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# The effect of diet on *Salmonella* survival in the bovine rumen and abomasum

## Key external stakeholders:
Beef farmers, beef processors, FSAI, DAFM and EFSA.

## Practical implications for stakeholders:
The aim of this study was to examine if diet could be used as a pre-harvest control strategy aimed at reducing the risk of *Salmonella* contamination at slaughter. This study concluded that dietary manipulation is not an effect means of reducing *Salmonella* carriage and shedding in beef animals.

## Main results:
Although the high grain diet resulted in significantly higher volatile fatty acid (VFA) concentrations in the rumen, overall diet did not affect *Salmonella* survival in the rumen, abomasum nor the faeces. Dietary manipulation is not an effective strategy for reducing Salmonella carriage and shedding in cattle.

## Opportunity / Benefit:
The hypothesis that diet may be manipulated to reduce *Salmonella* carriage and shedding in cattle thereby reducing carcass contamination levels, protecting public health, exports, etc. was tested in this project and shown to be ineffective. The results are available for interested parties.

## Collaborating Institutions:
UCD



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## 1. Project background:

There are an estimated 20,000 cases of salmonellosis in Ireland every year. Implicated foods include beef products. *Salmonella* carriage in cattle is asymptomatic and it is not possible to remove infected animals from the food chain before slaughter. Effective pre-harvest interventions to reduce *Salmonella* contamination along the beef chain culminating in fewer human infections.
For *Salmonella* to be excreted in bovine faeces it must first pass through the bovine gastrointestinal tract including passage through the rumen and abomasum. The VFAs in the rumen and low pH conditions in the abomasum should eliminate or reduce *Salmonella* thereby limiting passage into the hindgut from where they are excreted in the faeces. However, some studies suggest that low VFA concentrations in the rumen, as affected by diet, may reduce/eliminate *Salmonella*.

## 2. Questions addressed by the project:

- How does bovine diet effect rumen VFA concentration and pH?
- How does rumen VFA concentration and pH effect survival?
- Do the VFAs in the rumen trigger the acid tolerance response?
- If triggered, does this acid tolerance response facilitate survival through the abomasums?
- Would a high grain diet produce high levels of VFA resulting in the elimination/reduction of *Salmonella*?

## 3. The experimental studies:

Five lactating Holstein cows fitted with rumen fistulas were fed one of five different diets including grass; grass and concentrate; grass silage; hay and a high grain diet for 16 days. Cows fed diets 1 and 2 were kept outdoors and had uninterrupted access to their diet. Cows fed diets 3, 4 and 5 were housed indoors and fed once daily. All of the cows had free access to water. After 14 days acclimatisation to the diets, approximately 1 l of rumen fluid was collected through the fistulas twice daily at the same time (morning and afternoon) on day 15 and 16. The dietary treatment was repeated three times and feeding of the different diets was rotated so that no cow was fed the same diet twice. Rumen fluid harvested from the three treatments for each diet was pooled prior to use in the experiment. Each rumen pool was gauze-filtered and used undiluted. The pH of the harvested rumen fluids were measured immediately after collection. Samples were frozen, transported to the laboratory in cool boxes containing ice packs and stored at -20ºC until required. Prior to use, the samples were thawed at 4ºC overnight. The volatile fatty acid (VFA) concentration of the pooled rumen fluid from each diet was measured by gas liquid chromatography (GLC).

The *in vitro* survival of non-acid and acid adapted *Salmonella* cocktails (*Salmonella* serovars; Dublin, Enteritidis, Newport, Typhimurium and Typhimurium DT104) in rumen fluid and faeces, was examined at 6°C and 15ºC (faeces) and at 37ºC (rumen fluid). The harvested rumen fluid form each diet was also inoculated with non-acid and acid adapted 5-strain *Salmonella* cocktails. After 24 h incubation period, *Salmonella* were acid challenged to synthetic abomasum fluid (pH 2.5) for 5 h to determine their resistance to low pH.

## 4. Main results:

The main results were as follows:

- *Salmonella* survives in rumen fluid for up to 21 days, and in faeces for up to 84 days.
- *Salmonella* survival was not influenced by diet
- *Salmonella* does not survive in synthetic abomasum fluid although a high grain diet may increase the bacterial 'fitness' to survive the harsh conditions in the abomasum.
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## 5. Opportunity/Benefit:

This project evaluated dietary manipulation as a *Salmonella* control activity in cattle. The results clearly demonstrate that such a strategy would not be effective which should inform food safety regulatory policy (EFSA, FSAI & DAFM) as well as beef producers.



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## 6. Dissemination:

The information generated was however widely disseminated through oral and poster presentations at workshops (Centre for Foodborne Zoonomics workshop, UCD, 2008; Teagasc Agricultural Research Forum, Tullamore, 2009 and at the Relay workshop 'Pathogens of animal origin-implications for the food chain' Ashtown, 2011) and conferences (ProSafeBeef conference Ashtown, 2009 and at the Foodborne Zoonomics International Conference, Ashtown, 2009).

## Main publications:

Lenahan, M., Kelly, S., Fanning, S. and Bolton, D. J. (2010) The effect of bovine diet on Salmonella survival in synthetic abomasum fluid. *Journal of Applied Microbiology*, 109 (6), 2060-2068.

Bolton, D. J., Kelly, S., Lenahan, M. and Fanning, S. (2011) In vitro studies on the effect of pH and volatile fatty acid (VFA) concentration, as influenced by diet, on the survival of inoculated non-acid and acid adapted Salmonella in bovine rumen fluid and faeces. *Foodborne Pathogens and Disease*, 8(5), 609-614.

Rajtak, U., Boland, F., Leonard, N., Bolton, D. J. and Fanning, S. (2011) The role of diet and acid tolerance response on the survival of common *Salmonella* serotypes in faeces of finishing pigs. *Applied and Environmental Microbiology* 78 (1), 110-119.

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