Newsletter

BIOMIN Newsletter Vol. 10, No. 117

> EDITORIAL

Food safety is one of the top priorities in animal production today. Salmonella is a cause of bacterial food-borne disease in humans, and can often be attributed to contaminated food products. It is estimated that around 2.6 %, 10.6 %, 17.0 % and 56.8 % of human salmonellosis cases can be attributed to turkeys, broilers, laying hens and pigs, respectively. On-farm Salmonella control is an important issue which is directly linked to regulatory limits at slaughter and public health concerns. Effective Salmonella control on the farm is based on the prevention of Salmonella entering and spreading in a farm.
Salmonella is a common component of the gut microflora of animals and thus, can be found in the faeces of affected animals. Faecal pollution is the main culprit for the contamination of feed and water with Salmonella. Animals, especially poultry and swine can also become infected and act as reservoirs of Salmonella. In order to ensure a high level of animal performance, farmers should pay close attention to farm management and Salmonella prevention. Regular testing and observing the critical points of the production chain are necessary for prohibiting Salmonella occurrence and contamination.

You will find useful information in the following pages about the use of organic acids as an important tool for the control of Salmonella.

Enjoy reading!

Nataliya Roth

Biotronic® product line

Bacteria of the genus *Salmonella* are Gram-negative, facultatively anaerobic, non-spore forming, usually motile rods belonging to the family *Enterobacteriaceae*. *Salmonella* serotype *Typhimurium* and *Salmonella* serotype Enteritidis are the most common serotypes found in humans, but many others have been shown to cause diseases. Therefore, *Salmonella* associated with human foodborne diseases must be controlled. One possible area of control is the inclusion of organic acids in feed and water, which reduces the number of undesirable micro-organisms. Organic acids inhibit bacterial growth by decreasing the pH of feed, water and the upper part of the intestinal tract, interfere with bacterial metabolism by decreasing the cytoplasmatic pH, as well as inhibit enzymatic action and DNA synthesis.

The use of acidifiers in controlling *Salmonella*

Points of *Salmonella* control

The points of *Salmonella* control include: breeder houses, feed raw materials and compound feed, water, farm environment, vermin control and prohibiting cross-contamination.

Breeder houses

There must be adequate *Salmonella* monitoring and control at the hatchery for poultry and breeder farms. Control starts with getting healthy young animals to the farm. On arrival, the chicks and piglets should be *Salmonella* free. Samples from transport equipment and faeces should be taken to determine the *Salmonella* status.

Feed

Salmonella spp. is one of the major hazards leading to the microbial contamination of animal feed. Animal-derived protein and oil seed meal

are the major sources of risk among feed materials through which *Salmonella* may be introduced to industrial compound feed and feed mills.

International regulations require that food and feed are free from *Salmonella*. Appropriate process control and sanitation steps are needed during feed processing to reduce the contamination of feedstuffs and avoid the dissemination of contaminated feed to herds. It has been demonstrated in experimental settings that animals can become infected by consuming *Salmonella* contaminated feed, which can be transferred to products of animal origin.

It is important to check all raw materials, especially cereals and protein sources, for *Salmonella* contamination. It is also important to know that *Salmonella* colonies are naturally unevenly distributed in feed and therefore, their detection and quantification can be difficult. Therefore, preventing the spread of *Salmonella* is a recommended tool for *Salmonella* control.
The heat treatment of feed is a common means of feed sanitation. However, it should be conducted appropriately in order to reduce bacteria counts, taking into account, in particular, temperature, duration and initial bacterial counts. Moreover, heat treatment does not protect feed from recontamination during transportation and storage, as shown in *Figure 1*.

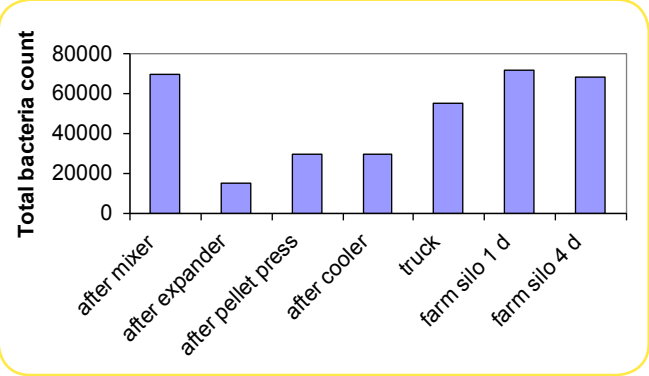

Figure 1. *Recontamination of feed without acidifier, Israelsen et al., 1996*

A multiple strategy encompassing heat and antimicrobial treatments, for instance, with organic acids, is required for the reduction of bacterial burden and improvement of feed hygiene. Acid treatments have a protective effect on feed, which reduces both the recontamination of feed as well as the contamination of milling and feeding equipment and the general environment. The efficacy of organic acids against *Salmonella* depends on the level of bacterial contamination.

Water

Salmonella can persist and grow in water given the right conditions. The diversity and concentration of *Salmonella* increases as temperatures rise. For better *Salmonella* control, the microbiological test of water is needed, especially if the source of water is a well or river. Water for animals should have the same high standards as that which is suitable for human consumption. The results of the two samples taken from the start and end points of the watering line, and the difference between them, gives a clear picture of whether improvements in water quality have to be made.

Water acidification can help prevent *Salmonella*. The supplementation of acids in drinking water reduces the pH level and bacterial counts. A very important feature of water acidification is the lowering of the pH level by adding acidifiers. Very often, farmers apply acids without knowing the pH level of the water. If the acidification is too strong, the pH level of the water goes below 4 and this has a negative impact on the equipment, water intake and growth performance of animals.

Farm hygiene and biosecurity

Contamination of the resident environment of animal housing can be a source of *Salmonella* infection. Keeping buildings clean and disinfecting farm equipment helps to minimize the danger of infection.
Improving farm personnel hygiene and the control of visitors are important factors for reducing the risk of *Salmonella*. Hand washing and disinfection as well as the cleaning of overalls and disinfection of boots before entering the stable are associated with decreased *Salmonella* prevalence. The relatively low cost incurred may be offset by the decreased transfer of other performance impairing pathogens.

Vertebrate and invertebrate species

Since all vertebrates are susceptible to *Salmonella* infection, contact with other species may pose an infection risk to animals. Pests (rodents, wild birds, and other wildlife species) have often been implicated as potential sources of *Salmonella*. It has been recognized that flies and beetles also serve as a potential reservoir and vectors for *Salmonella*. It is therefore important to ensure proper vermin and pest control on the farm.

Cross contamination

As animals have a lot of contact with each other, infections can spread very quickly. Aiming for small group sizes with minimal mixing of animals contributes a lot to the prevention of *Salmonella* spread. It is also important not to allow sick animals to return into the main production unit.

Salmonella prevention with natural feed additives

The addition of organic acids has been shown to contribute to environmental hygiene by preventing feed raw materials and compound feed from microbial and fungal deterioration. Moreover, it contributes to good farm management and interrupts the bacterial transmission in the animal-to-food chain. It is generally well-known that acids can reduce the number of *Salmonella* because of the reduction of the pH level and also because of their antimicrobial activity in the feed and gastrointestinal tract. The non-dissociated form of organic acids penetrates into the bacterial cell, prohibiting its replication. BIOMIN provides a family of products which combines synergistically acting organic acids, with each component strengthening the antimicrobial activity of the acids.

Reduction of *Salmonella* in feed

The *in-vitro* trial was conducted to determine the efficacy of an acidifier consisting of formic and propionic acids on Sequential Release Medium (SRM; Biotronic® SE forte) on *Salmonella* contamination in feed. Feed was artificially contaminated with a high level of *Salmonella* enteritidis. The metabolic activity of *Salmonella* is reduced in dry feed, which lowers their rate of replication. The feed was diluted with physiological saline solution and the cell count of *Salmonella* was determined right after the contamination, after one hour and 2.5 hours of incubation under optimal conditions. Results can be seen in *Figure 2*. *Salmonella* replication was steadily growing under optimal conditions while the number of *Salmonella* was decreased in the group containing the acidifier.

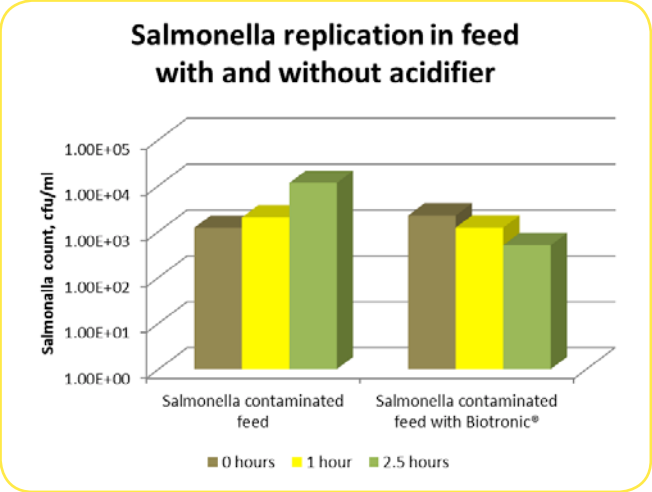

Figure 2. *Effect of acidification on Salmonella reduction in feed*

Reduction of *Salmonella* and *E. coli* in the gastrointestinal tract and its influence on animal performance

It has been shown that acid blends are even more effective than single acids in inhibiting pathogenic bacteria, due to their broader spectrum of activity compared to single acids. Combining acid blends with phytochemicals, which are in general defined as active-health compounds found in plants, could increase the effects of organic acids on the growth inhibition of pathogenic bacteria. It was shown in studies done *in vitro* that combining cinnamaldehyde with a blend of organic acids enhances the inhibition effects on the growth of pathogenic bacteria.
Gram-negative bacteria are surrounded by the outer membrane, which provides the bacteria with an inherent resistance to antimicrobial substances. Even if the outer membrane of the Gram-negative cell acts as a protective barrier for external agents, it is possible to weaken this barrier by using agents commonly characterised as permeabilisers.

Permeabilising substances act quite differently one from the other. Some remove stabilizing cations from the outer membrane, while others bind to the outer membrane, resulting in the loss of barrier function, etc. However, they all weaken the outer membrane of Gram-negative bacteria. This may increase the efficacy of other antimicrobials as it is easier for them to enter the cell and inhibit or destroy vital cellular functions. This means that when a permeabilising substance is added to a mixture of organic acids and a phytochemical, the effects on the inhibition of pathogenic bacteria by an organic acid-phytochemical might be enhanced by 10 to 70 %, depending on the bacteria strain.

A trial was carried out at the Research Center of Hunan Agricultural University in China, using 96 weaning pigs. Pigs (BW 10,5 kg; 35 days of age) were assigned to three treatments and fed commercial pig diets. The negative control group diet contained no feed additives, whereas the positive control group diet was supplemented with antibiotics (100 g/t Colistin and 100 g/t Chlortetracycline) and the diet of the trial group was supplemented with Biotronic® Top3 at the inclusion rate of 1.0 kg/t feed. The duration of the trial was 56 days. The weight and feed intake of the pigs were recorded at each diet change and feed conversion was calculated. Mortality and clinical symptoms for diarrhoea or other medical incidences were observed daily. At the end of the trial, the pigs were killed and the pH of digesta in the stomach and jejunum were measured. The content of the ileum was collected and analyzed for *E. coli*, *Salmonella*, *Lactobacilli* and *Bifidobacteria* populations.

Growth performance was improved due to the supplementation of the diets with the acidifier Biotronic® Top3. Body weight (BW), feed intake (FI) and daily weight gain (DWG) were significantly ($P<0.05$) improved at day 56, compared with the positive and negative control groups (*Table 1*).

Table 1. *Performance results*

	NC	PC	Biotronic® Top 3
No. animals	n = 32	n = 32	n = 32
Initial BW d35, kg	10.0	10.9	10.7
Final BW d91, kg	42.5[a]	43.2[a]	48.1[b]
FI, g/animal/day	1092[a]	1112[a]	1269[b]
DWG, g	580[a]	578[ab]	668[b]
FCR	1.88	1.92	1.90

[a,b] *Means with different superscripts differ significantly; P<0.05*

The pH in the stomach of pigs fed the negative and positive control diet were similar (4.28 vs. 4.30), while the pH in the stomach of pigs fed the diet supplemented with Biotronic® Top3 was significantly ($P<0.05$) lower compared with the stomach pH of pigs in the negative and positive control group. Also, the pH in the jejunum of pigs fed the diet supplemented with Biotronic® Top3 was lower

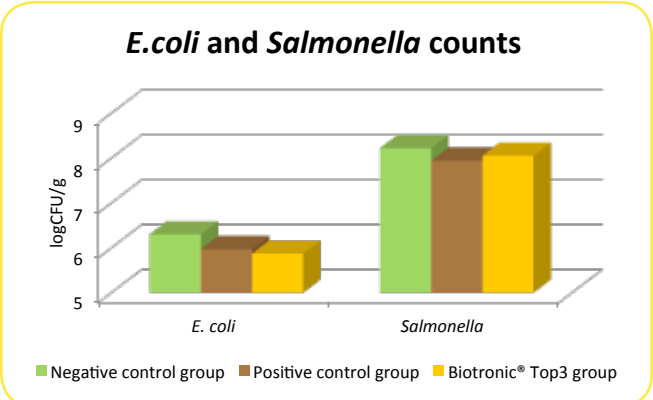

Figure 3. *E. coli and Salmonella counts (logCFU/g) in the ileum of pigs*

compared with the pH in the jejunum of pigs fed the negative and positive control diet. However, differences were not significant (P>0.05). *E. coli* counts found in the ileum of pigs fed the negative control diet were significantly higher (P<0.05) compared with *E. coli* counts found in the ileum of pigs fed the positive control and the Biotronic® Top3 diet (*Figure 2*). *Salmonella* counts in the ileum of pigs fed the negative control diet were significantly (P<0.05) higher compared to *Salmonella* counts found in the ileum of pigs fed the positive control and the Biotronic® Top3 diet.

Use of natural feed additives in artificial Salmonella challenge in broilers

The following trial shows the efficacy of natural feed additives in the challenge trial with *Salmonella* ser. Enteritidis. Eighty-four day-old broiler chicks (Ross) from the same origin were randomly divided into three treatment groups. The control group received no feed additives, whereas two trial groups received dietary supplementation with two different natural growth promoters. Trial group I received a diet supplementation with Biotronic® SE forte at inclusion levels of 3.0 kg per ton of feed. Trial group II received Biotronic® Top3 at an inclusion level of 1 kg per ton of feed.

At three days of age all chicks were orally inoculated, the challenged dose was 104 cfu/bird of Salmonella ser. Enteritidis. At 14 days post infection (dpi), the cecal content from 12 birds was taken and analyzed quantitatively and qualitatively for *Salmonella*.

At 11 dpi, the fecal samples were taken and analyzed qualitatively for *Salmonella*. The results of the present study showed that in the groups supplemented with the natural feed additives, *Salmonella* was neither detected in the quantitative and qualitative culture in cecal samples at 14 dpi nor in the qualitative culture in fecal samples at 11 dpi (*Table 2*).

Table 2. *Qualitative and quantitative Salmonella detection on 11 and 14 days post infection in cecal and fecal samples*

Groups	**Cecal samples** Salmonella positive/total 14 dpi	**Fecal samples** Salmonella positive/total 11 dpi
Control	2/12 (2.00 log10 cfu/g, 2.48 log10 cfu/g)	positive
Biotronic® SE forte	0/12	negative
Biotronic® Top3	0/12	negative

The present trial results are in accordance with scientific literature which has shown that organic acid blends are effective in preventing the cecal colonization of newly-hatched chicks by *Salmonella enterica* serovars Typhimurium, Enteritidis, Agona and Infantis (Iba & Berchieri Jr., 1995). This is consistent with previous findings by Hinton & Linton (1988), who reported that.

Conclusion

Salmonella control is key to preventing the introduction of *Salmonella* on the farm. Proper farm management, biosecurity measures, targeting small groups of animals and preventing the return of sick animals to the main production unit all contribute to the prevention of *Salmonella* spread.
Using natural feed additives in feed and water minimizes Salmonella infection and promotes gut health, thereby enhancing the performance of animals. Thus natural feed additives can be seen as one of management tool that improves animal growth performance and controls the growth of pathogenic bacteria such as *E. coli* and *Salmonella*, the main culprits in poultry and swine production. However, it cannot completely eliminate potential risk as several other factors such as management and diet also contribute to the occurrence of the disease.

References available upon request!

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MAG_NL117_BTR_EN_NRO

> IMPRESSUM
Newsletter is published by BIOMIN Holding GmbH. Editor: Natalyia Roth and Renata Urbaityte.
Industriestrasse 21, A-3130 Herzogenburg, Austria
Tel: +43 2782 803-0, Fax: +43 2782 803-40; e-Mail: office@biomin.net, www.biomin.net, Publisher: Erich Erber