



Food Institutional Research Measure

Final Report

'Marine compounds to enhance productivity and health in pigs (Sea-guard)

DAFM Project Reference No: 11/FP/403

Start date: 01/11/2012

End Date: 1/3/2014

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Please place one "x" below in the appropriate area on the research continuum where you feel this project fits

Basic/Fundamental			→	Applied		→	Pre Commercial	
1	2	3	4	5 X	6	7		

Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report;

Priority Area (s)	Sustainable Food Production
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Key words: (max 4)

Alternatives to antibiotics, pigs,

1. Rationale for Undertaking the Research

Post-weaning colibacillosis is the most common intestinal disorder of piglets in the immediate post-weaning period. Associated reduced feed intake, weight loss, dehydration, and death are common. In the past, these problems have been managed in a controlled fashion by the use of in feed antibiotics (Williams et al., 2001). Recent research (De Lange et al., 2010) showed that in feed antibiotics increased pig growth rate (from weaning to 30 kg) by +11% and improved feed efficiency by 3.4%. The use of these growth promoters have been banned in EU since 1st January 2006. Currently, there is no alternative available to infeed anti-biotics that gives the same level of animal performance. It is imperative that alternative strategies are developed to replace 'in-feed' antibiotics in pig and poultry diets. The outcome of this ban has been the growth in the use of large amounts of therapeutic and prescription antibiotics (De Lange et al., 2010). This is the worst possible outcome from a human health perspective and has arisen because of a lack of alternatives to antibiotics. Any good alternative must give a similar response to the use of the infeed antibiotics.

Salmonella spp. is regarded as one of the most important bacterial causes of acute, food-borne disease in humans. Salmonella intervention methods can be made through general diet formulation or the addition of expensive additives. Heat treatment can reduce microbial loads in feed materials and compound feed. Heat treatment for more than 30 seconds at more than 75°C can achieve a 1000 fold reduction of salmonella. However, the risk of recontamination is substantial and a lot of home mixers do not have heat treatment facilities available to them. Organic acids, such as formic, acetic and propionic acids have also been shown to have an inhibitory effect on salmonella growth. However, the results are very variable (Sweeney et al., 2011, funded in RSF 06 326). Currently, there is no good alternative method available on farms to eliminate salmonella infection in pigs.

2. Research Approach

This was a multidisciplinary project including animal nutritionists and molecular biologists from UCD. In this project we investigated the enhancement of the immune system of the early neonatal pig through maternal sources. In task 1, we investigated the effect of feeding laminarin to sows and how it enhances the piglet's immune response while suckling. Following weaning we experimentally challenged the weaned pigs with enterotoxigenic E.coli and examined its effect on intestinal health and performance. Sixty hybrid sows of mixed parity were fed a basal gestation and lactation diet or a basal gestation and lactation diet supplemented with 1 g seaweed extract/d (SWE) from day 107 of gestation until weaning (day 20). The SWE was extracted from *Laminaria* spp. and sourced from Bioatlantis Ltd. (Kerry Technology Park, Tralee Co. Kerry, Ireland). At weaning four piglets per litter were selected for the ETEC challenge. Piglets remained in the same treatment group as defined by their dams. They were subdivided into 4 treatment groups. Lactation diet (LT) and the post-weaning diet (PW) defined these treatment groups. The groups were arranged as follows: (1) BB (basal fed sows - basal fed pigs); (2) BS (basal fed sows – SWE supplemented pigs); (3) SB (SWE supplemented sows – basal fed pigs), (4) SS (SWE supplemented sows – SWE supplemented pigs). Individual feed intakes, weight gain and faecal scores for consistency were measured during the post-weaning period. At day 10 post-weaning, the ETEC was orally administered at a dose of 10⁸ CFU/mL. The pigs were checked for diarrhoea to evaluate their status before and after challenging with ETEC K88. Severity of diarrhoea was characterised by using the faecal consistency score system 1) hard firm faeces; 2) slightly soft faeces; 3) soft, partially formed faeces; 4) loose, semi-liquid faeces (mild diarrhoea); and 5) watery, mucous-like faeces (severe diarrhoea). Faeces were scored for consistency at challenge and six, twelve, 24, 36 and 48 hours afterwards. At day 12 post-weaning, pigs were humanely sacrificed. Samples for microbiological, morphological and immunological analysis were collected at slaughter and gut pH was assessed.

In task 2, we investigated the effect of feeding laminarin to sows while suckling. Following weaning an experimental challenge with *Salmonella* Typhimurium was investigated. Forty eight hybrid sows of mixed parity were fed a basal gestation and lactation diet or a basal gestation and lactation diet supplemented with 1 g seaweed extract/d (SWE) from day 107 of gestation until weaning (day 20). The SWE was extracted from *Laminaria* spp. and sourced from Bioatlantis Ltd (Kerry Technology Park, Tralee Co. Kerry, Ireland).

At weaning, four piglets per litter were selected. Pigs remained in treatment groups as defined by their dams. Pigs were subdivided into two groups of two pigs so that resulted in four experimental groups. Two factors, lactation diet (LT) and post-weaning diet (PW), were arranged in a 2 x 2 factorial design to provide 4 treatment groups as follows: (1) BB (basal sows - basal pigs); (2) BS (basal fed sows – SWE supplemented pigs); (3) SB (SWE supplemented sows - basal pigs); (4) SS (SWE supplemented sows – SWE supplemented pigs).

At day 14 post-weaning, the *Salmonella* Typhimurium challenge was orally administered at a dose of 10^8 CFU/mL. Feed intakes, weight gain and faecal scores for consistency were measured during the post-infection period. Faecal samples were collected 5 days prior to *Salmonella* challenge and on days 2, 4, 7, 14 and 21 post-infection. Animals were humanely sacrificed on day 21 and digesta samples were taken from the caecum, colon and the rectum to quantify *Bifidobacteria*, *Lactobacilli* and *Salmonella* spp. present at each sample site, as well as volatile fatty acids. Samples from the caecal, mesenteric and ileo-caecal lymph node, tonsils, liver, spleen and carcass samples were analysed to quantify the *Salmonella* Typhimurium contamination after slaughter.

3. Research Achievements/Results

Task 1. The results from this task indicate that dietary laminarin supplementation reduced faecal Enterobacteriaceae numbers in sows at parturition and was accompanied by a reduced colonic *E. coli* population in piglets at weaning. The results demonstrate that laminarin supplementation during lactation influence growth performance of pigs. Pigs weaned from laminarin supplemented sows had a greater ADG compared with pigs weaned from non- laminarin extract supplemented sows.

Task 2: All pigs were experimentally challenged with *Salmonella* Typhimurium. A maternal supplementing effect is seen on the *Salmonella* spp. abundance on the overall post-challenge period. Maternal supplementation of laminarin resulted in a decreased overall faecal abundance of *Salmonella* spp.

4. Impact of the Research

Task 1

The results from this task indicate that dietary laminarin supplementation reduced faecal Enterobacteriaceae numbers in sows at parturition and was accompanied by a reduced colonic *E. coli* population in piglets at weaning. The results demonstrate an important immunomodulatory role of maternal laminarin supplementation on piglet digestive health and selected intestinal microflora at weaning that may help alleviate the negative impact of an ETEC challenge at weaning. The results also demonstrate that laminarin supplementation during lactation influence growth performance of pigs. Pigs weaned from SWE-supplemented sows had a greater ADG compared with pigs weaned from non-SWE supplemented sows post weaning.

Task 2

The results demonstrate an important immunomodulatory role of maternal laminarin supplementation on piglet digestive health and selected intestinal microflora at weaning that may help alleviate the negative impact of salmonella challenge at weaning. A maternal supplementing effect is seen on the *Salmonella* spp. abundance on the overall post-challenge period. Maternal supplementation of laminarin resulted in a decreased overall faecal abundance of *Salmonella* spp.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

Based on this work, UCD was part of a Horizon 2020 funding application (Oceans4 health) which was submitted in March 2014. This application was led by a Norwegian consortium. However it was unsuccessful and was not funded.

(ii) Outcomes where new products, technologies and processes were developed and/or adopted

These results provide new insights into the protective activity of maternal laminarin supplementation, making it a good alternative for improving the health status of pigs with ETEC and salmonella associated disease.

(iii) Outcomes with economic potential

This research proves that laminarin is effective in protecting pigs when challenged by pathogens such as *E.coli*. and salmonella and that maternal application of laminarin can enhance the immune system of the piglets and protect them when challenged by pathogens such as *E.coli* and salmonella. It also proves that that pigs can be reared economically without the use of infeed growth promoting antibiotics.

(iv) Outcomes with national/ policy/social/environmental potential

We have discovered a replacement for in feed antibiotics

4 (b) Summary of Research Outputs

(i) Peer-reviewed publications, International Journal/Book chapters.

We have nothing published yet. However we do expect 2 publishable papers from this work. They are in preparation

Bouwhuis, M.A., Sweeney, T., McDonnell, M. and J.V. O' Doherty
Effects of maternal feeding of laminarin on performance and intestinal microflora of weaned piglets experimentally challenged with Salmonella Typhimurium. (Submitted to Journal of Animal Science)

Bouwhuis, M.A., Sweeney, T., McDonnell, M. and J.V. O' Doherty

Effects of maternal feeding of laminarin on performance and intestinal microflora of weaned piglets experimentally challenged with enterotoxigenic E.coli (Submitted to Journal of Animal Science)

(iii) Popular non-scientific publications and abstracts including those presented at conferences

We intend to present two papers from the work at the Digestive physiology in pigs meeting in Poland (May 2015).

Bouwhuis, M.A., Sweeney, T., McDonnell, M. and J.V. O' Doherty
Effects of maternal feeding of laminarin on performance and intestinal microflora of weaned piglets experimentally challenged with Salmonella Typhimurium.

Bouwhuis, M.A., Sweeney, T., McDonnell, M. and J.V. O' Doherty
Effects of maternal feeding of laminarin on performance and intestinal microflora of weaned piglets experimentally challenged with enterotoxigenic E.coli.

(iv) National Report
None

(v) Workshops/seminars at which results were presented

Prof O Doherty spoke at Irish pig Health society meeting in Mullingar in April 2014. His talk was based partly on this work.

(v) Intellectual Property applications/licences/patents

This project confirms the results obtained from a previous Stimulus research project (RSF 06 636). A patent was granted from RSF 06 636, based on the results of this work entitled "Improvement of gastrointestinal health, immunity and performance by dietary". European Patent Application Number: 06809743.5, US Patent Application Number 12094383, PCT 2506 May 2009. This work on sea weed extracts has been licensed by UCD to Bioatlantis Ltd, Tralee, Co Kerry. These extracts are now classified as feed materials under EU guidelines and are approved for sale in the EU. Bioatlantis are now beginning to market these sea weed extracts as feed ingredients.

(vi) Other

5. Scientists trained by Project

Total Number of PhD theses: 0

Total Number of Masters theses: 1 MSc student (Meike Bouwhuis)

This work has become part of a PHD thesis. Meike Bouwhuis initially registered for an MSc. However after completing this project, she continued on for a PhD. She will submit her PHD in December 2015. This work will make up 2 chapters in her PhD thesis.

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
UCD	2	0.2
Total	2	0.2

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers		
PhD students		
Masters students	1	1
Temporary researchers		
Research assistant	1	0.5
Total	2	1.5

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended
N/A	

9. Project Expenditure

Total expenditure of the project: €99,614.98

Total Award by DAFM: €99,387

Other sources of funding including benefit in kind and/or cash contribution(specify): €0

Breakdown of Total Expenditure

Category	UCD	Name Institution 2	Name Institution 3	Name Institution 4	Total
Contract staff	19329.77				
Temporary staff					
Post doctorates					
Post graduates	25000				
Consumables	32001.14				
Travel and subsistence	296.00				
Sub total	76626.91				
Durable equipment					
Other					
Overheads	22988.07				
Total	99,614.98				

10. Leveraging

Based on this work, UCD was part of a Horizon 2020 funding application (Odens4 health) which was submitted in March 2014. This application was led by a Norwegian consortium. However, it was unsuccessful and was not funded.

11. Future Strategies

This work on sea weed extracts has been licensed by UCD to Bioatlantis Ltd, Tralee, Co Kerry. These extracts are now classified as feed materials under EU guidelines and are approved for sale in the EU. Bioatlantis Ltd has begun marketing these feed ingredients to pig producers/millers in Europe.