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

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Knowledge partners

Industrial partners

Supporting partner

IPOP CN team of

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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

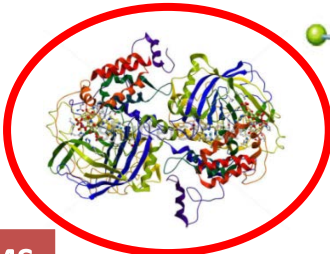
- To assess functional properties of new/alternative protein sources using animal models (mice and pigs)

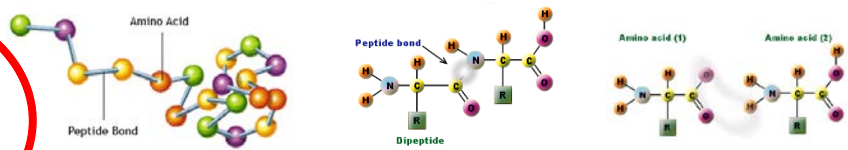
Topics of today's presentation

- 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

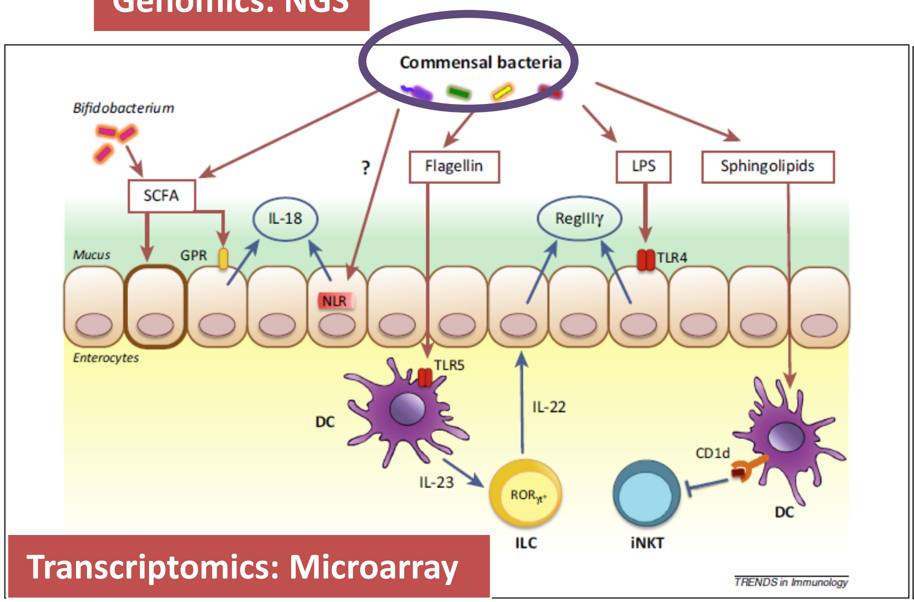
Digestion of proteins in gastro-intestinal tract



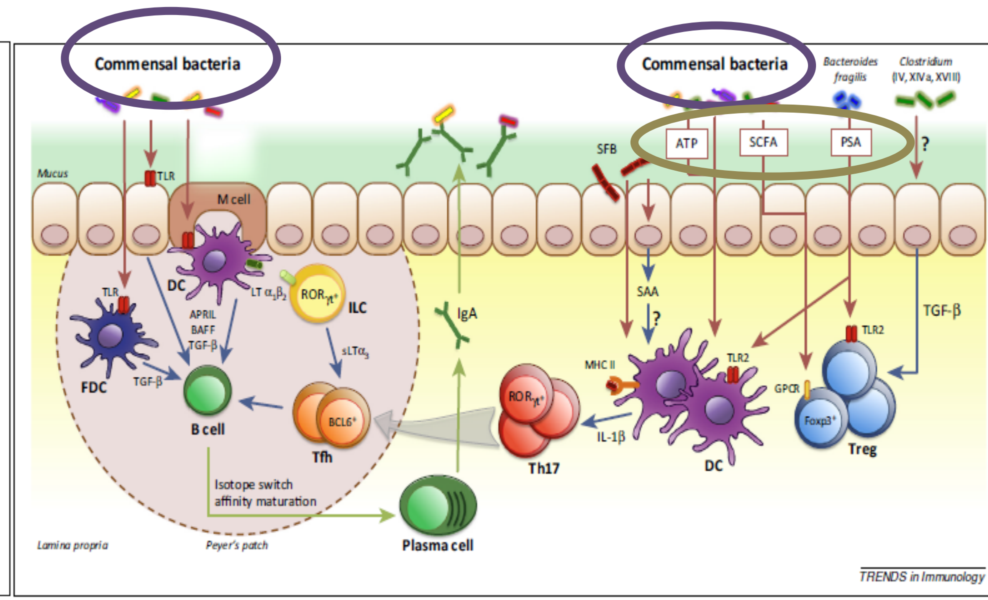


Proteomics: MS

Genomics: NGS

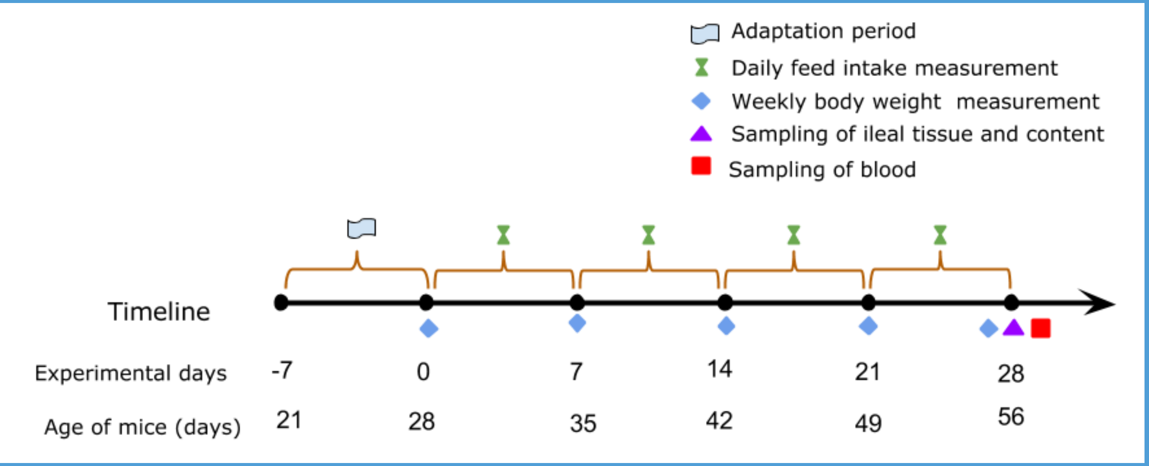


Transcriptomics: Microarray



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

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Kar et al. 2016 (submitted)

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)

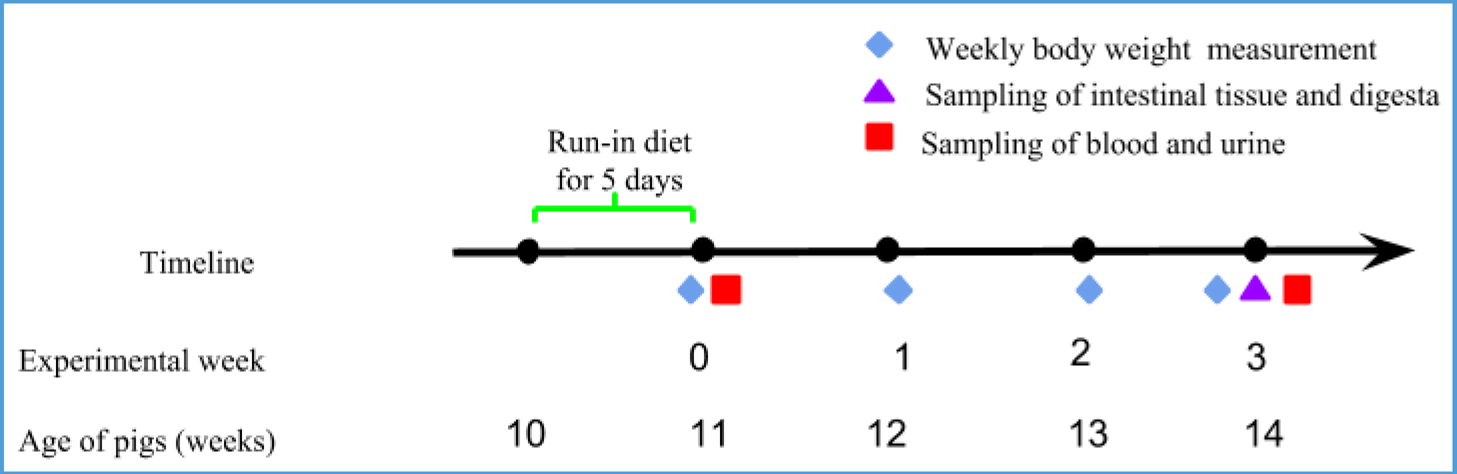
Housing: Individually

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

	Diet				
Item	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 grounded 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

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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

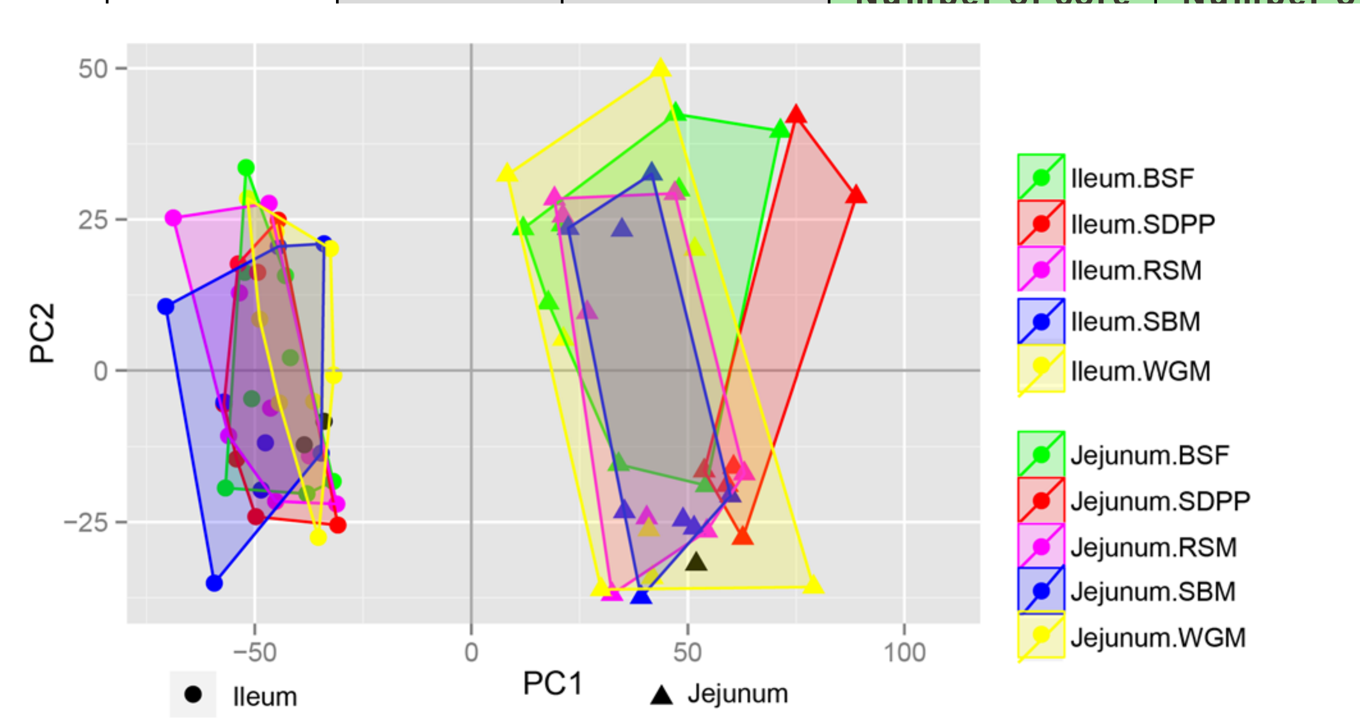
 Significant differences were observed

Pig study: Intestinal gene expression

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

Location: Jejunum and Ileum

Loc	Diets with	Number of	Number of	Number of core	Number of core genes ulated
Jeju					
Ile					



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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

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  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

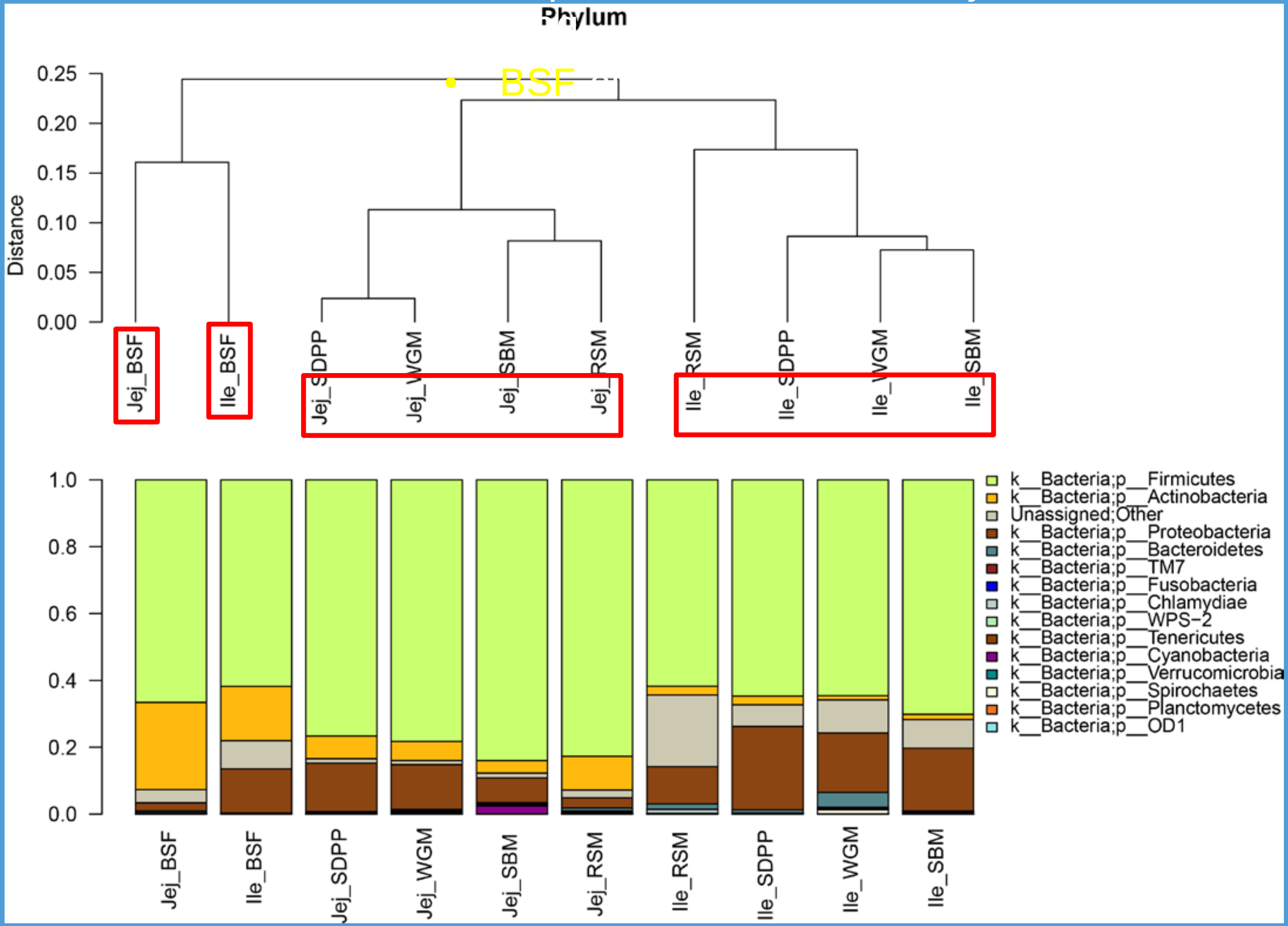
Analysis method: Community scale analysis by 16S RNA gene sequencing
SBM used as the reference

Location: Jejunum and Ileum

Pig study: Intestinal microbiota

RESULTS AND CONCLUSION

- Clear separation of location by hierarchical



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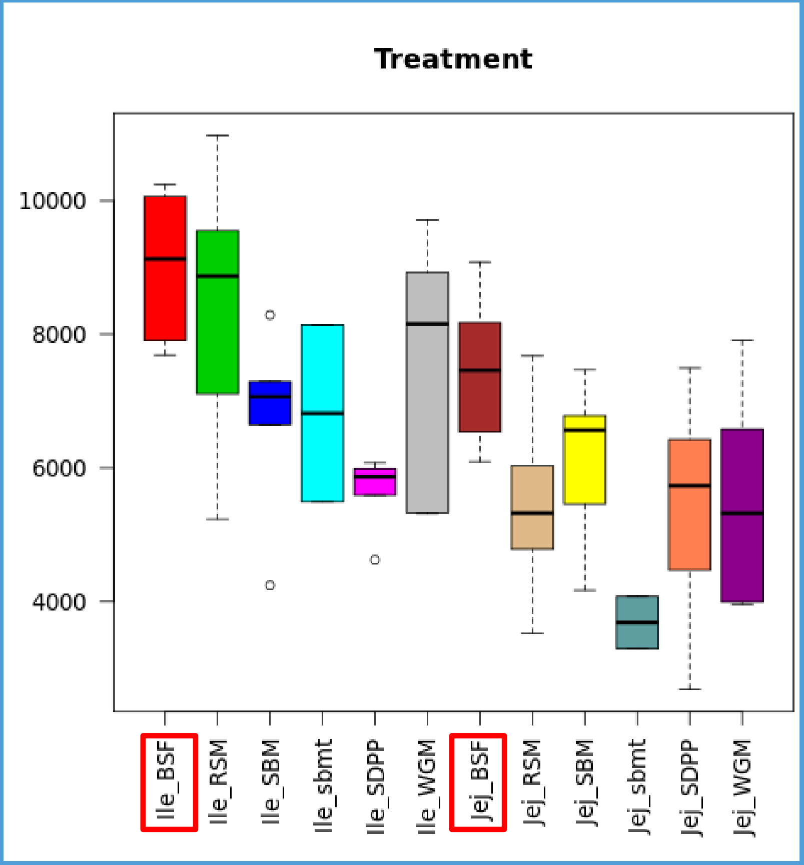
Pig study: Intestinal microbiota

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



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Pig study: Intestinal microbiota

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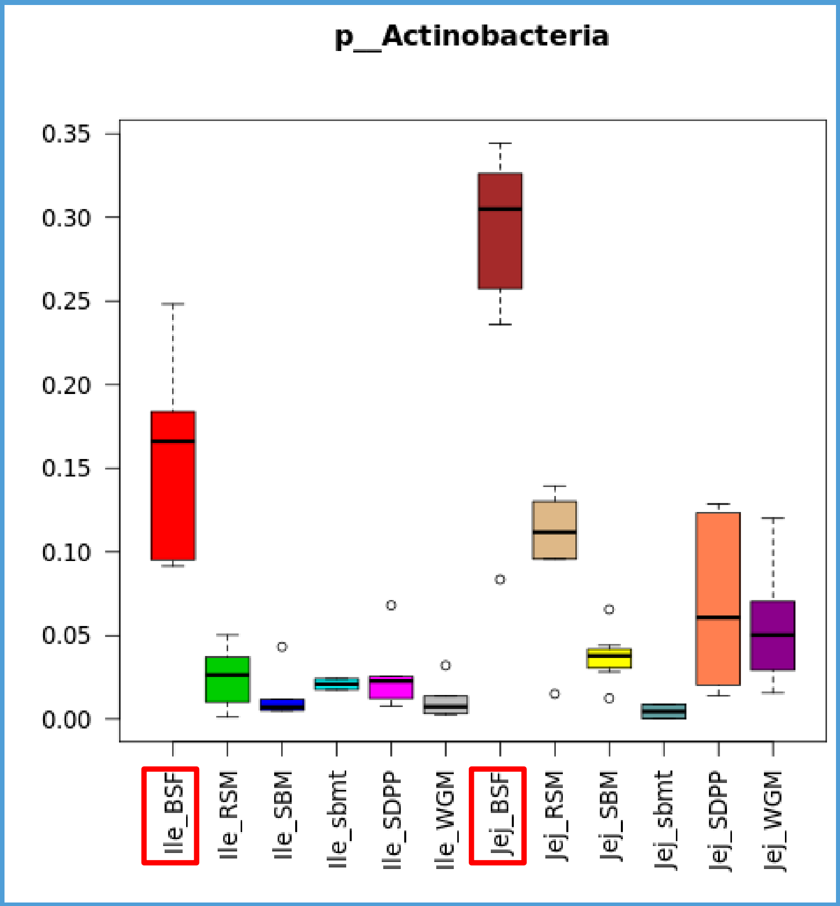
      - Higher abundance of *Actinobacteria* for BSF



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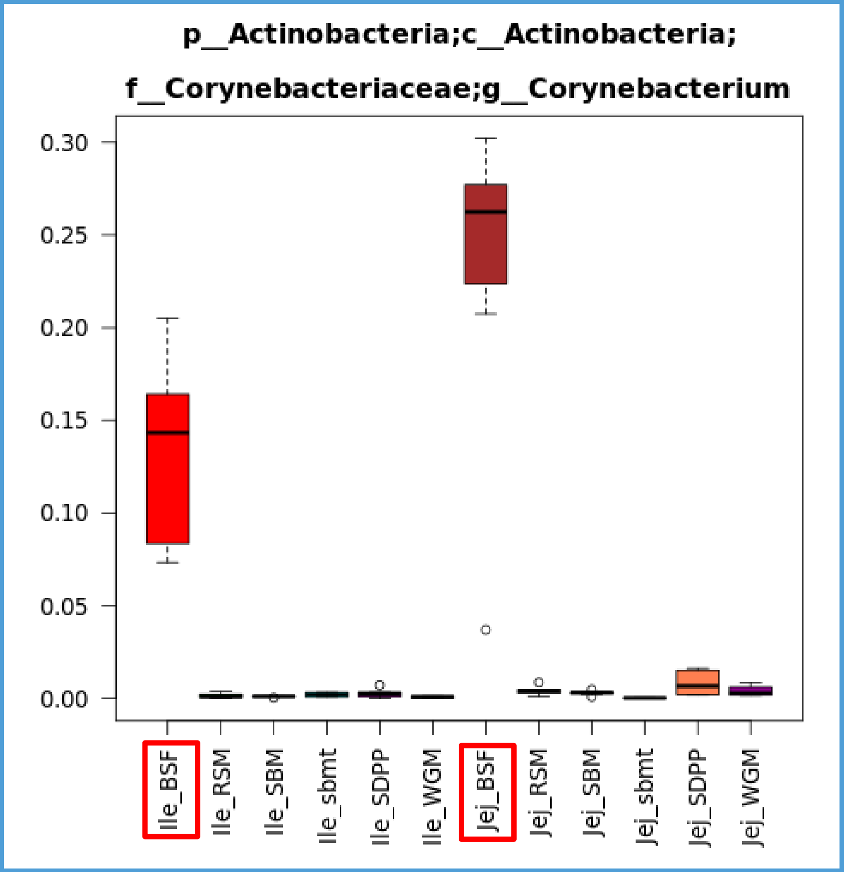

- Higher diversity in comparison to all the other treatments (in both location) by chao1 (alpha diversity): BSF
- Higher abundance of *Actinobacteria* for BSF
- Higher abundance of *Corynebacterium* for BSF

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Intestinal microbiota: Mice vs Pigs

Comparison of salient results of mice and pigs study

- <u>Mice:</u>
  - SBM deviated strongly from the other diets: ↑ abundance of *Bacteroidales* Family *S24-7*
- <u>Pigs:</u>
  - 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, IFNγ, 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

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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.

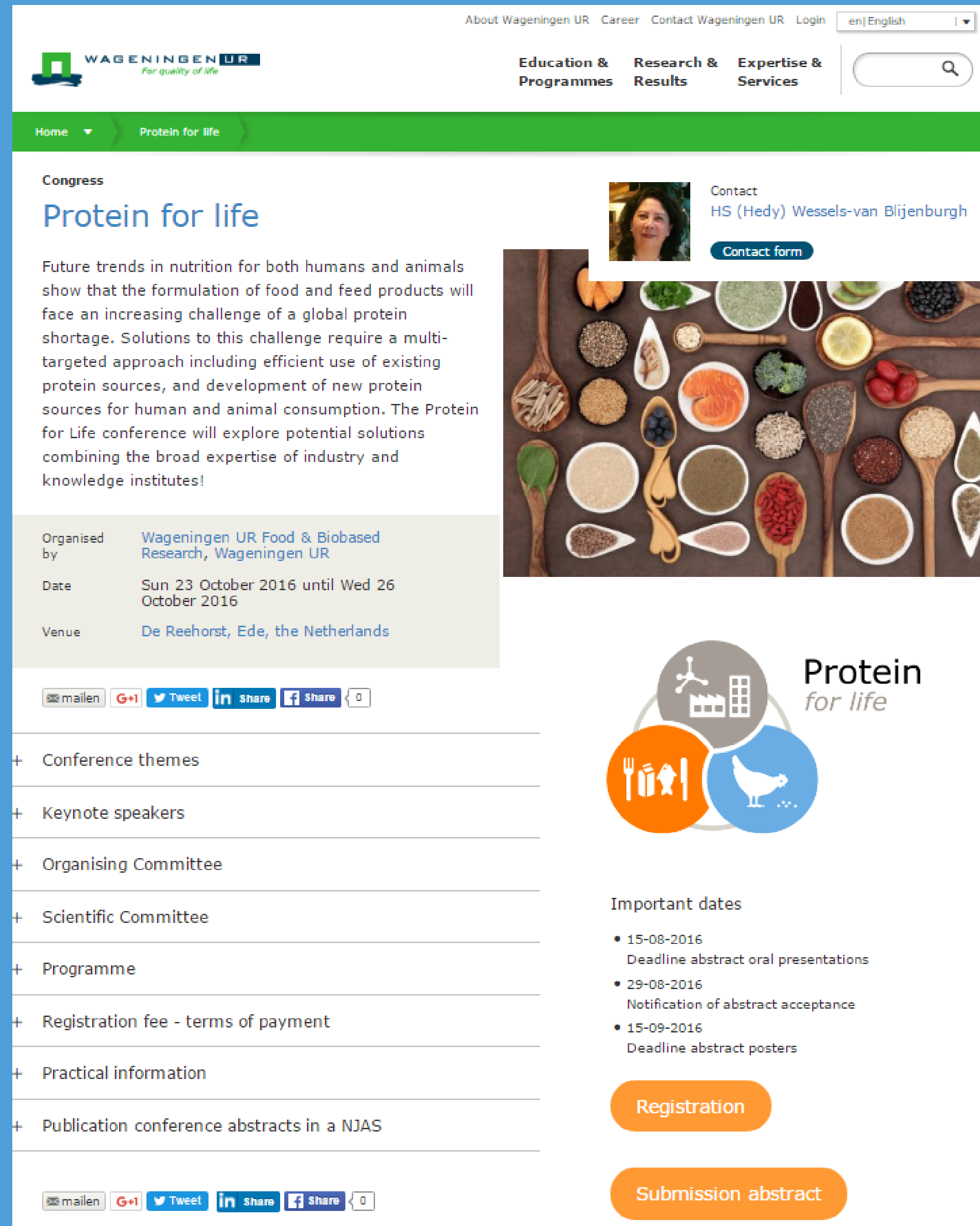
Bonus slide: Upcoming conference

Conference name: Protein for life

Date: 23rd – 26th of Oct 2016

Venue: Ede, The Netherlands

For more information, visit the official website



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