Assessing the effect of dietary inulin and resistant starch on gastrointestinal fermentation in pigs

B. U. Metzler-Zebeli
Potential prebiotics in pig nutrition

**Inulin**
- **Natural sources**: fruits and vegetables
- **Purified sources**: extracted from chicory
  - *Lactobacillus* + *Bifidobacterium* ↑
  - Growth of enterotoxigenic *Escherichia coli* ↓
  - Post-weaning diarrhoea ↓
  - Fermentation acids ↑ ➔ gut development ↑

**Resistant starch (RS)**
- **Natural sources**: cereals, legumes
- **Purified sources**: extracted from corn, potato, tapioca, rice, …
  - Amylolytic and butyrogenic bacteria ↑
  - Propionate, butyrate ➔ gut development ↑
  - Gut integrity and immunity ↑

- Varying results across research studies
- Qualitative reviews cannot consider changes in direct (type and dose) and indirect factors (e.g., age of the animal)
Objective

- To evaluate the capability of inulin and RS to modify intestinal fermentation, pH and gut health-related bacteria in pigs.
- Inulin and resistant starch type 2 (RS2) were separately assessed using a meta-analytical approach.
Literature search

- Public search generators: Pubmed, Google Scholar, Web of Science, and Scopus

**Effect of inulin & RS on microbial activity**

**Research articles on controlled experiments**

- IN: 2000 - 2016
- RS: 2000 – 2017

**Search terms**

- inulin, chicory, chicory root, Jerusalem artichokes
- resistant starch type 2, high-amylose starch, slowly digestible starch
- pig, piglet, swine, gut, intestine, gastrointestinal tract, large intestine or individual segments, small intestine or individual segments, stomach, fermentation, microbial metabolites, volatile fatty acids (VFA) and short-chain fatty acids (SCFA), lactic acid, neutral and anionic forms of fermentation acids, bacteria, microbiota, microflora, and microbiome
Construction of databases

**Predictor variables**
- level and source of inulin / RS (purified concentrate or natural source)
- dietary composition
- details on pig (breed, age, BW, age, sex, production stage)
- housing condition
- number of pigs within treatment groups
- duration of the experimental period
- experimental design including randomization of treatment groups
- description of statistical analysis
- intra-study error (SE or SD)

**Response variables**
- stomach, ileum, cecum, proximal, mid and distal colon and feces (rectum)
- bacterial abundances
- microbial metabolites (i.e., SCFA and lactate)
- pH values
Quality assessment criteria

▪ only *in vivo* studies were included
▪ 3 studies as minimum requirement to quantify the combined effect size (Lipsey and Wilson, 2001)
▪ 10 single observations (treatment means) as minimum requirement per dependent variable as well as the respective SEM of each variable

Predictor variables and dependent variables of interest were not always available across all studies or ill-defined → leading to a large number of missing data

Additional predictor variables to consider maturational changes from weaned to finisher pigs & interactions with other dietary components:
▪ “age” and “start BW”
▪ “dietary fiber / carbohydrate composition” and “crude protein”
# Construction of databases

**Inulin**
- 33 (out of 45) articles met eligibility criteria
- Dietary level: 0.1 to 25.8%
- Pig’s start BW: 21.8 kg (mean)

**Resistant starch type 2**
- 24 (out of 35) articles met eligibility criteria
- Dietary level: 0 to 78.0%
- Pig’s start BW: 30.4 kg (mean)

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**Original dataset including all studies**

**Sub-datasets for the individual dependent variable categories:**
- bacterial abundances
- pH values
- microbial metabolites including SCFA and lactate
Data analysis

- MEANS procedure of SAS for descriptive statistics
- Mixed modeling analysis using the MIXED procedure (St-Pierre, 2001):
  - Estimates, root mean square error (RMSE) and $R^2$ were computed and used to evaluate the goodness of fit
  - Significance: $P \leq 0.05$; Trend: $0.05 < P \leq 0.10$

**Inulin**
- **Predictor variables:** start BW, dietary crude protein & dietary inulin
- **Random effects:** slope and intercept by study, start BW, dietary crude protein & dietary inulin

**Resistant starch**
- **Predictor variables:** start BW, duration of experiment & dietary RS content
- **Random effects:** slope and intercept by study, start BW, duration of experiment & dietary inulin

- Backward elimination analysis:
  - Simultaneous evaluation of the predictor variables on the response variables
  - Variance inflation factor (VIF) < 10 to avoid model overparameterization
Effect of inulin on gastric pH

Y = 3.51 – 0.04*X
RMSE = 0.115
R² = 0.81
P < 0.001
n_Treat = 12

3% inulin decrease gastric pH by 0.12 log units
Effect of inulin on bacterial abundances

Y = 9.31 – 0.46*X  
RMSE = 1.218  
R² = 0.41  
P < 0.001  
n_Treat = 26

Y = 7.64 + 0.13*X  
RMSE = 1.343  
R² = 0.26  
P = 0.07  
n_Treat = 13

Y = 6.96 – 0.06*X  
RMSE = 0.259  
R² = 0.66  
P = 0.001  
n_Treat = 15

Introduction

Materials & Methods

Results

Conclusion

3% inulin …

↓ Lactobacilli by 1.7 log units

↑ Bifidobacteria by 0.4 log units

↓ Escherichia coli by 0.2 log units

6 - 8% inulin needed to effectively decrease Escherichia coli

6 → 8% inulin needed to effectively decrease Escherichia coli

6 - 8% inulin needed to effectively decrease Escherichia coli
Best-fit equations for influence of all predictor variables on response parameters using backward elimination technique - inulin

<table>
<thead>
<tr>
<th>Response variable (Y)</th>
<th>Factor (X)</th>
<th>n&lt;sub&gt;Treat&lt;/sub&gt;</th>
<th>Parameter estimates</th>
<th>Model statistics</th>
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<td>Gastric pH</td>
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<td>dietary CP (%)</td>
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<td>Inulin (%)</td>
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<td>Inulin (%)</td>
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<tr>
<td>Fecal bifidobacteria</td>
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<td>BW (kg)</td>
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<td>dietary CP (%)</td>
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<tr>
<td>Inulin (%)</td>
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<tr>
<td>Fecal <em>Escherichia coli</em></td>
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<td>BW (kg)</td>
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<td>dietary CP (%)</td>
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<tr>
<td>Inulin (%)</td>
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</table>
**Effect of RS2 on intestinal pH**

- RS2 decreased luminal pH especially in distal large intestine
- Minimum of 15 to 30% RS to decrease pH by 0.2 to 0.6 log units
**Effect of RS2 on molar proportions of SCFA**

<table>
<thead>
<tr>
<th>Response variable (Y)</th>
<th>nTreat</th>
<th>Parameter estimates</th>
<th>Model statistics</th>
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<tbody>
<tr>
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<td>Intercept</td>
<td>Slope</td>
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<tr>
<td><strong>Mid colon</strong></td>
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<tr>
<td>Total SCFA (µmol/g)</td>
<td>10</td>
<td>73.8</td>
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<tr>
<td>mol/100 mol total SCFA</td>
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<tr>
<td>Acetate</td>
<td>10</td>
<td>59.3</td>
<td><strong>-0.36</strong></td>
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<tr>
<td>Propionate</td>
<td>10</td>
<td>26.7</td>
<td><strong>0.24</strong></td>
</tr>
<tr>
<td>Butyrate</td>
<td>10</td>
<td>15.4</td>
<td><strong>-0.16</strong></td>
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</tbody>
</table>

- RS2 promotes propionate fermentation
- Minimum of 20% RS to increase propionate by 5% in mid-colonic digesta
## Effect of RS2 on molar proportions of SCFA

<table>
<thead>
<tr>
<th>Response variable (Y)</th>
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<th>Parameter estimates</th>
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<tr>
<td></td>
<td></td>
<td>Intercept</td>
<td>Slope</td>
</tr>
<tr>
<td><strong>Mid colon</strong></td>
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</tr>
<tr>
<td>Total SCFA (µmol/g)</td>
<td>10</td>
<td>73.8</td>
<td>-0.95</td>
</tr>
<tr>
<td>mol/100 mol total SCFA</td>
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<tr>
<td>Acetate</td>
<td>10</td>
<td>59.3</td>
<td>-0.36</td>
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<tr>
<td>Propionate</td>
<td>10</td>
<td>26.7</td>
<td>0.24</td>
</tr>
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<td>Butyrate</td>
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<td><strong>Distal colon</strong></td>
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<td>mol/100 mol total SCFA</td>
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<td>Propionate</td>
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<td>Butyrate</td>
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<td>13.76</td>
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Effect of RS2 on bacterial abundances

- RS2 promoted lactic acid-producing bacteria
- Minimum of 10% RS to increase bacteria by 0.5 log units
Best-fit equations for influence of all predictor variables on response parameters using backward elimination technique - resistant starch type 2

<table>
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<tr>
<th>Response variable</th>
<th>Parameter estimates</th>
<th>Model statistics</th>
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<td>n_{Treat}</td>
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<td>Fecal lactobacilli</td>
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<td>experimental period (days)</td>
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<td>squared RS content (%)</td>
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<td>Fecal bifidobacteria</td>
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<td>experimental period (days)</td>
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<td>squared RS content (%)</td>
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</table>
Assessing the effect of dietary inulin supplementation on gastrointestinal fermentation, digestibility and growth in pigs: A meta-analysis

B.U. Metzler-Zebeli,1,2,3, P. Trevisi4, J.A.M. Prates5, S. Tanghe,6, P. Bosi,6, N. Canibe,6, L. Montagne,6, J. Freire6, Q. Zebeli6

1 University Clinic for Swine, University of Veterinary Medicine Vienna, 1210 Vienna, Austria
2 Department of Agricultural and Food Science (DISTAL) — University of Bologna, 40127 Bologna, Italy
3 CISA, Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, Alto da Ajuda, 1300-077 Lisbon, Portugal
4 Nutritional Solutions Division, Nutrition Sciences N.V., 9031 Gent, Belgium
5 Department of Animal Science, Aarhus University, 8830 Tjele, Denmark
6 PEGASE, Agrocampus Ouest, INRA, 35500 Saint-Gilles, France
7 ILAS, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal
8 Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, Vetmeduni Vienna, Veterinärplatz 1, 1210 Vienna, Austria

ABSTRACT

Inulin has been reported to improve the homeostasis in the gastrointestinal modulating the intestinal microbiota and fermentation. The present study evaluated the relationship between dietary inulin and microbial response variables in the jejunum of weaned, growing and finishing pigs using a meta-analytical approach. The effect of dietary inulin on the coefficients of ideal (GIT) and digestibility (CTTAD) of nutrients and ADG. Pig's starting body weight was inclusion criterion. Missing information about explanatory variables and response variables reduced the number of studies included. From the 33 studies, all studies between 2000 and 2016, individual sub-datasets for fermentation, digestion and inclusion criterion.

Resistant starch reduces large intestinal pH and promotes fecal lactobacilli and bifidobacteria in pigs

B. U. Metzler-Zebeli,1,2, N. Canibe6, L. Montagne3, J. Freire4, P. Bosi5, J. A. M. Prates6, S. Tanghe6 and P. Trevisi4

1 Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, Vetmeduni Vienna, 1210 Vienna, Austria
2 Department of Animal Sciences, Aarhus University, 8830 Tjele, Denmark
3 PEGASE, Agrocampus Ouest, INRA, 35500 Saint-Gilles, France
4 ILAS, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal
5 Department of Agricultural and Food Science (DISTAL), University of Bologna, 40127 Bologna, Italy
6 CISA, Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, Alto da Ajuda, 1300-077 Lisbon, Portugal
7 Institute of Animal Nutrition and Functional Plant Compounds, Nutrition Sciences N.V., 9031 Gent, Belgium

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Dietary resistant starch (RS) may have prebiotic properties but its effects on fermentation and the microbial population are inconsistent. This meta-analysis aimed to quantify the relationship between RS type 2 (RS2) and intestinal short-chain fatty acids (SCFA) and pH as well as certain key bacterial taxa for intestinal health in pigs. From the 24 included articles with sufficient information about the animal, and dietary and physiological measurements published between 2000 and 2017, individual sub-data sets for fermentation metabolites, pH, bacterial abundances and apparent total tract digestibility were built and used to parameterize prediction models on the effect of RS2, accounting for inter- and intra-study variability. In addition, the effect of pig's BW at the start of the experiment and duration of the experimental period on response variables were also evaluated using backward elimination analysis. Dietary RS levels ranged from 0% to 78.0% RS, with median and mean RS levels of 28.8% and...
Conclusion

Meta-regressions support that dietary inulin and resistant starch type 2 may have some favorable effects on gut homeostasis in pigs

- **Inulin:**
  - gastric pH ↓, fecal *Escherichia coli* ↓, but fecal lactobacilli ↓
  - To achieve physiologically relevant changes: dietary inulin content > 3 to 5%
- **Resistant starch type 2:**
  - hindgut pH ↓, fecal lactic acid-producing bacteria ↑
  - To achieve physiologically relevant changes: dietary RS content > 10 to 15%

- For many response variables, low numbers of treatment comparisons were available.
- Due to missing information, influential effects of other dietary fractions on the prebiotic effect could not be weighted.
- Established relationships are more applicable for growing pigs and are universal trends.
Thank you for your attention!
Maintaining gut homeostasis in pigs

Demand to reduce use of antibiotics
Demand for meat from healthy animals
High production efficiency
Intensive rearing systems

Challenge for the gut homeostasis

- Weaning at an immature stage
- Immature gut microbiota & digestive functions

- Abrupt diet changes
- New housing
- New groups

Functional dietary ingredients and supplements, e.g. prebiotics
Definition:
Resistant starch (RS) includes all starch and starch degradation products that are not digested in the small intestine.

- RS is divided into 5 categories:
  - RS1 = physically inaccessible starch
  - RS2 = native granular starch consisting of ungelatinized granules and high amylose starch
  - RS3 = retrograded amylose starch
  - RS4 = cannot be found in nature and represents starch being chemically modified by esterification, crosslinking or transglycosylation
  - RS5 = amylose-lipid complexes

Lattimer and Haub (2010) Nutrients 2:1266