DIFFERENTIATED AND BIOFUNCTIONAL FOODS DEPARTMENT OF FOOD SCIENCE
IMMUNOLOGY AND MICROBIOLOGY, DEPARTMENT OF ANIMAL SCIENCE

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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NORFEED A/S

ICROFS Organic RDD

Miljø- og Fødevareministeriet gudp

AARHUS UNIVERSITY
SCIENCE AND TECHNOLOGY

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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.

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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- ekstract) – 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

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PLANT MATERIAL

Cultivated/collected – known origin
Chemical characterization of antibacterial compounds

FREEZE DRIED MILLED PLANT POWDERS STORED AT -20°C.

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

Knowledge on chemical concentrations as a basis for correct and safe dose

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IN VITRO ANTIMICROBIAL TEST

Stomach- or small intestine content from slaughtered pigs

Incubation with enteroxigenic *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

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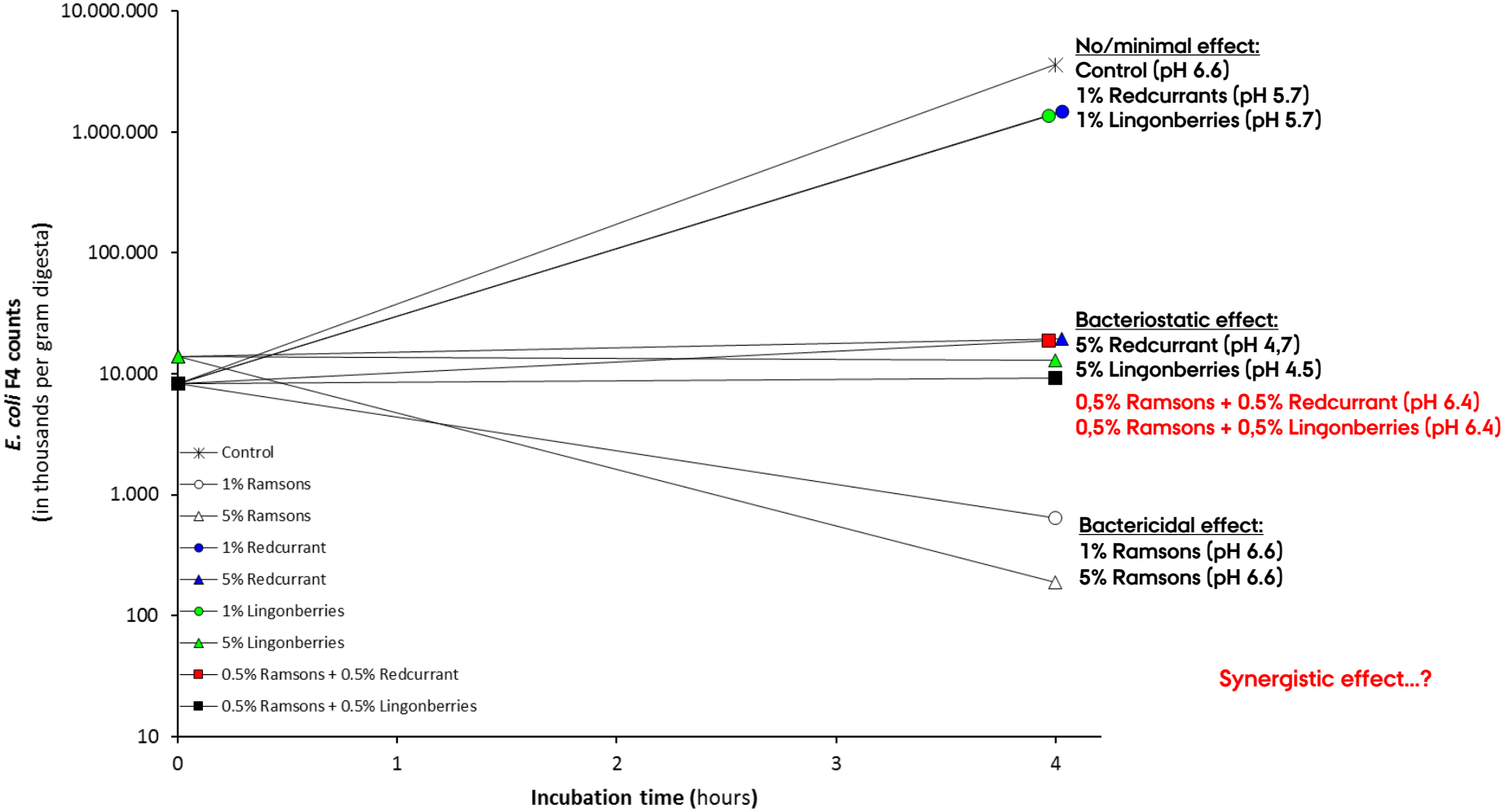
E. coli F4 growth/survival in small intestine content (pH 6.6)



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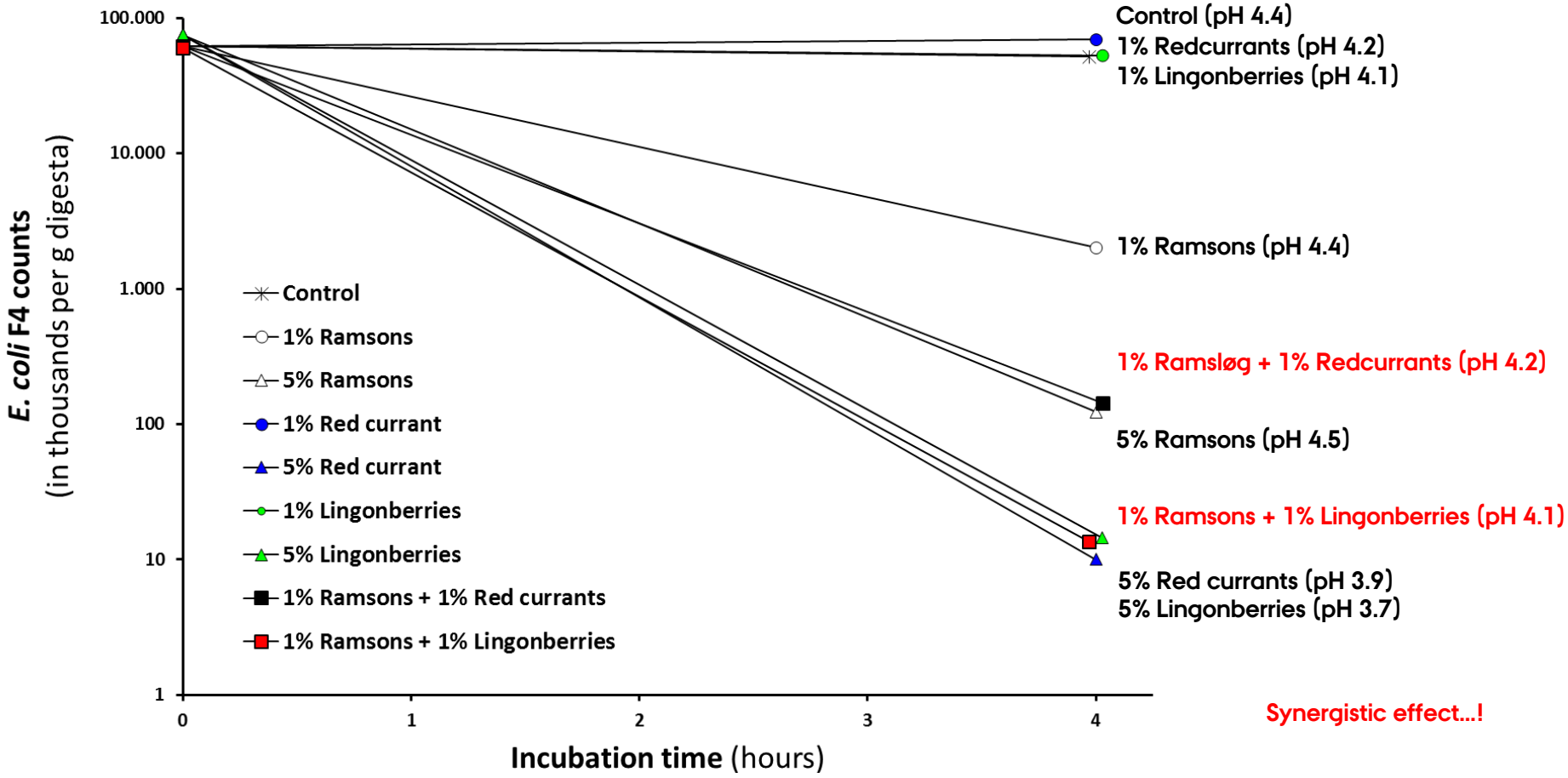
E. coli F4 survival in stomach content (pH 4.4)



Synergistic effect...!

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What have we observed *in vitro*...?

- Ramsons (freeze dried powder, concentrations 1-5%) have very strong antimicrobial effect *in vitro* against enterotoxigenic *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 *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: http://icrofs.dk/forskning/dansk-forskning/organic-rdd-22/maffra/

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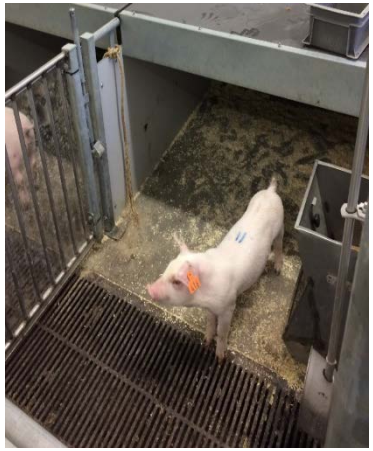
In vivo pilot trial

❑ Purpose:

- will the pigs eat it?
- Will the plant powders reduce coli bacteria in the GI-tract?



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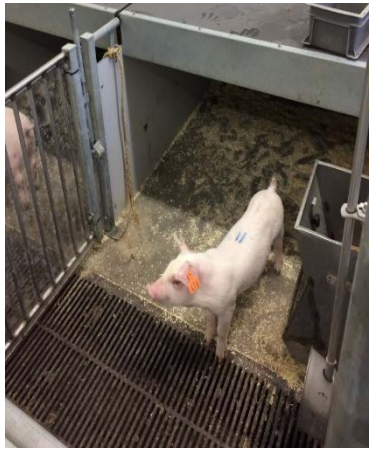
In vivo pilot trial

- **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

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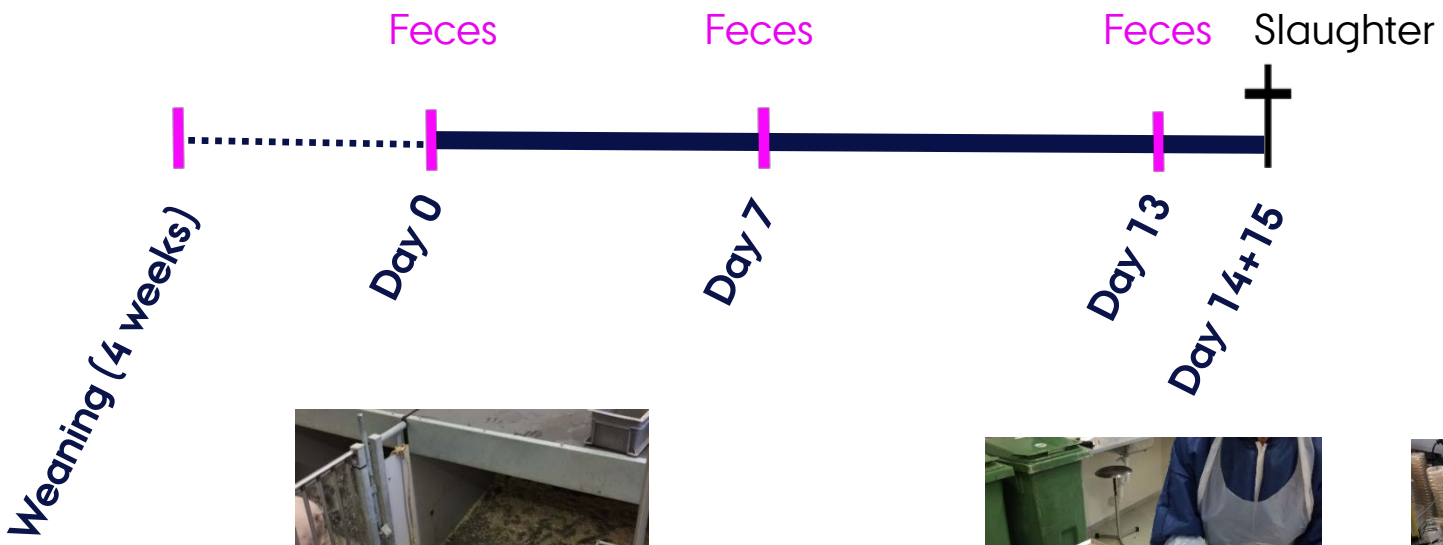
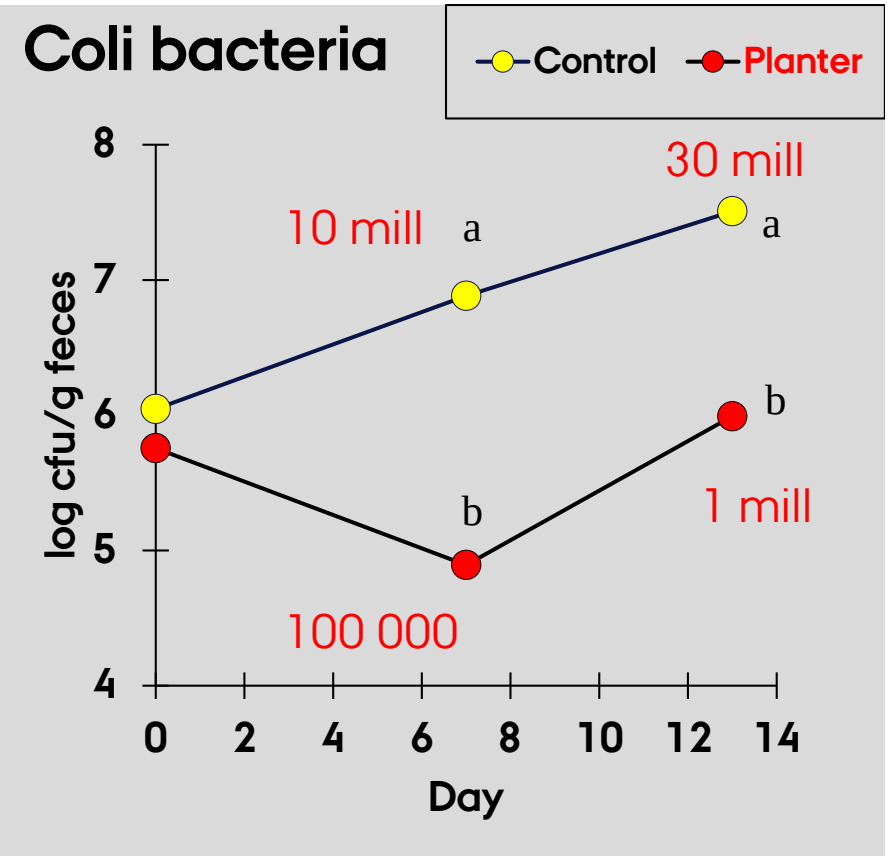
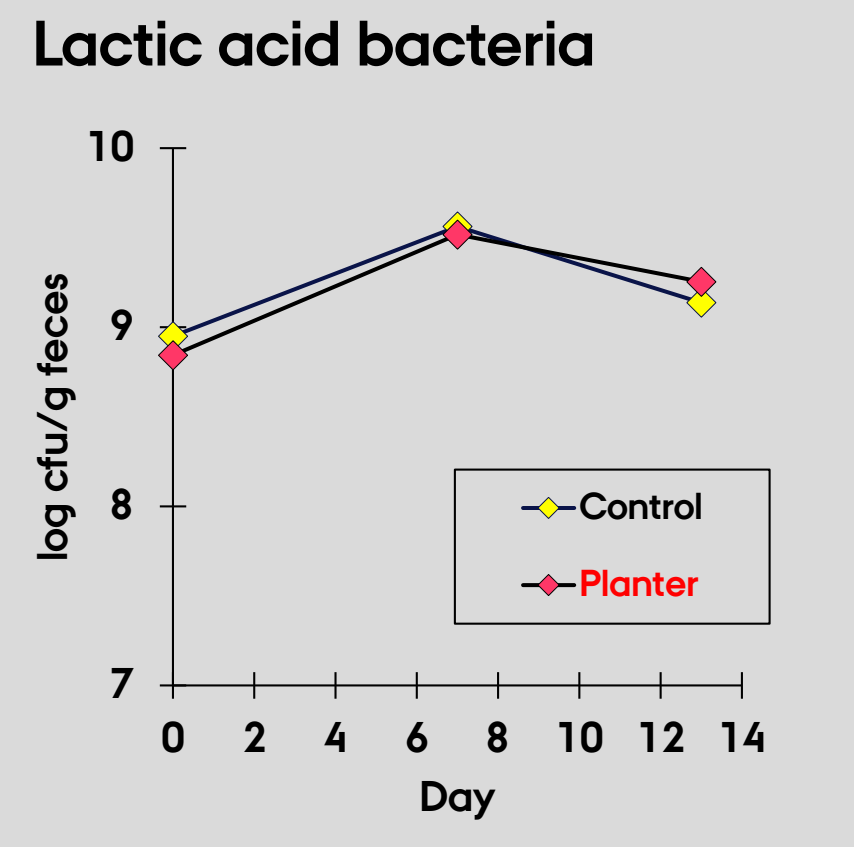
In vivo pilot trial

❑ Protocol:



In vivo pilot trial (feces)





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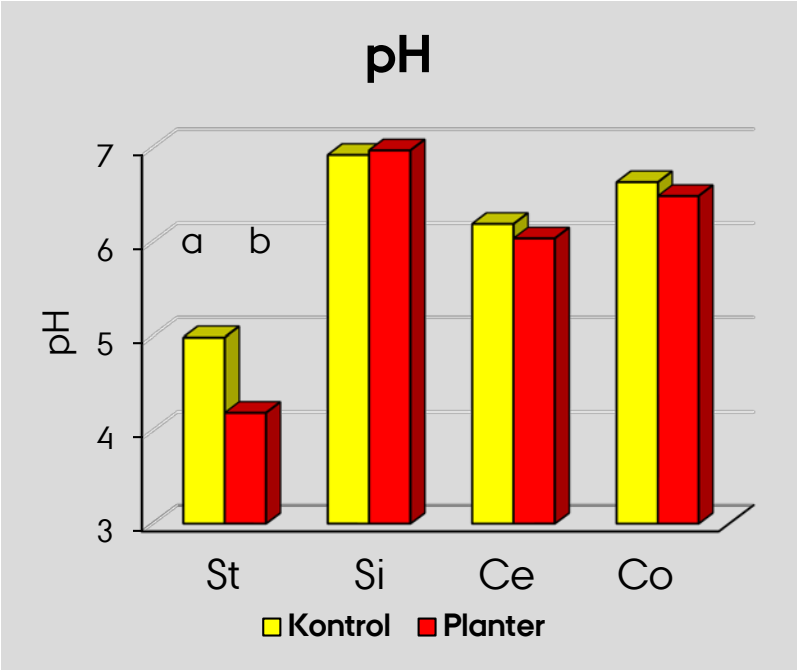
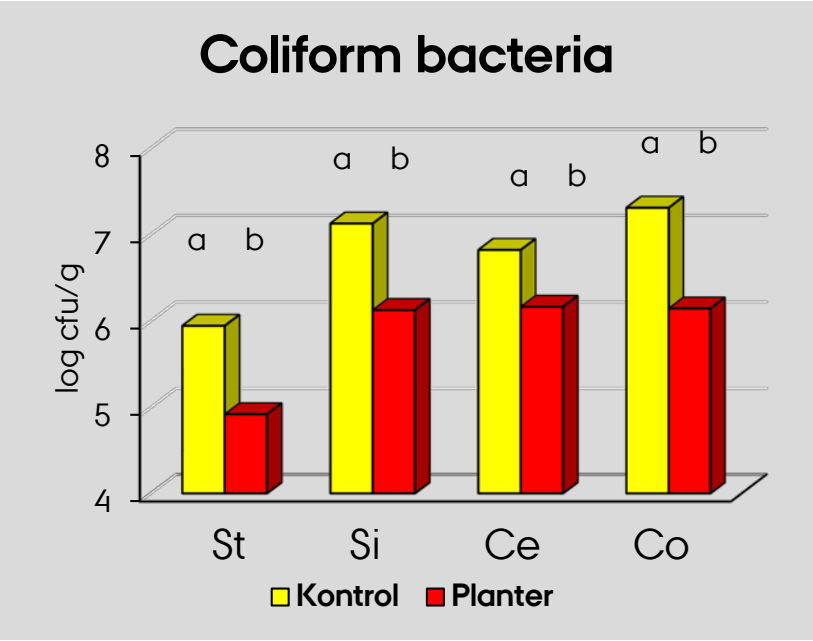
In vivo pilot trial (digesta)





St: Stomach Si: Small intestine Ce: Cecum Co:Colon

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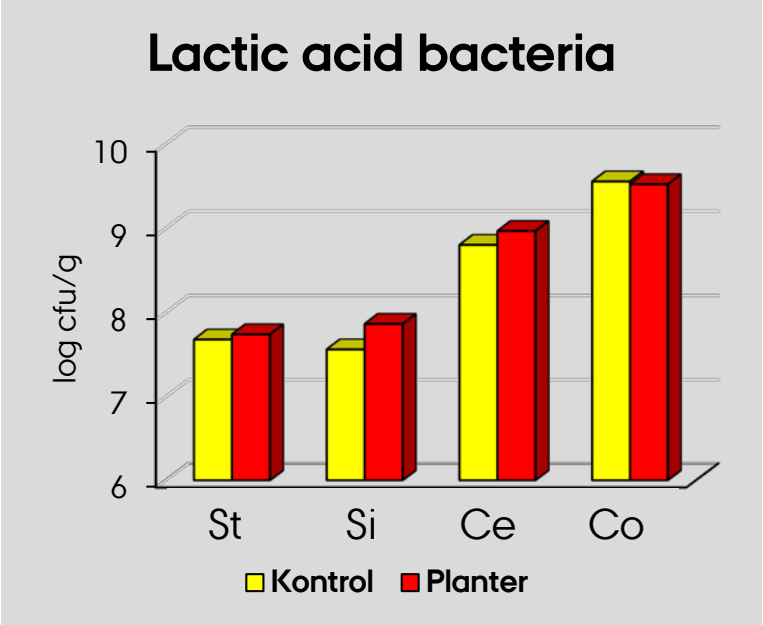
In vivo pilot trial (digesta)



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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

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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

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