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

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Potential prebiotics in pig nutrition

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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)

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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

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- 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

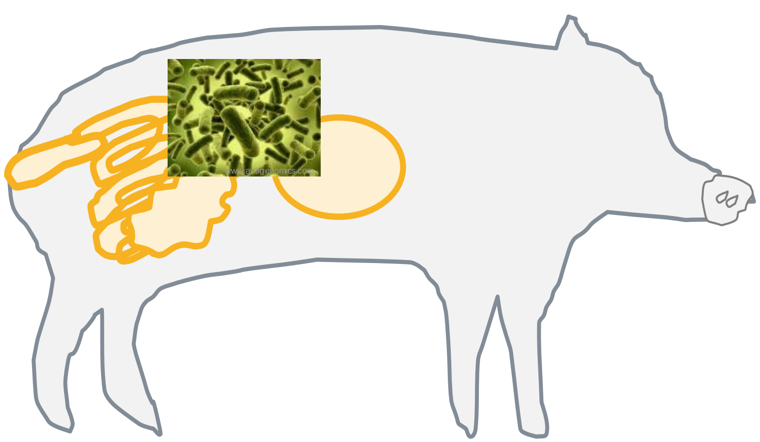
→

Search terms

inulin, chicory, chicory root, Jerusalem artichokes

resistant starch type 2, high-amylose starch, slowly digestible starch

pig, piglet, swine, gut, intestine, gastro-intestinal 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

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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

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- 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

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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)

↓

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

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- 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

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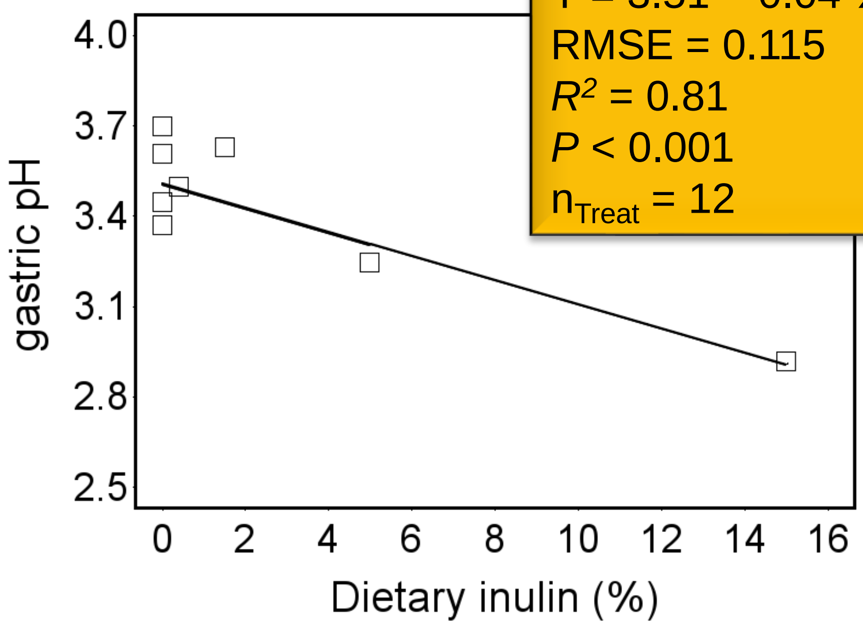
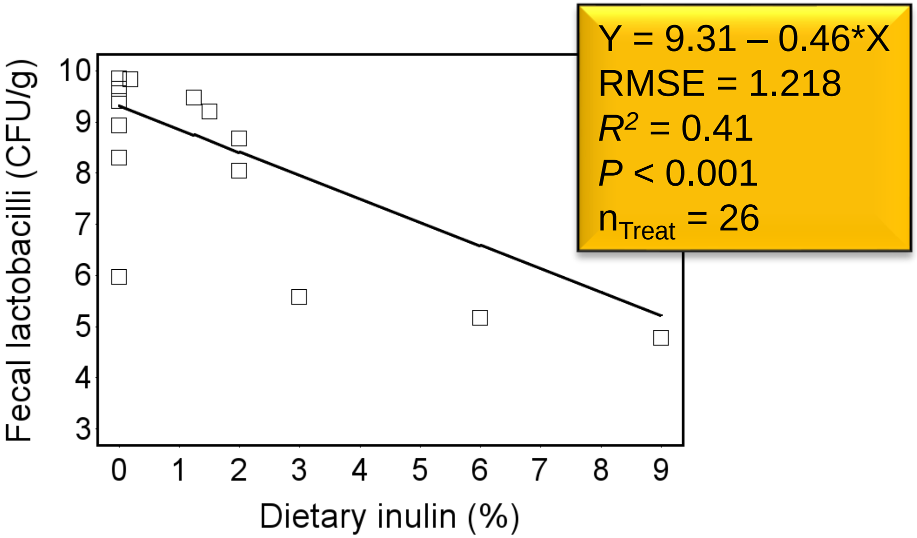
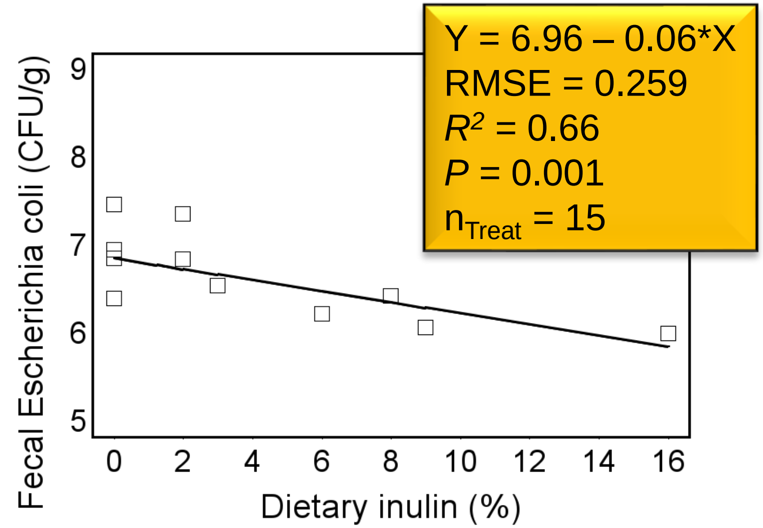


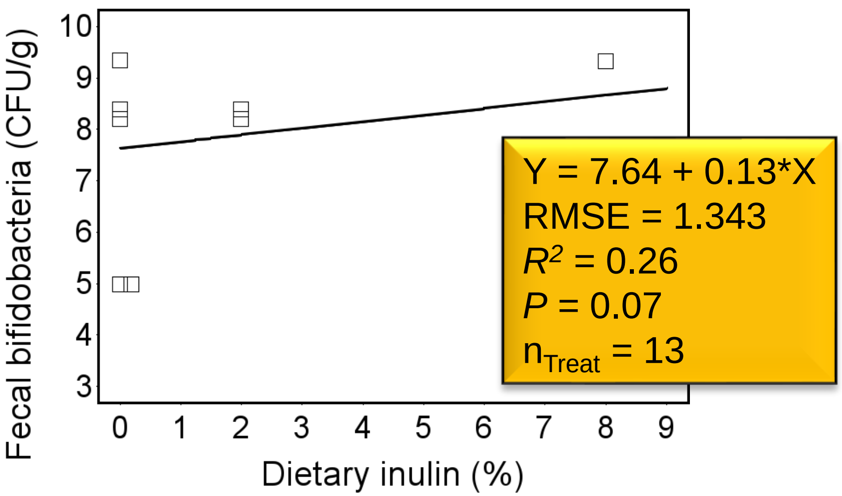
Y = 3.51 – 0.04*X
RMSE = 0.115
R^2 = 0.81
P < 0.001
n_{Treat} = 12

3% inulin decrease gastric pH by 0.12 log units

Effect of inulin on bacterial abundances







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*

Best-fit equations for influence of all predictor variables on response parameters using backward elimination technique - inulin

			Parameter estimates		Model statistics			
Response variable (*Y*)	Factor (*X*)	n_{Treat}	Intercept	Slope	RMSE	R^2	VIF	*P*-value
Gastric pH		15	5.28		0.037	0.98		
	BW (kg)			-0.14			1.52	0.002
	dietary CP (%)			-0.02			1.52	0.003
	Inulin (%)			-0.03			1.09	<0.001
Fecal Lactobacilli		26	10.30		0.763	0.79		
	BW (kg)			-0.045			1.22	<0.001
	Inulin (%)			-0.255			1.22	0.004
Fecal bifidobacteria		13	5.52		0.096	1.00		
	BW (kg)			0.129			1.64	<0.001
	dietary CP (%)			0.549			1.64	<0.001
Fecal *Escherichia coli*		19	2.59		0.216	0.80		
	BW (kg)			0.063			6.10	<0.001
	dietary CP (%)			0.134			6.31	0.012
	Inulin (%)			-0.044			1.67	0.004

Effect of RS2 on intestinal pH

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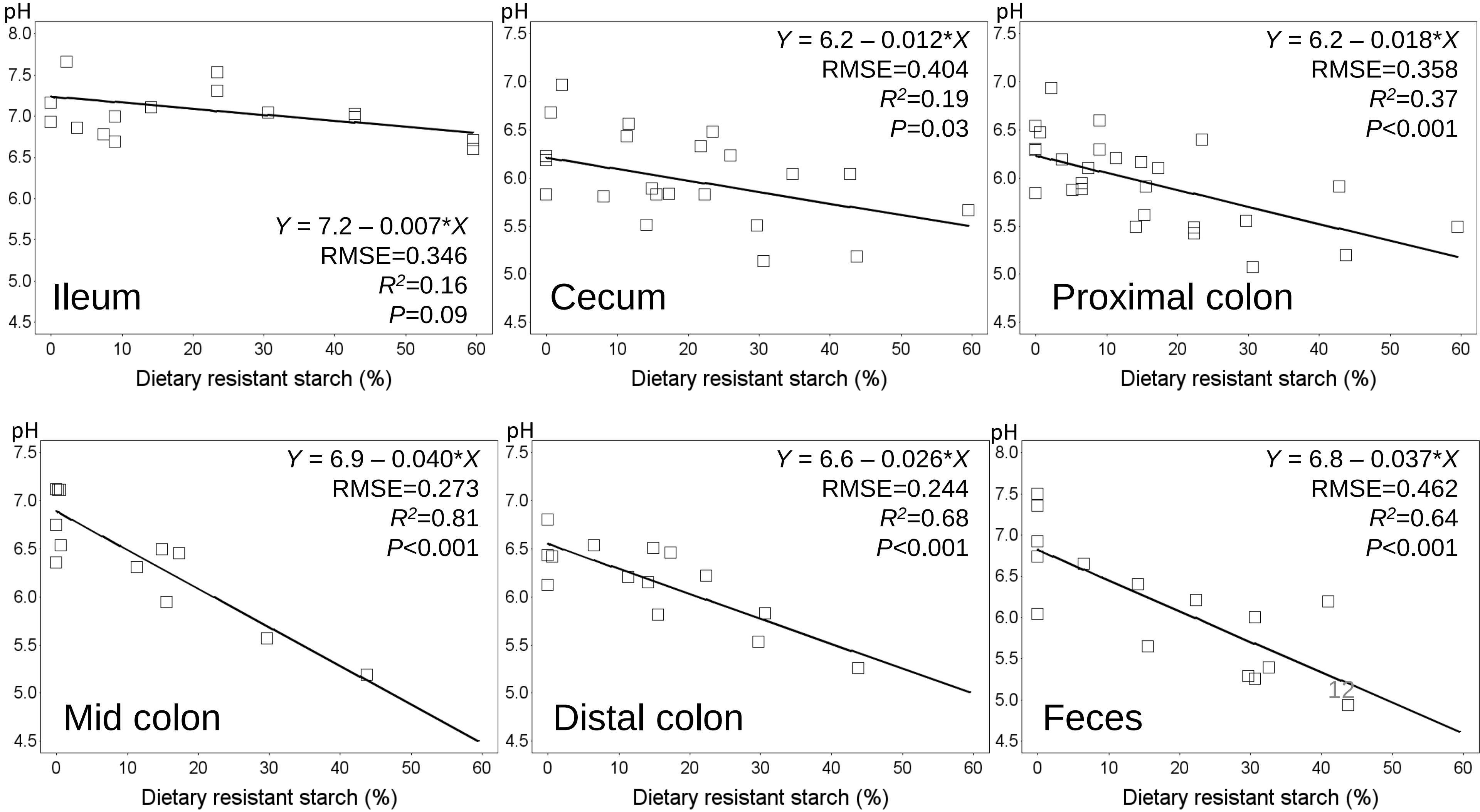


- RS2 decreased luminal pH especially in distal large intestine
- Minimum of 15 to 30% RS to decrease pH by 0.2 to 0.6 log units

Effect of RS2 on molar proportions of SCFA

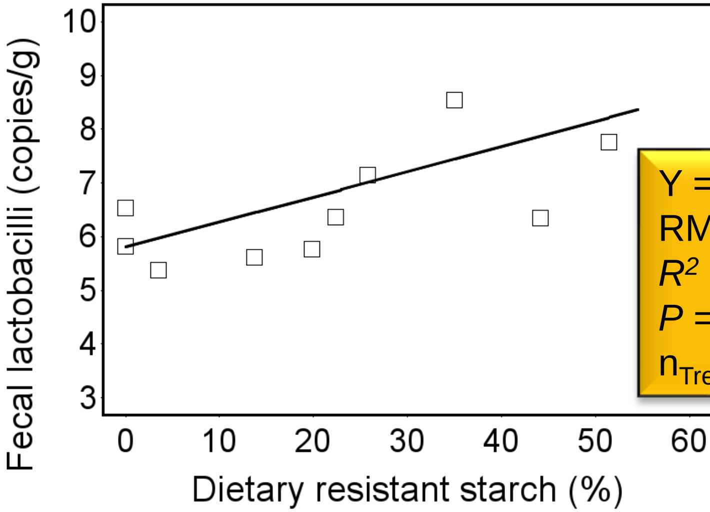
		Parameter estimates		Model statistics		
Response variable (*Y*)	n_{Treat}	Intercept	Slope	RMSE	R^2	*P*-value
Mid colon						
Total SCFA (µmol/g)	10	73.8	-0.95	52.16	0.07	0.45
mol/100 mol total SCFA						
Acetate	10	59.3	-0.36	4.79	0.57	0.01
Propionate	10	26.7	0.24	3.29	0.56	0.01
Butyrate	10	15.4	-0.16	2.72	0.44	0.04

- RS2 promotes propionate fermentation
- Minimum of 20% RS to increase propionate by 5% in mid-colonic digesta

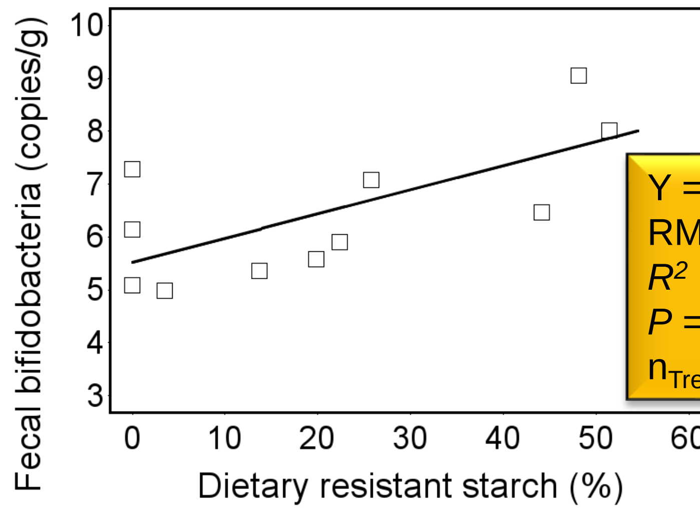
Effect of RS2 on molar proportions of SCFA

		Parameter estimates		Model statistics		
Response variable (*Y*)	n_{Treat}	Intercept	Slope	RMSE	R^2	*P*-value
Mid colon						
Total SCFA (µmol/g)	10	73.8	-0.95	52.16	0.07	0.45
mol/100 mol total SCFA						
Acetate	10	59.3	-0.36	4.79	0.57	0.01
Propionate	10	26.7	0.24	3.29	0.56	0.01
Butyrate	10	15.4	-0.16	2.72	0.44	0.04
Distal colon						
Total SCFA (µmol/g)	17	69.2	-0.69	33.03	0.08	0.28
mol/100 mol total SCFA						
Acetate	10	57.09	-0.150	5.66	0.14	0.28
Propionate	10	29.96	-0.025	8.49	0.00	0.90
Butyrate	10	13.76	-0.154	2.34	0.50	0.02

Effect of RS2 on bacterial abundances



Y = 5.81 + 0.05*X
RMSE = 0.926
R^2 = 0.46
P = 0.008
n_{Treat} = 12



Y = 5.52 + 0.05*X
RMSE = 0.950
R^2 = 0.52
P = 0.008
n_{Treat} = 12

- 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

Response variable (*Y*)	Factor (*X*)	n_{Treat}	Parameter estimates: Intercept	Parameter estimates: Slope	Model statistics: RMSE	Model statistics: R^2	Model statistics: VIF	Model statistics: *P*-value
Fecal lactobacilli		14	5.43		0.452	0.88		
	experimental period (days)			0.024			1.03	<0.001
	squared RS content (%)			0.001			1.03	0.001
Fecal bifidobacteria		12	7.32		0.689	0.78		
	experimental period (days)			-0.094			1.00	0.04
	squared RS content (%)			0.001			1.00	<0.001

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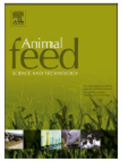

Assessing the effect of dietary inulin supplementation on gastrointestinal fermentation, digestibility and growth in pigs: A meta-analysis

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ABSTRACT

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

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

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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.

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Thank you for your attention !

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Maintaining gut homeostasis in pigs

Demand to reduce use of antibiotics

Weaning at an immature stage

Demand for meat from healthy animals

Immature gut microbiota & digestive functions

High production efficiency

Intensive rearing systems

Challenge for the gut homeostasis

- Abrupt diet changes
- New housing
- New groups

Functional dietary ingredients and supplements, e.g. prebiotics

Resistant starch

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