

Effect of dietary protein sources on intestinal and systemic responses of pigs

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WAGENINGEN **UR**
For quality of life



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Acknowledgement

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Knowledge partners



Industrial partners



Supporting partner



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Aim of project

- To characterize the protein component of new/alternative protein sources using proteomics
- To predict functionality of protein sources using bioinformatics
- To assess functional properties of new/alternative protein sources using animal models (mice and pigs)



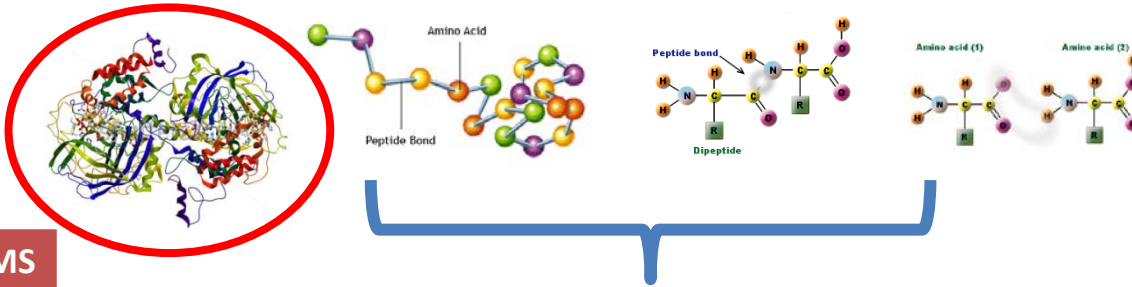
Topics of today's presentation

- To characterize the protein component of new/alternative protein sources using proteomics
- To predict functionality of protein sources using bioinformatics
- To assess functional properties of new/alternative protein sources using animal models (mice and pigs)



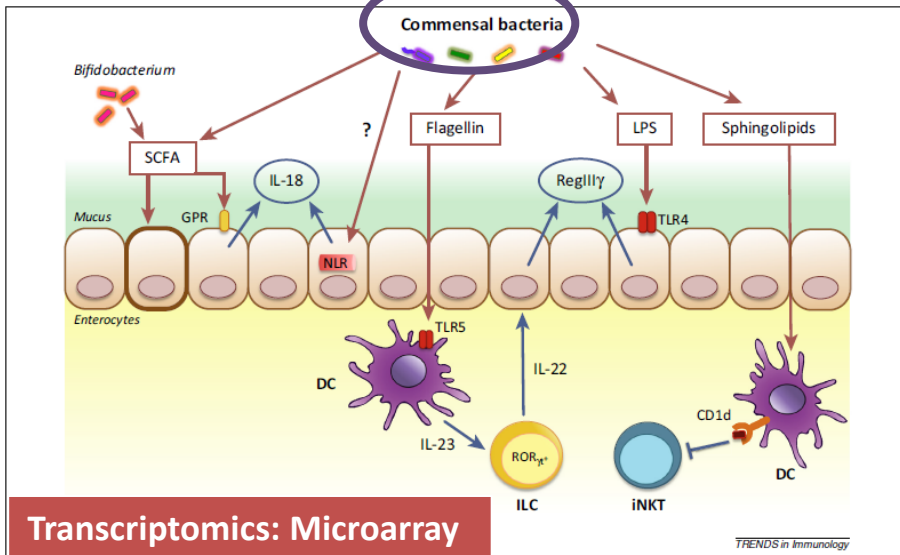
In vivo digestion process

Digestion of proteins in gastro-intestinal tract

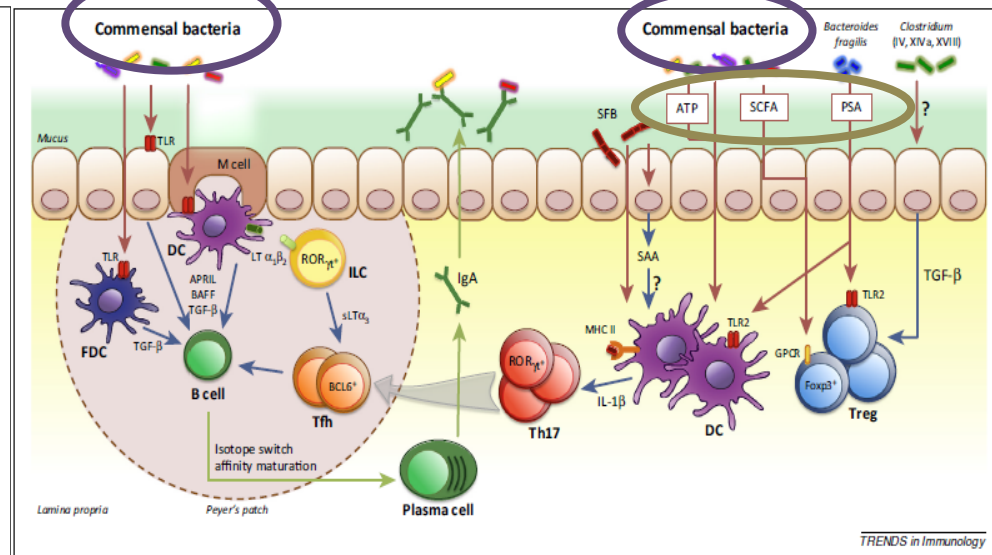


Proteomics: MS

Genomics: NGS



Transcriptomics: Microarray



ELISA

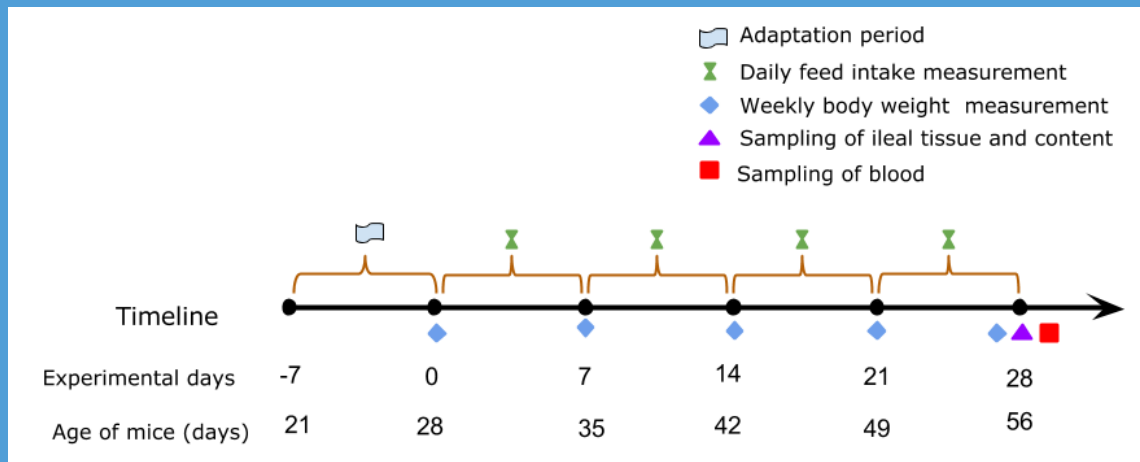
Cytokines

Systemic effects

Metabolites

MS/ GC

Previous study: Mice



Experimental diets:

SBM: Soybean Meal
CAS: Casein (feed grade)
DWP: Delactosed Whey Powder
SDPP: Spray Dried Plasma Protein
WGM: Wheat Gluten Meal
YMW: Yellow Meal Worm

Local effect : Ileum

Gene expression : ↓ mTOR signalling pathways **SBM**

Microbiota: ↑ abundance of *Bacteroidales* Family *S24-7* **SBM**

Systemic effect: Serum & Urine

Cytokines and chemokines: ↑ granulocyte-colony stimulating factor (G-CSF) **SBM**

Biogenic amines metabolites: ↑ 1-Methylhistidine **YMW**

Pig study: Experimental design & set-up



Age: 10-11 weeks

Average Body weight: 33 ± 0.5 kg

Blocking: Litter (8 sows and 5 barrows were distributed over 5 dietary groups)

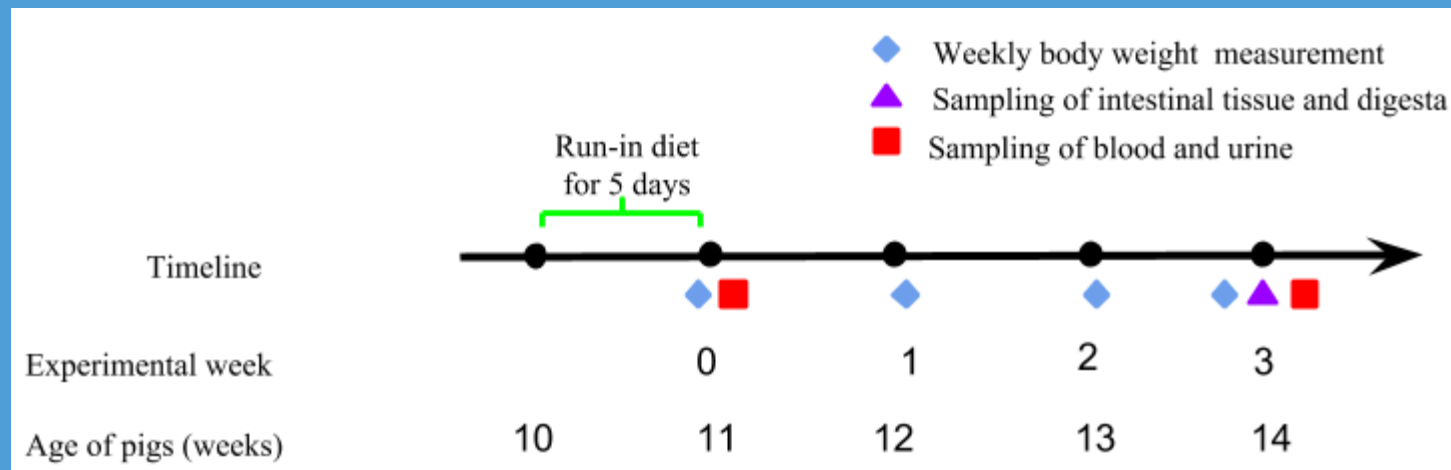
Housing: Individually

Number of animals/group: 8

Genetic background: Topigs 20 x Tempo

Sex: Male

Status: Specific pathogen free (SPF)



Pig study: Experimental diet

Experimental diet:

Protein sources included in the experimental diets at a level of 150-500 g/kg in a way that the crude protein content of the diets is about 160 g/kg.

- Twice a day in equal amount
- 2.5 times the maintenance requirement for energy
- Ad libitum water



| Item | Diet | | | | |
|----------------------------|------------|------------|------------|------------|------------|
| | SBM | WGM | RSM | SDPP | BSF |
| Ingredients, g/kg | | | | | |
| Maize starch | 376.2 | 527.9 | 258 | 521.4 | 451 |
| Sugar | 100 | 100 | 100 | 100 | 100 |
| Dextrose | 50 | 50 | 50 | 50 | 50 |
| Arbocel | 50 | 50 | 50 | 50 | 50 |
| Soybean oil | 43.3 | 17.6 | 30 | 32.6 | 6.4 |
| Finely ground chalk | 14.4 | 15.9 | 5.1 | 15.7 | 0 |
| Mono sodium phospahte | 10.1 | 13.6 | 4.6 | 14.2 | 11.7 |
| Salt | 4.1 | 2 | 4 | 0 | 0 |
| Sodium bicarbonate | 1.4 | 3.9 | 1.2 | 0 | 6.7 |
| Calcium carbonate | 0 | 10.6 | 0 | 11.2 | 5 |
| Calcium chloride | 0 | 0 | 0 | 0 | 4.8 |
| Premix (growth) | 5 | 5 | 5 | 5 | 5 |
| Titanium di-oxide | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| L-Lysine HCl | 0 | 5.8 | 0 | 0 | 0 |
| DL-Methionine | 0.3 | 0 | 0 | 1.4 | 1.3 |
| L-Threonine | 0 | 0.5 | 0 | 0 | 0 |
| L-Tryptophan | 0 | 0 | 0 | 0 | 0.6 |
| Soybean meal | 342.7 | 0 | 0 | 0 | 0 |
| Wheat gluten meal | 0 | 194.7 | 0 | 0 | 0 |
| Rape seed meal | 0 | 0 | 489.6 | 0 | 0 |
| Proglobulin 80 P | 0 | 0 | 0 | 196 | 0 |
| Black-soilder fly (larvae) | 0 | 0 | 0 | 0 | 305 |
| Total | 1000 | 1000 | 1000 | 1000 | 1000 |
| Composition, g/kg | | | | | |
| Dry matter | 904 | 904 | 911 | 912 | 919 |
| Crude protein | 166 | 156 | 163 | 158 | 158 |
| Sugar | 175 | 152 | 199 | 162 | 150 |
| Starch | 296 | 453 | 236 | 431 | 385 |
| Fat | 31 | 19 | 48 | 20 | 45 |
| Ash | 53 | 45 | 52 | 57 | 48 |

Pig study: Nutritional, clinical, systemic signatures

Nutritionally associated clinical signatures

- 15 serum biomarkers: no signs of pathological symptoms

Gross clinical signs and symptoms

- Animals appeared to be healthy  Apparently healthy animals
- No significant differences in body weight gain  Significant differences were observed

Cytokines and chemokines

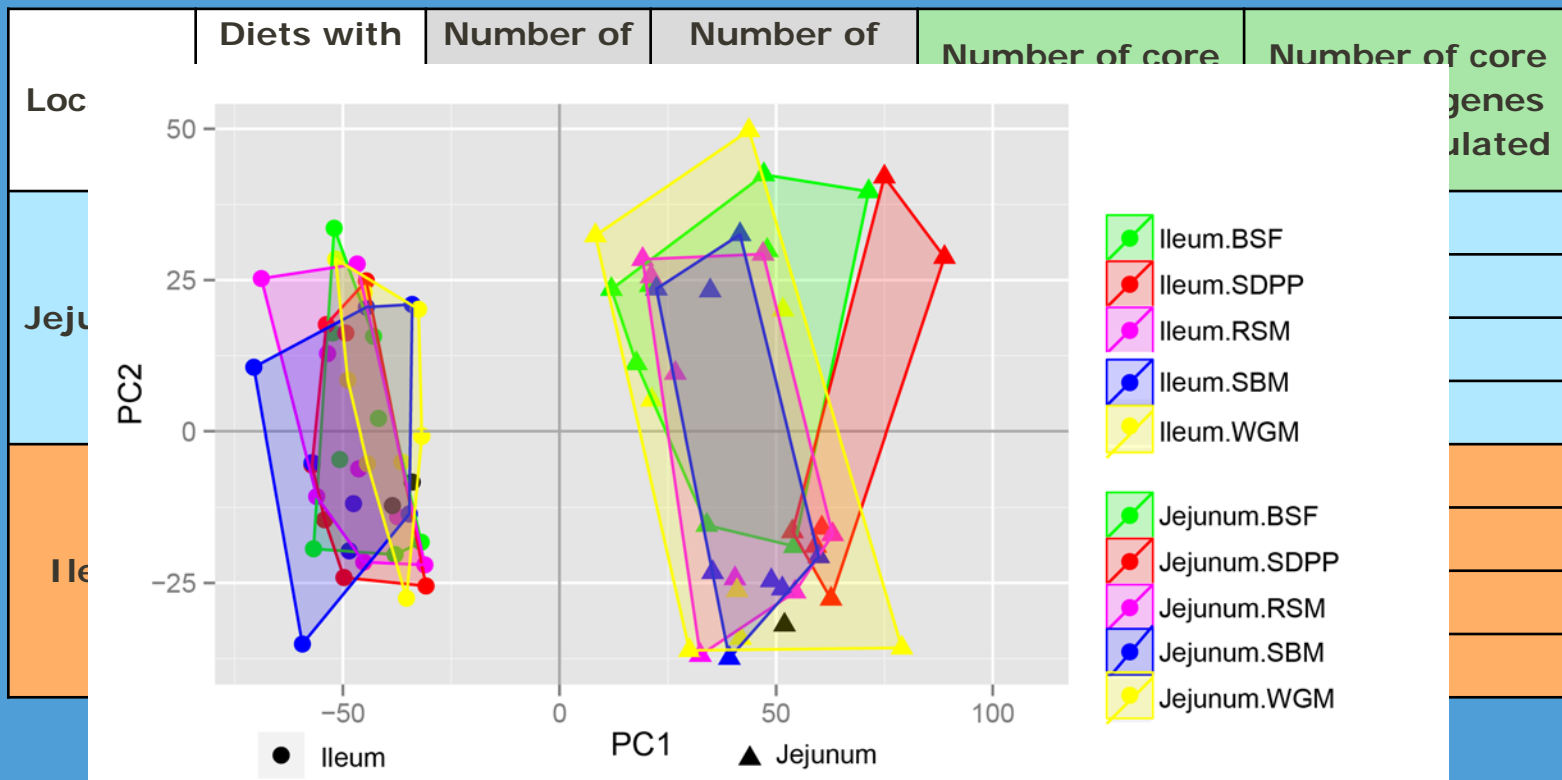
- No significant effects on nine measured blood immune parameters  Significant differences were observed



Pig study: Intestinal gene expression

Analysis method: Microarrays
 SBM used as reference
 Gene set enrichment analysis (GSEA)

Location: Jejunum and Ileum



Pig study: Intestinal gene expression

CONCLUSION

- Jejunum is more responsive than ileum.

| Tissue | Diets with different protein sources | Number of Gene-sets up-regulated | Number of Gene-sets down-regulated | Number of core enriched genes up-regulated* | Number of core enriched genes down-regulated |
|---------|--------------------------------------|----------------------------------|------------------------------------|---|--|
| Jejunum | BSF | 1 | 11 | 6 | 118 |
| | SDPP | 0 | 36 | 0 | 340 |
| | RSM | 3 | 0 | 12 | 0 |
| | WGM | 8 | 0 | 99 | 0 |
| Ileum | BSF | 2 | 8 | 31 | 55 |
| | SDPP | 0 | 0 | 0 | 0 |
| | RSM | 0 | 0 | 0 | 0 |
| | WGM | 7 | 22 | 50 | 152 |



Pig study: Intestinal gene expression

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Functionalities of SDPP and BSF down-regulated genes: **barrier functions** and **immune signalling**

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- Compared to SBM, more down-regulated genes in jejunum.
 - Functionalities of SDPP and BSF down-regulated genes: **barrier functions** and **immune signalling**
- Functionalities of RSM and WGM up-regulated genes: **metabolism of bio-molecules (xenobiotics, retinol and tryptophan)**

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Intestinal gene expression: Mice vs Pigs

Comparison of salient results of mice and pigs study

- Mice:
 - SBM deviated strongly from the other diets: down-regulation of mTOR pathway genes
- Pigs:
 - Diet-specific effects
 - Jejunum is more responsive than ileum



Pig study: Intestinal microbiota

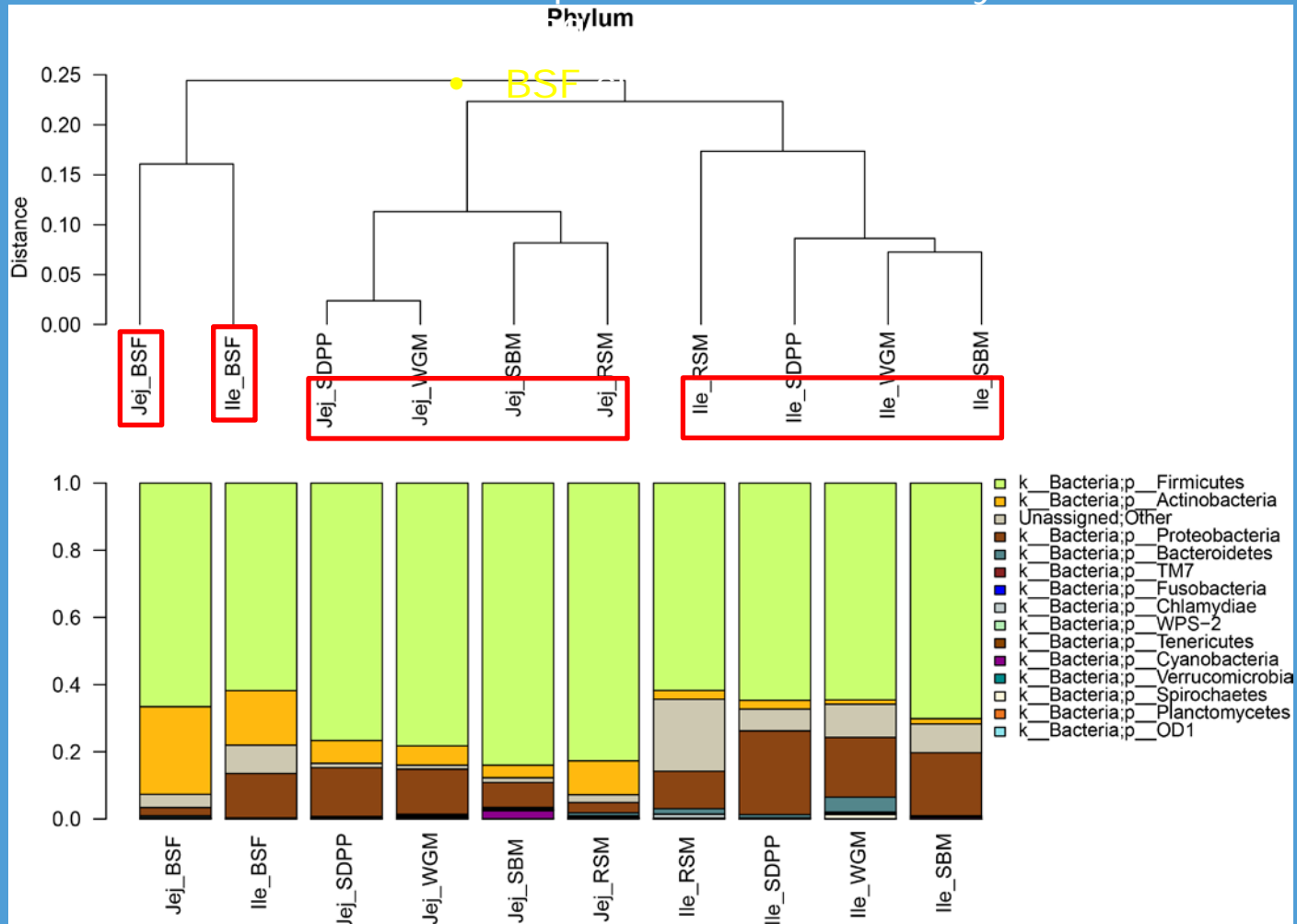
Analysis method: Community scale analysis by 16S RNA gene sequencing
SBM used as the reference

Location: Jejunum and Ileum



Pig study: Intestinal microbiota

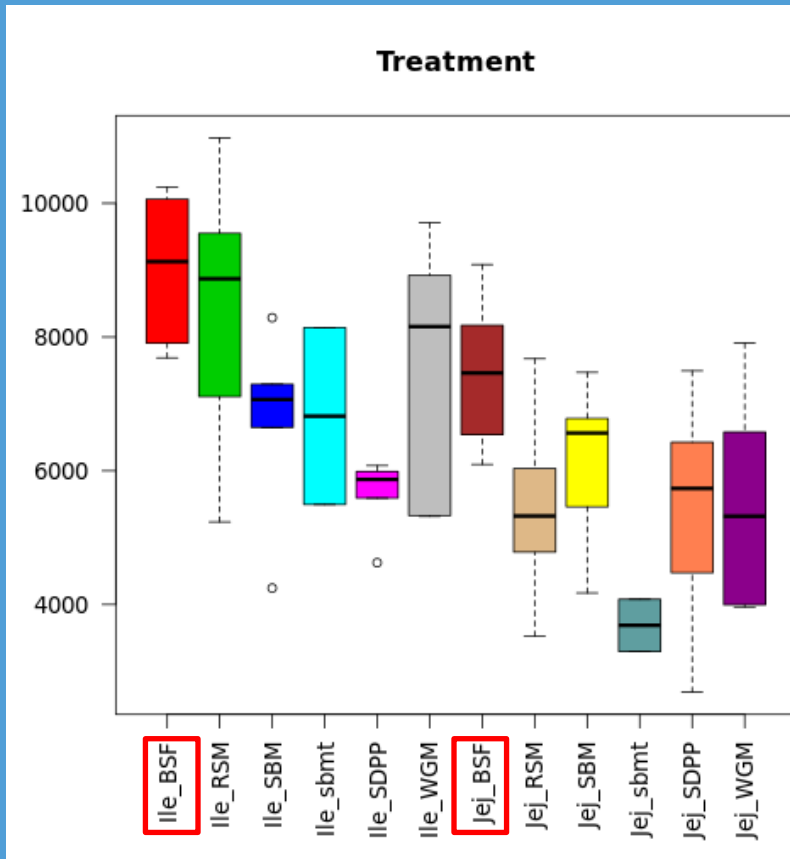
RESULTS AND CONCLUSION • Clear separation of location by hierarchical



Pig study: Intestinal microbiota

RESULTS AND CONCLUSION • Clear separation of location by hierarchical clustering

- **BSF** clusters separately

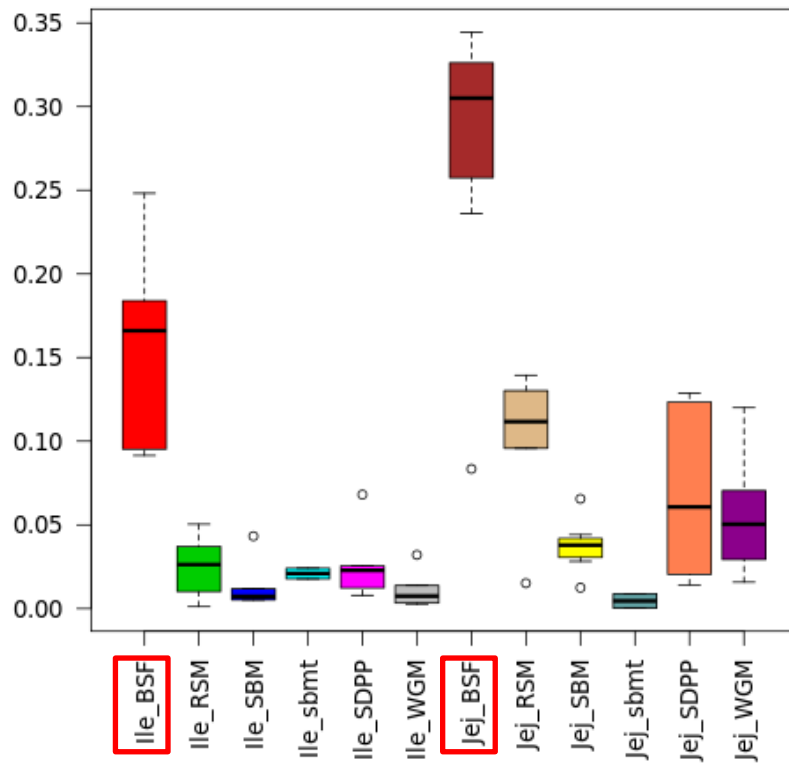


- Higher diversity in comparison to all the other treatments (in both location) by chao1 (alpha diversity): **BSF**

Pig study: Intestinal microbiota

- RESULTS AND CONCLUSION
- Clear separation of location by hierarchical clustering
 - **BSF** clusters separately

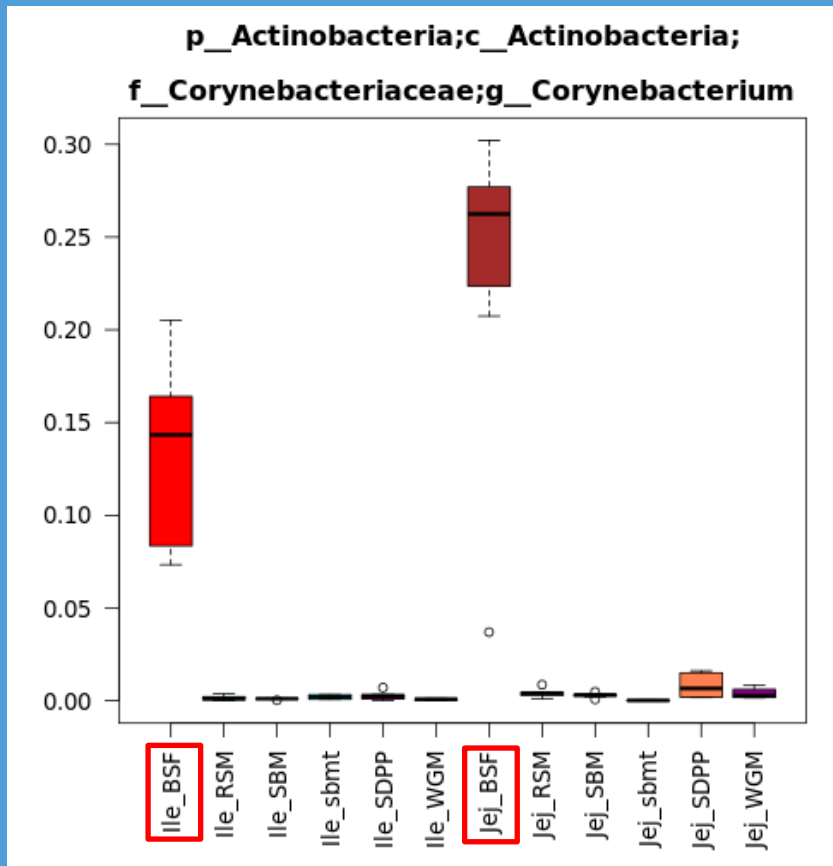
p_Actinobacteria



- Higher diversity in comparison to all the other treatments (in both location) by chao1 (alpha diversity): **BSF**
- Higher abundance of *Actinobacteria* for **BSF**

Pig study: Intestinal microbiota

- RESULTS AND CONCLUSION
- Clear separation of location by hierarchical clustering
 - **BSF** clusters separately



- Higher diversity in comparison to all the other treatments (in both location) by chao1 (alpha diversity): **BSF**
- Higher abundance of *Actinobacteria* for **BSF**
- Higher abundance of *Corynebacterium* for **BSF**

Intestinal microbiota: Mice vs Pigs

Comparison of salient results of mice and pigs study

- Mice:
 - SBM deviated strongly from the other diets: ↑ abundance of *Bacteroidales* Family *S24-7*
- Pigs:
 - BSF deviated strongly from the other diets at both the small intestinal location
 - ‘ diversity and abundance of *Actinobacteria* especially *Corynebacterium*



Overall conclusion

1) Host responses of dietary protein sources, as measured in mice, do not predict responses of pigs

For example: SBM induced inhibition of mTOR regulated immune parameters, as seen in mice, totally absent in pigs

2) Host systemic immune response to dietary protein sources

Mice: Present (modulation of G-CSF, IFN γ , Eotaxin, GM-CSF, IL6, IL2, IL 12p70, IL13, MCP, MIP 1b)

Pigs: Totally absent

3) Dietary proteins affect microbial composition and diversity

Mice: SBM deviates strongly (‘ abundance of *Bacteroidales* Family *S24-7*)

Pigs: BSF deviates strongly in both jejunum and ileum (‘ diversity and abundance of *Actinobacteria* Genus *Corynebacterium*)

4) Effect on metabolites in blood and urine

Mice: YMW (‘ 1-Methylhistidine)

Pigs: Results awaiting



Take away home message

Changes in gut microbiota and mucosal gene expression might have short-term and/or long-term consequences for (intestinal) health.



Therefore, the potential health effects of protein sources apart from their nutritional values, should be taken into account when preparing animal diets.

Bonus slide: Upcoming conference

Conference name: Protein for life

Date: 23rd – 26th of Oct 2016

Venue: Ede, The Netherlands

For more information,
visit the official
website

The screenshot shows the Wageningen UR website for the 'Protein for life' conference. The header includes the Wageningen UR logo and navigation links for 'About Wageningen UR', 'Career', 'Contact Wageningen UR', and 'Login'. The main navigation bar features 'Home' and 'Protein for life'. The page content includes a 'Congress' section with the title 'Protein for life' and a description of the conference's focus on future trends in nutrition and protein sources. A contact card for HS (Hedy) Wessels-van Blijenburgh is visible, along with a 'Contact form' button. A large image of various grains and seeds in wooden spoons is featured. Below the main text, there is a table with details: Organised by Wageningen UR Food & Biobased Research, Wageningen UR; Date: Sun 23 October 2016 until Wed 26 October 2016; Venue: De Reehorst, Ede, the Netherlands. Social media sharing options for mail, Google+, Twitter, LinkedIn, and Facebook are provided. A list of conference themes and speakers is shown with expandable sections. A logo for 'Protein for life' is displayed, featuring icons for a factory, a fork and knife, and a chicken. 'Important dates' are listed, including deadlines for abstract presentations and posters. Two prominent orange buttons for 'Registration' and 'Submission abstract' are located at the bottom right. The footer of the website includes the Wageningen UR logo and the tagline 'For quality of life'.

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Protein for life

Future trends in nutrition for both humans and animals show that the formulation of food and feed products will face an increasing challenge of a global protein shortage. Solutions to this challenge require a multi-targeted approach including efficient use of existing protein sources, and development of new protein sources for human and animal consumption. The Protein for Life conference will explore potential solutions combining the broad expertise of industry and knowledge institutes!

Contact
HS (Hedy) Wessels-van Blijenburgh
[Contact form](#)

Organised by Wageningen UR Food & Biobased Research, Wageningen UR

Date Sun 23 October 2016 until Wed 26 October 2016

Venue De Reehorst, Ede, the Netherlands

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- + Conference themes
- + Keynote speakers
- + Organising Committee
- + Scientific Committee
- + Programme
- + Registration fee - terms of payment
- + Practical information
- + Publication conference abstracts in a NJAS

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Protein for life

Important dates

- 15-08-2016
Deadline abstract oral presentations
- 29-08-2016
Notification of abstract acceptance
- 15-09-2016
Deadline abstract posters

Registration

Submission abstract

Thank you



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