Reducing colonisation of the intestinal tract by Salmonella enteritidis

Human illness due to salmonella continues to be reported globally. Many cases are associated with the consumption of contaminated poultry meat, including an incident in Brazil involving contamination of broiler meat. This has had a devastating effect on the country's export market. In the second part of 2018, the EU announced a ban on the importation of broiler meat from 20 Brazilian plants.

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In the United States, human outbreaks of salmonella associated with the consumption of raw turkey products surfaced during late 2018 and early 2019. Over 200 people were reported to have been affected.

Approximately 110,000kg of raw turkey meat was recalled due to potential contamination of several different raw turkey product lines. This cost turkey producers up to $1 million.

Salmonella entry points

The contamination of broiler or turkey meat by salmonella has several potential entry points. Salmonella strains are known to colonise the intestinal tract and internal organs like the liver.

Salmonella typhimurium, Salmonella enteritidis and Salmonella heidelberg are three of the most prevalent serovars historically implicated in human food poisoning events. Most often these strains do not cause disease in poultry. Yet humans can be severely affected with disease if these bacteria are consumed. The source of the salmonella in raw meat products can often be linked to contamination of the product during processing of the carcases.

In order to reduce the potential of salmonella contamination of meat products, it is essential to reduce both the number of birds carrying salmonella in their intestinal tract as well as the amount of salmonella present. This two-fold intervention strategy has proven to reduce product contamination as well as decrease potential internal organ infection. The reduction of salmonella contamination in egg products is equally important.

Several outbreaks of salmonella illness in humans were reported in Australia after the consumption of eggs. Some 28 cases of Salmonella enteritidis were reported throughout Australia in September 2018. An outbreak in March 2019 affected an undetermined number of people.

A Salmonella braenderup outbreak associated with the consumption of shell eggs in the United States resulted in illness of 45 people and over 17 million dozen eggs being recalled.

Since the 1980s the global commercial egg industry has been on the vanguard against Salmonella enteritidis (SE) contamination of table eggs. Many interventions have been used to mitigate the possibility of SE contaminating eggs for human consumption.

A well-researched, food safety intervention in broilers is the use of feed additive probiotics. Less is known of live production intervention strategies in commercial hens.

There are a couple of possible modes of entry of SE in commercial laying eggs:

- The first is direct contamination of the eggshell during egg laying. It is proposed that the SE penetrates from the exterior into the internal components of the egg during the cooling of the egg.
- The second route of entry is direct contamination of the egg albumin or yolk from an infected ovary.

Both modes of contamination involve the intestinal tract. This would indicate that a reduction in the population of salmonella in the intestinal tract would directly result in reduced risk of transmission into consumable eggs.

The research trial

Recently Chr. Hansen investigated the effect of the dietary use of Gallipro Fit and the resulting SE colonisation of the intestinal tract, most specifically colonisation of the caeca, where the highest concentration of salmonella is thought to harbour.

Gallipro Fit is the triple strain poultry probiotic selected to boost prevention programmes and contribute to food safety, while also delivering profitability. It is currently being commercially used in the United States, Australia and South Africa.

The probiotic contains two unique Bacillus subtilis strains and a Bacillus amyloliquefaciens strain. The recommended inclusion rate is 500g per metric ton of feed.

The trial was conducted in the US at the Southern Poultry Research Group facilities using 196 robust W-36 pullets. The birds were housed at 10 weeks of age, and allowed to acclimatise for one week.

At 11 weeks, 98 of the birds were fed Gallipro Fit at the 500g/ton equivalent level. The other 98 birds were fed the same ration formulation, only without the inclusion of Gallipro Fit. Six weeks after the birds had continuously consumed the feeds, they were orally challenged with a high level of a nalidixic-acid resistant Salmonella enteritidis.

It is proven that continuous consumption of the Gallipro Fit would influence the intestinal microbiome, ultimately influencing the ability of the SE to colonise the intestine.

The reason for using a nalidixic acid-resistant SE is in order for bacterial identification using a selective growing environment. This will distinguish the ability of a 'known' salmonella to colonise the intestine after the prescribed amount of time. Finally, an oral challenge at 17 weeks of

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age was used to investigate the susceptibility of the pullets after experiencing the stress of sexual maturation.

One and two weeks after the oral challenge with the SE, caecal samples were harvested from a respective number of the study animals. Two types of analysis were performed. The presence or absence of the nalidixic-acid resistant salmonella were determined from each caecal sample.

Assessing SE populations of infected caeca

The results of the prevalence of caeca populated with the nalidixic acid- resistant salmonella are shown in Fig. 1.

The results of the numerical populations of nalidixic acid-resistant SE in caeca positive for SE corresponded with the prevalence results. In birds known to be infected with SE, a significant reduction in the total number of SE colonising the caeca was detected from those pullets receiving Gallipro Fit in the diet.

The numbers were determined by the proven microtiter MPN (most probable number) technique of quantifying bacteria.

The average population of SE found in the caeca from birds fed Gallipro Fit was 7.76 SE bacteria per gram of caeca.

The average population of SE found in the birds not fed Gallipro Fit was 11.48 per gram of caeca. These results are the culmination of all 98 birds sampled per treatment group.

Although this reduction may look insignificant, the results are profound in that both aspects of SE colonisation were reduced. Both the number of birds colonised by the bacteria and the actual number of SE were reduced in birds fed diets containing Gallipro Fit.

Faecal shedding of SE

An additional analysis of the study was the utilisation of boot swabs. These samples were obtained by an individual walking under the cages. Samples were obtained at 16 weeks of age just before the oral infection of SE. All these boot samples were negative.

A second boot swab sampling was taken at 18 weeks of age. All 18 week samples were positive for salmonella. This indicates that the SE was being shed in the faeces and could serve as a source of bacteria and possibly contaminate eggs.

Commercial pullet and hen diets

Two major results were observed in this study.

- Less birds were colonised with salmonella in the group receiving a diet containing Gallipro Fit.
- In the group receiving a diet containing Gallipro Fit, the number of SE colonising the intestine was less than birds not receiving a diet containing the probiotic.

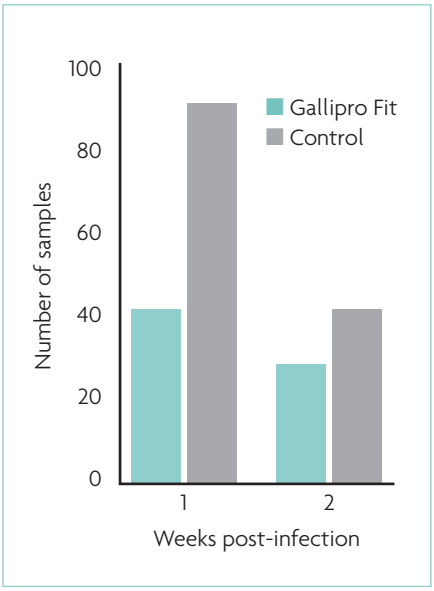

Fig. 1. Caecal samples positive for nalidixic acid-resistant SE at the respective ages.

The presence of a 100% detection rate for salmonella in the boot swabs indicates that the artificial challenge of the SE by oral inoculation sufficiently produced infection. That infection resulted in the contamination of faecal contents, which could ultimately provide a route of entry into the egg.

It is clear that the control of salmonella during the live production phase is a multi-factor effort. There is no one intervention that will provide 100% protection against the bacteria. Each intervention builds on the other to create a stronger barrier against entry.

In the seminal paper published in 1973 by Nurmi and Rantala, it was identified that the oral inoculation of young chicks with caecal contents from the mother hens, provided a reduction in the number of chicks susceptible to salmonella colonisation.

That paper did not go the extra step in numbering the salmonella in the intestinal tracts of those chicks.

The results of this trial support those early findings and build on the results by showing the additional reduction in caecal populations – all of which build on that barrier to prevent salmonella contamination of food products.

Gallipro Fit has shown properties of inhibiting salmonella growth in laboratory experiments. The purpose of this trial is to substantiate laboratory findings with live production results.

With this in mind, Gallipro Fit is a perfect natural mitigation tool toward food safety control programmes involving pathogens like salmonella. ■

References are available from the authors on request