USE OF ANTIBACTERIAL PLANTS AS FEED ADDITIVE TO REDUCE DIARRHEA IN WEANING PIGLETS

Project: MAFFRA
Multicomponent antibacterial feed additives to prevent piglet post-weaning diarrhea
Organic RDD2 (ICROFS- GUDP)
Start 1/1 2016 – end 31/12 2017

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What is the problem? (Background)

- Gastro-intestinal diseases and weaning diarrhea represent ethical and economic challenges in organic pig production reducing animal welfare, decreasing growth and with high mortality.

- A high use of antibiotics in pig production may induce bacterial resistance and result in unwanted accumulation of zink and copper in agricultural soils. Zink is phased out and political wishes of reducing use of antibiotics to pigs.

- Natural alternatives to antibiotics, zink and copper is of interest - especially in organics.

- Weaning diarrhea is associated primarily with pathogenic *E. coli* (F4, F18).

- In 2 previous projects (BERRYMEAT and REPLACE) a number of plant species were shown to have antibacterial activity against a.o. *E.coli* in laboratory in vitro tests.

- The hypothesis was that these may be used as a preventive feed additive and contribute to reduce weaning diarrhea and thus reduce the use of antibiotics, zink and copper.
What was the purpose and content of the project?

• Identify and cultivate food plant species and cv’s with antibacterial effect.
• Process into dry, milled and stable powders (not-extract) – as possible feed additive
• Characterize content of main antibacterial compounds
• Investigate inhibition effect *in vitro* against *E. coli* (F4 og F18) og *C. perfringens*
• Investigate single plants and plant combinations (possible ‘cocktail effects’))
• Point out the most promising combinations to be tested in a future challenge trial
• The preliminary effect in vivo on *E. coli* in GI tract in a pilot experiment
## PLANT MATERIAL

Cultivated/collected – known origin
Chemical characterization of antibacterial compounds

<table>
<thead>
<tr>
<th>Species: Danish name</th>
<th>Latin name</th>
<th>Plant part</th>
<th>Primary antibacterial compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peberrod</td>
<td>Armoracia rusticana</td>
<td>roots</td>
<td>isothiocyanate (120 mg/g (allyl isothiocyanate))</td>
</tr>
<tr>
<td>Rabarber</td>
<td>Rheum rhabarbarum</td>
<td>stems/petioles</td>
<td>phenolics, oxalic acid (pH 3.2)</td>
</tr>
<tr>
<td>Ramsløg</td>
<td>Allium ursinum</td>
<td>bulbs</td>
<td>allicin (5 - 11 mg/ g DM)</td>
</tr>
<tr>
<td>Ribs</td>
<td>Ribes rubrum</td>
<td>berries</td>
<td>organic acids (pH 3).</td>
</tr>
<tr>
<td>Tyttebær</td>
<td>Vaccinium vitis-idaea</td>
<td>berries</td>
<td>phenols, benzoic acid, organic acids (pH 2.7)</td>
</tr>
<tr>
<td>Oregano</td>
<td>Origanum vulgare ssp. hirtum</td>
<td>shoots and leaves</td>
<td>carvacrol and thymol (1.3 % E. oil)</td>
</tr>
<tr>
<td>Salvie</td>
<td>Salvia officinalis</td>
<td>shoots and leaves</td>
<td>kamfer and thujon (1 % E. oil)</td>
</tr>
<tr>
<td>Sommersar</td>
<td>Satureja hortensis</td>
<td>shoots and leaves</td>
<td>carvacrol, terpinene (1.4 % E. oil)</td>
</tr>
<tr>
<td>Rosmarin</td>
<td>Romarinus officinalis</td>
<td>shoots and leaves</td>
<td>kamfer, pinene, eucalyptol (2.5% oil)</td>
</tr>
<tr>
<td>Sort sennep</td>
<td>Brassica nigra</td>
<td>shoots and leaves</td>
<td>isothiocyanates (180 mg/g allyl isothiocyanate)</td>
</tr>
<tr>
<td>Humle</td>
<td>Humulus lupulis</td>
<td>flower buds</td>
<td>phenolics, alfa and beta acids</td>
</tr>
<tr>
<td>Vintereg</td>
<td>Quercus petrea</td>
<td>fruits/acorns</td>
<td>phenolics, tannins</td>
</tr>
</tbody>
</table>

Knowledge on chemical concentrations as a basis for correct and safe dose
IN VITRO ANTIMICROBIAL TEST

Stomach- or small intestine content from slaughtered pigs
Incubation with enterotoxigenic *E. coli* (F4 og F18) and plant material in different concentrations and combinations

Incubation in water bath (37°C, 4 hours)

Live counts (CFU) of *E. coli* on MacConkey agar plates
**E. coli** F4 growth/survival in small intestine content (pH 6.6)

- **No/minimal effect:**
  - Control (pH 6.6)
  - 1% Redcurrants (pH 5.7)
  - 1% Lingonberries (pH 5.7)

- **Bacteriostatic effect:**
  - 5% Redcurrants (pH 4.7)
  - 5% Lingonberries (pH 4.5)
  - 0.5% Ramsons + 0.5% Redcurrant (pH 6.4)
  - 0.5% Ramsons + 0.5% Lingonberries (pH 6.4)

- **Bactericidal effect:**
  - 1% Ramsons (pH 6.6)
  - 5% Ramsons (pH 6.6)

**Synergistic effect...?**

*Graph showing the growth/survival of E. coli F4 in small intestine content with different treatments and pH values.*
E. coli F4 survival in stomach content (pH 4.4)

- Control (pH 4.4)
- 1% Redcurrants (pH 4.2)
- 1% Lingonberries (pH 4.1)
- 1% Ramsons (pH 4.4)
- 1% Ramsløg + 1% Redcurrants (pH 4.2)
- 5% Ramsons (pH 4.5)
- 1% Ramsons + 1% Lingonberries (pH 4.1)
- 5% Red currants (pH 3.9)
- 5% Lingonberries (pH 3.7)

Synergistic effect...!
What have we observed \textit{in vitro}...?

- Ramsons (freeze dried powder, concentrations 1-5%) have very strong antimicrobial effect \textit{in vitro} against enterotoxigenic \textit{E. coli} (F4 and F18)

- Acidic berries, in concentrations (e.g. 5%) that can decrease pH $< 4$, also show strong antimicrobial effects

- Other tested species were observed to show less or no effect

- A mixture of ramsons and acidic berries show synergistic effect and can, in relatively low doses, inhibit \textit{E. coli in vitro} in pig stomach and small intestine content

- Ramsons alone or in combination with acidic berries looks promising

See further website for the MAFFRA-project: \url{http://icrofs.dk/forskning/dansk-forskning/organic-rdd-22/maffra/}
In vivo pilot trial

- Purpose:
  - Will the pigs eat it?
  - Will the plant powders reduce coli bacteria in the GI-tract?
In vivo pilot trial

- **Treatments:**
  - **Control:** standard pig feed
  - **Plants:** standard pig feed + 3% **ramsons** + 3% **lingonberries**
    - 16 g ramsons powder (allicin 9.7 mg/g DM)/piglet/day
    - 16 g lingonberry powder (pH 2.7) /piglet/day

- **Pigs:**
  - 8 per treatment
  - Weaned at 4 weeks of age
  - Trial start at 5 weeks of age
**In vivo pilot trial**

**Protocol:**

- **Weaning (4 weeks)**
- **Day 0**
- **Day 7**
- **Day 13**
- **Day 14+15**

Feces Feces Feces Slaughter
**In vivo** pilot trial (feces)

### Coli bacteria

- **Control**
- **Planter**

![Diagram showing the log cfu/g feces over days for Coli bacteria with comparison between Control and Planter groups.](Diagram)

### Lactic acid bacteria

- **Control**
- **Planter**

![Diagram showing the log cfu/g feces over days for Lactic acid bacteria with comparison between Control and Planter groups.](Diagram)
**In vivo pilot trial (digesta)**

St: Stomach  Si: Small intestine  Ce: Cecum  Co: Colon
In vivo pilot trial (digesta)

St: Stomach    Si: Small intestine    Ce: Cecum    Co: Colon
CONCLUSION IN VIVO PILOT TRIAL

- Ramsons and lingonberries (3% + 3% in feed) reduced coliform bacteria in the gastrointestinal tract of weaned piglets without reducing lactic acid bacteria
- Piglet feed intake was NOT influenced negatively
NEXT STEP

➢ Will the approach reduce diarrhea...? Challenge trial needed.

➢ Will the approach work under organic production conditions (e.g. 7 weeks weaning age, organic feed, etc.)...? Applied demonstration needed.

➢ How low can we go in plant powder dosage and still obtain sufficient prevention effect...? And how can we ensure reproducibility?

➢ Further research in the plant material is crucial in relation to
  ➢ Low cost and adequate supply of plants– possible use of side-streams with similar bioactive components
  ➢ Optimize cultivation adapted to use as feed additive
  ➢ Minimize costs related to processing and formulation

➢ Economic sustainability of use in pig production