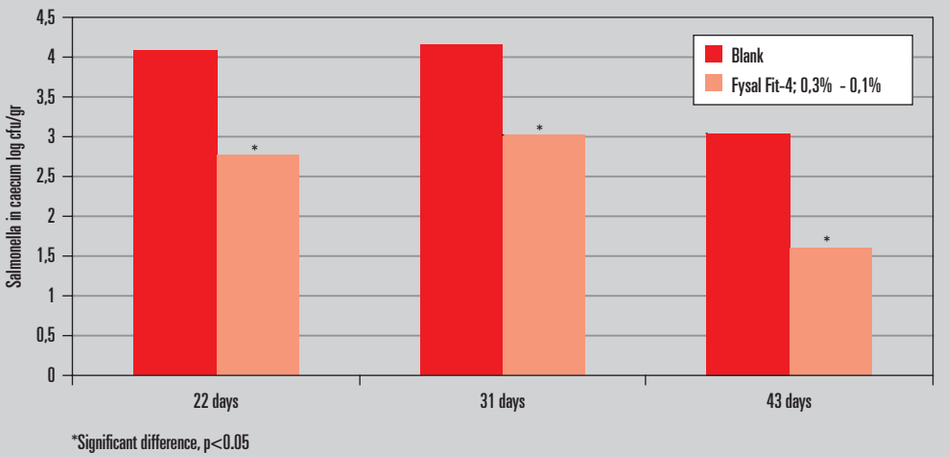


Figure 4 - Salmonella counts at day 22, 31 and 43 in caecum of broilers fed diets with a blend of organic acids, MCFA, butyrate and mannobiose (Fysal Fit-4) at a level of 0.30% from 0-21 days and 0.10% from 22-42 days. Birds were infected (*S. enteritidis*) with 1 ml inoculum (10^6 cfu/g) at days 7 and 8.



*Significant difference, p<0.05

Synergy of combinations

The differences in mode and site of action of specific organic acids, butyrate, MCFA and mannobiose offer opportunities for a "multi-hurdle" approach in which the growth, adhesion and invasion of Salmonella is inhibited. Organic acids and MCFAs have a bacteriostatic action on Salmonella in the crop and proximal intestinal tract. Mannobiose blocks the attachment of Salmonella to the gut wall and butyrate and MCFA decrease the risk that Salmonella will become invasive. Therefore, Nutreco recently tested the efficacy of combinations of these feed additives to decrease Salmonella colonisation in broilers. In the first study, broilers were fed a control diet, a diet that contained either 0.1% coated butyrate, 0.1% of a specific hydrolysed copra meal containing mannobiose, 0.1% of a commercial galactomannan preparation, or a combination of the latter two with butyrate. Birds were infected at days 7 and 8 with a 1 ml inoculum, containing 10^3 cfu/g *Salmonella enteritidis*. In total, 20 birds per treatment were randomly selected and sacrificed at day 28, and another 20 birds at day 35 of age. The caecal contents of two birds were pooled before determination of Salmonella counts.

Reduced colonisation

The combined use of coated butyrate and mannobiose significantly reduced the colonisation of *S. enteritidis* in the caeca of broilers, whereas the effect of the single components was not statistically significant (*Figure 3*). Synergistic effects of butyrate and mannobiose were demonstrated in the study, and that a "multi-hurdle" approach is valid.

The combination of butyrate with mannobiose formed the basis for a follow-up study in which the efficacy of an extended multi-hurdle concept was tested. In addition to butyrate and mannobiose, organic acids and MCFA were added to the feed additive combination. This supplement (Fysal Fit-4, Selko Feed Additives) was fed to broilers in a Salmonella challenge study at 0.3% from 0-21 days and at 0.1% from 21-42 days. Birds receiving this extended multi-hurdle treatment had significantly lower caecal Salmonella levels than the control group at 22, 31 and 43 days (*Figure 4*). Numerically, caecal Salmonella levels were reduced by 96% in birds treated with the extended multi-hurdle concept. These results indicate that this multi-hurdle concept is very effective in reducing intestinal colonisation of broilers with Salmonella.

Transmission study

In parallel, a transmission study was carried out. In this trial, at days 7 and 8, a total of 3 birds were orally inoculated with 10^6 cfu/ml of *S. enteritidis* and placed in a pen with non-inoculated birds. The transmission was studied by determining the Salmonella status in the caeca of the non-inoculated birds at day 22, 31 and 43. It was seen at these days that, compared to the control, respectively 50, 30 and 65% fewer birds receiving the multi-hurdle concept were contaminated with Salmonella, demonstrating the reduced risk of transmission. Although a number of the birds became infected in the multi-hurdle treatment groups, it should be kept in mind that the experimental set-up included an artificial challenge by birds inoculated with a relatively high level of Salmonella. In practical conditions, such a high infection pressure is not realistic, and control of Salmonella is likely to be even more successful.

References are available on request

MORE INFORMATION

- www.efsa.europa.eu
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