The use of seaweed extracts in pig diets as an alternative to in-feed medication

Professor John O’ Doherty,
School of Agriculture and Food Science,
University College Dublin.
Concerns grow over farm drugs used like 'sweets'

By Matt McGrath
Environment correspondent, BBC News
Weaning

- Loss of protective maternal milk antibodies
- Change in diet from digestible milk proteins and CHO to solid feed with complex nutrients
- Rise in cortisol due to social stress factors
- Villus atrophy, reducing nutrient absorption and allowing nutrients to pass down to the colon
- Inflammation
- Allow proliferation of *E.coli*, *Salmonella* etc. that produce toxins
- Diarrhoea, decreased feed intake and growth
Antibiotic growth promoters (AGP)

Traditional measures → ameliorate weaning associated intestinal dysfunctions

- Reduce pathogenic bacteria
- Improve feed intake and growth rate

In-feed AGP → development of antibiotic resistance

In-feed ZnO → accumulation of Zn in the environment
### Danish research on feed additives (Piglets 7-30 kg BW)

<table>
<thead>
<tr>
<th>Additive Type</th>
<th>No of studies</th>
<th>% change in daily gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td>15</td>
<td>+11</td>
</tr>
<tr>
<td>Organic acids</td>
<td>40</td>
<td>+7.1</td>
</tr>
<tr>
<td>Aromatic compounds</td>
<td>19</td>
<td>+2.6</td>
</tr>
<tr>
<td>Enzymes</td>
<td>9</td>
<td>+2.1</td>
</tr>
<tr>
<td>Microbial cultures</td>
<td>14</td>
<td>+1.0</td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>2</td>
<td>+3.1</td>
</tr>
</tbody>
</table>

Search continues for sustainable alternatives (Thacker et al., 2013)
Overall Research Objective

To identify and characterize reliable natural alternatives to replace antimicrobial growth promoters and Zinc Oxide during weaning in the piglet.
Novel sugars from seaweed

• Seaweed supplementation

Minerals, vitamins, fatty acids, laminarin, fucoidan, alginites, tannins, phenols, etc
UCD pipeline to define bioactive compounds

Anti-inflammatory hits

In-vitro screen
Caco 2 cell lines

Ex-vivo screen

In-vivo screen
### Results *(In-vitro, Caco 2 cell lines)*

<table>
<thead>
<tr>
<th>Species</th>
<th>Extract</th>
<th>IL8 conc.(pg/ml)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>121.0</td>
<td>-</td>
</tr>
<tr>
<td><em>F. serratus</em></td>
<td>CW</td>
<td>73.9</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>HW</td>
<td>92.6</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td>EW</td>
<td>50.3</td>
<td>0.001</td>
</tr>
<tr>
<td><em>F. spiralis</em></td>
<td>CW</td>
<td>71.9</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>HW</td>
<td>44.5</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>EW</td>
<td>40.5</td>
<td>0.001</td>
</tr>
<tr>
<td><em>F. vesiculosus</em></td>
<td>CW</td>
<td>25.8</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>HW</td>
<td>107.9</td>
<td>0.454</td>
</tr>
<tr>
<td></td>
<td>EW</td>
<td>33.2</td>
<td>0.001</td>
</tr>
<tr>
<td><em>A. nodosum</em></td>
<td>CW</td>
<td>58.9</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>HW</td>
<td>36.7</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>EW</td>
<td>42.7</td>
<td>0.001</td>
</tr>
<tr>
<td><em>P. palmata</em></td>
<td>CW</td>
<td><strong>208.2</strong></td>
<td>0.001</td>
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<tr>
<td></td>
<td>HW</td>
<td><strong>261.2</strong></td>
<td>0.001</td>
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<tr>
<td></td>
<td>EW</td>
<td><strong>172.8</strong></td>
<td>0.005</td>
</tr>
</tbody>
</table>

*(Bahar et al., 2012 JAS)*
Ex vivo: IL8 gene expression

Relative gene expression

- F. serratus
- A. nodosum
- F. spiralis
- F. vesiculosus

Cold
Hot
Ethanol

NS

P < 0.001

(Bahar et al., 2012 JAS)
Effect of crude Asc Nod seaweed extracts on *E. coli* populations

![Graph showing the effect of crude Asc Nod seaweed extracts on *E. coli* populations. The graph displays the log10 cfu/ml digesta across different treatments: 0 g/kg, 3 g/kg, 6 g/kg, and 9 g/kg. The treatment with 6 g/kg shows a 1 log reduction in *E. coli* populations compared to the control (0 g/kg).](image)

SWE  P <0.05

(Gardiner et al., 2008)
Effect of crude Asc Nod seaweed extracts on daily gain

Due to high content of **alginites and tannins**

(Gardiner et al., 2008)
Effect of crude seaweed extracts* on daily gain in weaned pigs

Due to high content of alginites and mannitol
Lead to the search for bioactive compounds

* includes laminarin, fucoidan, alginate and mannitol
Laminarin

- water soluble polysaccharides
- low molecular weight (5kDa)
- composed of β-(1-3) linked glucans with β-(1-6) linked side chains of various distribution and length

Properties:
- Anti-inflammatory (Sweeney et al., 2012)
- antibacterial (McDonnell et al., 2010)
Fucoidan

- Water soluble polysaccharides
- Molecular weight 40-1400 kDa
- $\alpha(1-3)$ linkages, fucose sulphated at position 4

**Properties:**
- antiviral (Damonte et al., 2004)
- immunomodulatory (Sweeney et al., 2012)
- antibacterial (Walsh et al., 2013)
- potential prebiotic agents (Lynch et al., 2009)
Objective 1

Can supplementation of the piglet diet with laminarin or fucoidan at weaning influence subsequent performance and health of the piglet?
Effect of laminarin and fucoidan on average daily gain (d 0-35 pw)

**Laminarin**

- Control
- 150 ppm
- 300 ppm (P<0.05)

**Fucoidan**

- Control
- 240 ppm

~14% (Walsh et al., 2013 BJN)
The effect was lost when laminarin and fucoidan were combined (gain to feed ratio)

Lam*Fuc: P<0.05

What is the mode of Action?

(Walsh et al., 2013)
The effect was lost when laminarin and fucoidan were combined

faecal score d0-8

Log of AEEC gene copy number/g of digesta

(Walsh et al., 2013)
Duodenal morphology (villous height)

~18%

~20%

(Walsh et al., 2013)
Colonic inflammatory gene expression

(Walsh et al., 2013)
Carbohydrate & fatty acid transport

Lumen

Glucose Galactose

Fructose

Long chain fatty acids

Epithelial cell

Apical membrane

Glucose Galactose Fructose

Basolateral membrane

Basement membrane

Interstitial fluid

Blood

Enterocyte

SGLT1

GLUT1

GLUT7

GLUT5

CD36

FABP2

Tight junction
Laminarin increases the expression of glucose transporters

**SGLT1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mRNA Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.896</td>
</tr>
<tr>
<td>FUC</td>
<td>1.153</td>
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<tr>
<td>LAM</td>
<td>1.687</td>
</tr>
<tr>
<td>LAM + FUC</td>
<td>1</td>
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</table>

* Significantly different from control (p < 0.05)

**GLUT1 and GLUT2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mRNA Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.034</td>
</tr>
<tr>
<td>FUC</td>
<td>1.283</td>
</tr>
<tr>
<td>LAM</td>
<td>2.147</td>
</tr>
<tr>
<td>LAM + FUC</td>
<td>1.043</td>
</tr>
</tbody>
</table>

* Significantly different from control (p < 0.05)

(Heim et al., 2014 BJN)
Nutrient digestibility

Gross energy

Nitrogen

(* < P 0.05)

(Heim et al., 2014)
Objective 2
To compare the effects of laminarin to ZnO at weaning on performance and health of the piglet
Average daily gain (0-28d)

* P< 0.05

Control: 280 g/day
LAM: 353 g/day
ZnO: 308 g/day

(Heim et al., 2014)
Ability of Sea weed* extracts to reduce *Salmonella.* Typhimurium in finisher pigs.

* 340 ppm fucoidan, 180 ppm laminarin
Salmonella Typhimurium in colonic digesta

hilA in Colon, 17 days later

1 log reduction

(Bouwhuis et al., 2016 Animal)
Can sow nutrition influence lifetime performance and health of the pig

Most research to date has focused on feeding the young animal to enhance its own immune system.

Another less researched novel approach is to enhance the immune system of the young via maternal colostrum and milk.
Objective 3
Can maternal supplementation with SWE* or fish oil influence lifetime performance and health of the piglet?

Day 109 of gestation  birth  weaning

Supplementation diet

No Supplementation

SWE

Fish oils

SWE/Fish oil

*laminarin and fucoidan
Colostral IgG concentrations (2 hours post farrowing)

(Leonard et al., 2010 JAS)
Piglet serum IgG conc. (d 5)

Maternal SWE supplementation

Maternal FO supplementation

~21%

(Leonard et al., 2010 JAS)
Enterobacteria in sow faeces and piglet digesta at weaning

Sow faeces

Piglet colonic digesta

(Log10 cfu/ml digesta)

Basal

SWE

Basal

SWE

Treatment

Treatment

(Leonard et al., 2010 JAS)

(* P<0.05)
Caecal *E. coli* (9 dpw)

FO x SWE P < 0.05

(Leonard et al., 2010 BJN)
Overall daily gain (0 – 28 dpw)

Maternal SWE supplementation

Maternal FO supplementation

SWE P<0.05

NS P>0.05

~13%

(Leonard et al., 2010 BJN)
Objective 4
Can maternal supplementation with SWE influence the response of the piglet to an ETEC (K88 challenge)?
Effect of maternal dietary and ETEC challenge (0-72 hr)

Sow x ETEC P = 0.03
a,b P<0.05

(Heim et al., 2014)
Meat Quality Problems
Treatments

T1: Basal diet (20 ppm Vit E)

T2: Basal diet + 200ppm Vitamin E

T3: Basal diet + Seaweed extracts* (Raujaria et al., 2016)

* 340 ppm fucoidan, 180 ppm laminarin

(Raujaria et al., 2016)
Microbiological analysis (Total Viability Counts)

Storage time (days at 4ºC)

Log\(\text{cfu/g}^*\)

- Control
- SWE
- Vitamin E
Effect of seaweed extract on lipid oxidation

Lipid oxidation, TBARS

Storage time (days at 4°C) vs. Lipid oxidation inhibition (µg MDA equivalent)

Control, SWE, and Vit E compared.
Antioxidant analysis

DPPH scavenging assay

Storage time (days at 4°C)
The pig as a model for digestive health in humans

Similarities;
1. Anatomy
2. Gut microbiota
3. Gut size
4. Inflammatory mediators
Inflammatory bowel disease;

- Inflammatory bowel disease (IBD) - a group of debilitating conditions affecting the gastrointestinal tract
- Incidence of IBD is increasing in adults and children
- Pathogenesis involves immune cell infiltrate, denudation of the lumen mucosa, epithelial disruption, lesions, and oedema.
- Symptoms include weight loss, lethargy, diarrhoea, abdominal pain, bloody stool

• Dextran Sodium Sulphate model with the pig
*IL-6* gene expression in the proximal colon

(O Shea et al., 2016)
Spectrum of pathology in proximal colon transverse section stained with haematoxylin and eosin (10x)

<table>
<thead>
<tr>
<th>Normal</th>
<th>Normal with reduced cell infiltrate</th>
<th>Focal evidence of UC</th>
<th>Diffuse UC</th>
</tr>
</thead>
</table>

![Pathology images](image1.png)

Pathology score in the proximal colon:

<table>
<thead>
<tr>
<th>pathology score</th>
<th>Control</th>
<th>Control + DSS</th>
<th>FUC + DSS</th>
<th>LAM+DSS</th>
<th>LAMFUC + DSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1) normal with reduced cell infiltrate
2) normal
3) focal UC
4) multi-focal UC
5) diffuse UC

(O Shea et al., 2016)
Conclusions

• Seaweed extracts have a variety of biological properties that support gut health comparable to ZnO

• The seaweed species, method of extraction and characteristics of the extract are fundamental, as well as pig maturity

• Supplementing the maternal diet with SWE through the latter part of gestation and lactation provides the best benefit to the offspring post-weaning
Acknowledgements

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THANKING YOU FOR YOUR ATTENTION

“God made the world, sea weed made the field” Bull McCabe
### Results (*In-vitro*, mouse 3T3-L1 cell)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Description</th>
<th>Anti-adipogenic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISCG 032</td>
<td>Fucus serratus</td>
<td>Yes</td>
</tr>
<tr>
<td>ISCG 033-1</td>
<td>Alaria esculanta</td>
<td>No</td>
</tr>
<tr>
<td>ISCG 033-2</td>
<td>Alaria esculanta</td>
<td>No</td>
</tr>
<tr>
<td>ISCG 023</td>
<td>Ascophyllum nodosum</td>
<td>Yes</td>
</tr>
<tr>
<td>ISCG 041</td>
<td>Chondrus crispus</td>
<td>No</td>
</tr>
<tr>
<td>ISCG xxx</td>
<td>Chitosan</td>
<td>-</td>
</tr>
<tr>
<td>ISCG xxx</td>
<td>Chitosan- crabshell</td>
<td>-</td>
</tr>
<tr>
<td>ISCG 081</td>
<td>Chitosan- prawn shell</td>
<td>-</td>
</tr>
<tr>
<td>ISCG 067</td>
<td>Ascophyllum nodosum</td>
<td>Yes</td>
</tr>
<tr>
<td>ISCG 068</td>
<td>Fucus serratus</td>
<td>Yes</td>
</tr>
<tr>
<td>ISCG 072</td>
<td>Fucus vesiculosus</td>
<td>Yes</td>
</tr>
<tr>
<td>UL1-UL16</td>
<td>Macro-algal protein extract</td>
<td>No</td>
</tr>
</tbody>
</table>