

# Alternative protein sources for monogastrics: composition and functional assessment

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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)
- To elucidate underlying mechanisms



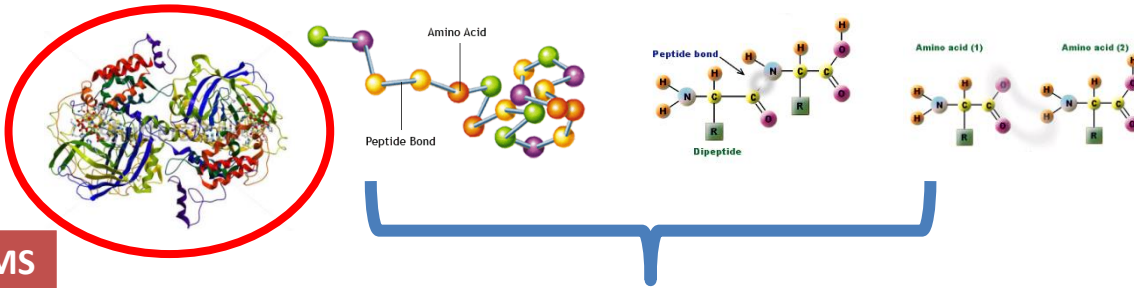
# Topics of today's presentation

- 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)
- To elucidate underlying mechanisms



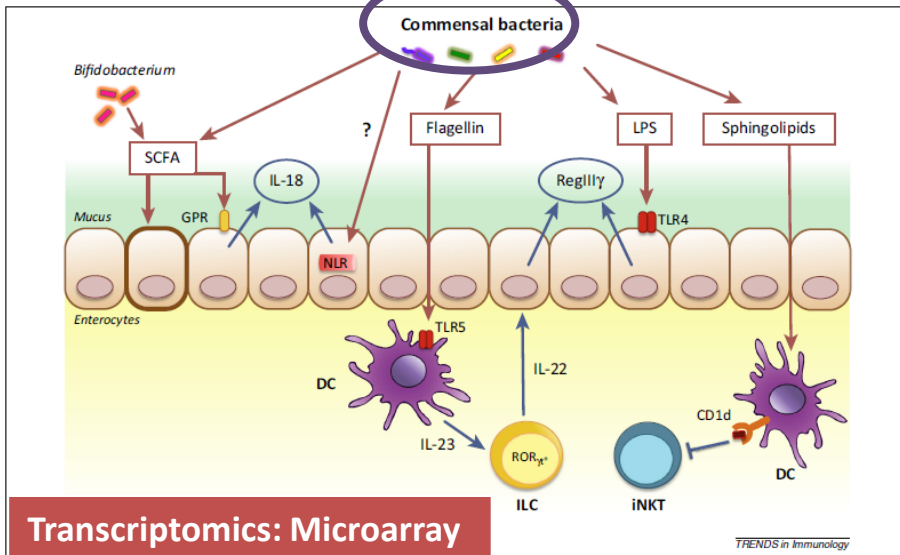
# In vivo digestion process

## Digestion of proteins in gastro-intestinal tract

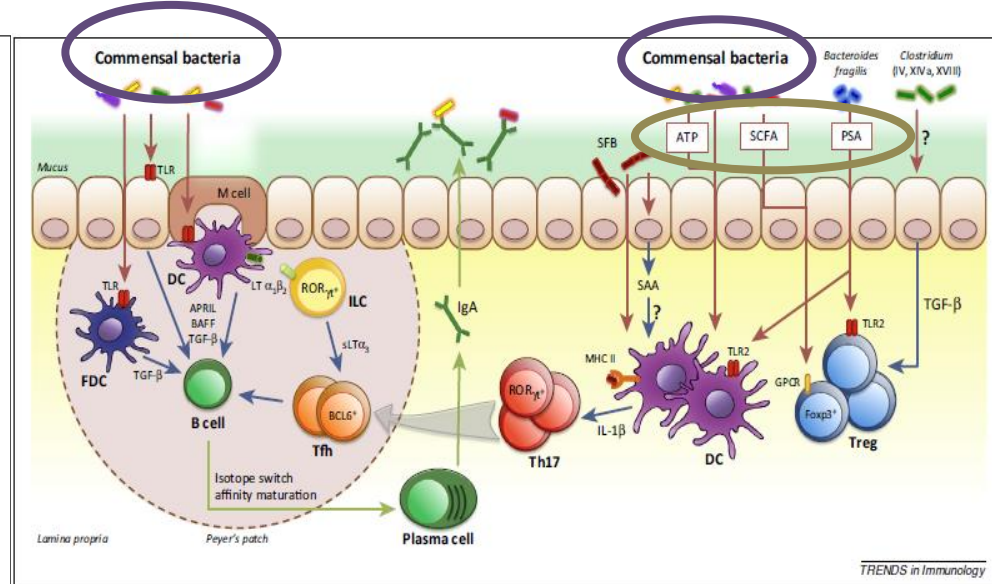


Proteomics: MS

Genomics: NGS



Transcriptomics: Microarray



ELISA

Cytokines

Systemic effects

Metabolites

MS/ GC

# Proteomic analysis of feed ingredients

Feed  
Ingredients

Proteomics  
analysis  
MS

Bioinformatic  
analysis

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

Protein Sample  
(Filter Aided  
Sample  
Preparation)

Fragmented using  
enzyme

Spectrum of  
fragment  
generated

Identified  
peptides  
and  
proteins

Results

Library

Match

Genomics driven database



# Bioinformatic analysis of feed ingredients

List of identified peptides and proteins

Selected top 90% of the of the total calculated protein content

Amino acid composition (ACC) prediction

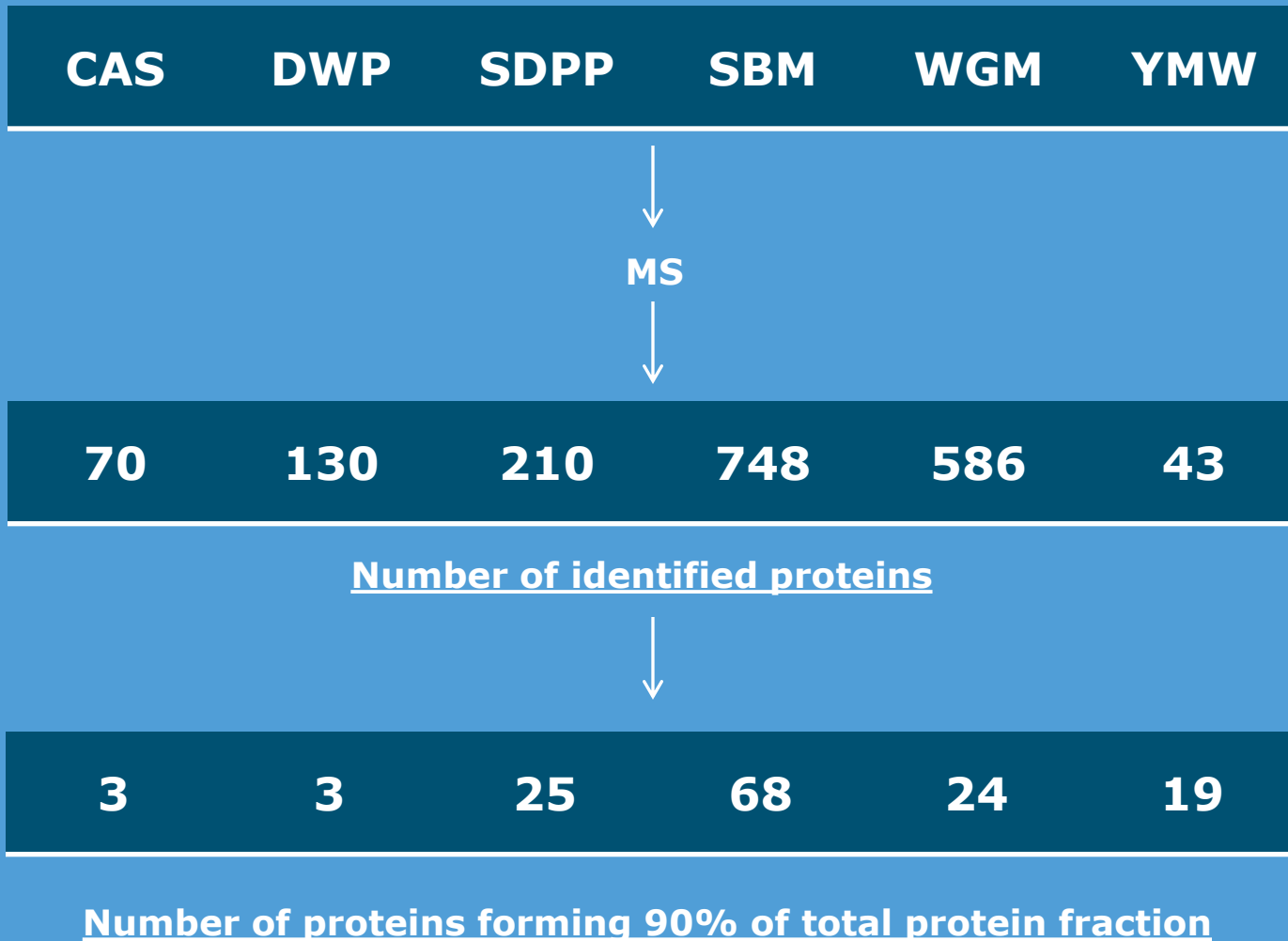
	A	B	C	D
1	CAS			
2				
3	Protein IDs	Proteins count	Peptide counts (all)	iBAQ
4	P02668	1	3	9.7E+08
5	P02663	1	14	6.25E+08
6	P02662	1	11	3.98E+08
7	P02666	1	5	1
8	P02754	1	10	4
9	P01888	1	3	1
10	F1MCF8;F	2	5	
11	F1MXX6;C	3	11	
12	P10790;F1	4	6	
13	P00711	1	4	6577300
14	P81265;F1	4	14	2057700
15	F1MUT3;P	2	15	1430200
16	P24627	1	15	1249900
17	P11151	1	5	826010
18	F1N1N6;Q	4	2	635070
19	Q0P569	1	4	471980
20	P80025	1	4	464300
21	E1BGX8	1	7	423930
22	G8JXX4;P	9	2	376620
23	G3X7A5;Q	2	15	359580
24	F1N726	1	7	299000
25	A6QLZ7	1	3	290000

*In silico* digestion with pepsin, trypsin and chymotrypsin

Prediction of bioactivity

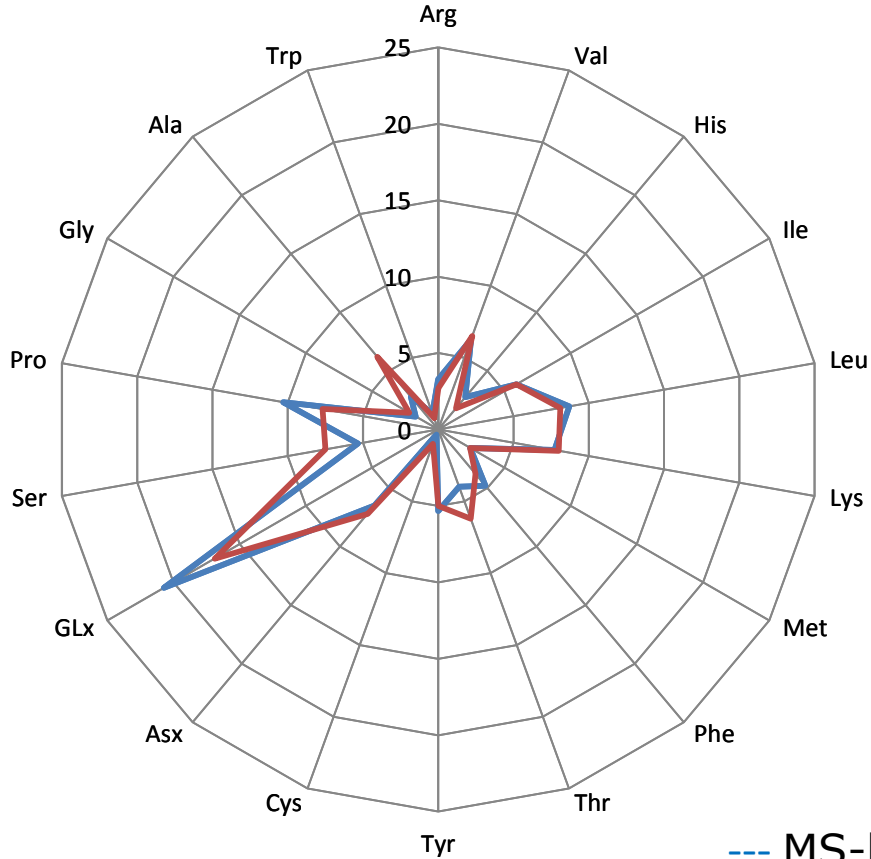
Compare ACC values obtained with conventional analytical method

# Results: proteomic analysis



# Results bio-informatic analysis: amino acid composition

CAS



r: 0.94

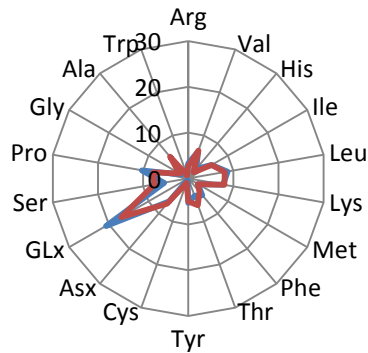
--- MS-based predicted ACC  
--- Chemically defined ACC



# Results bio-informatic analysis: amino acid composition

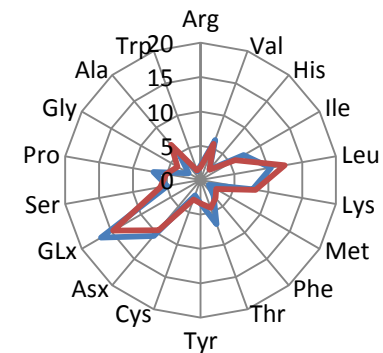
CAS

r: 0.94



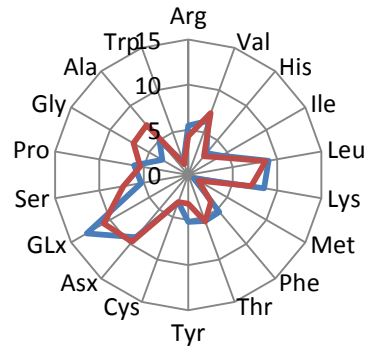
DWP

r: 0.94



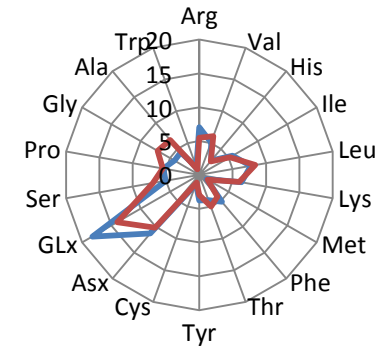
SDPP

r: 0.86



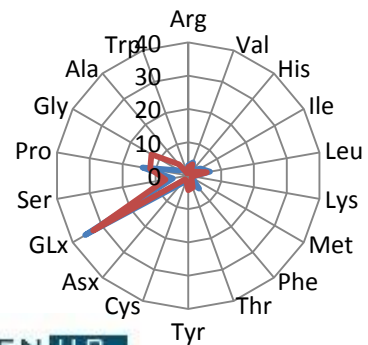
SBM

r: 0.92



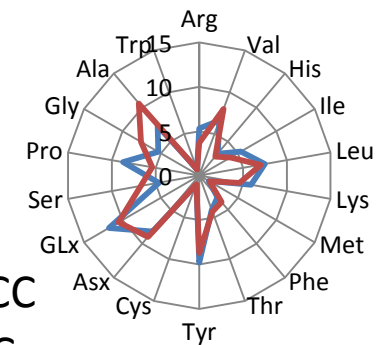
WGM

r: 0.94



YMW

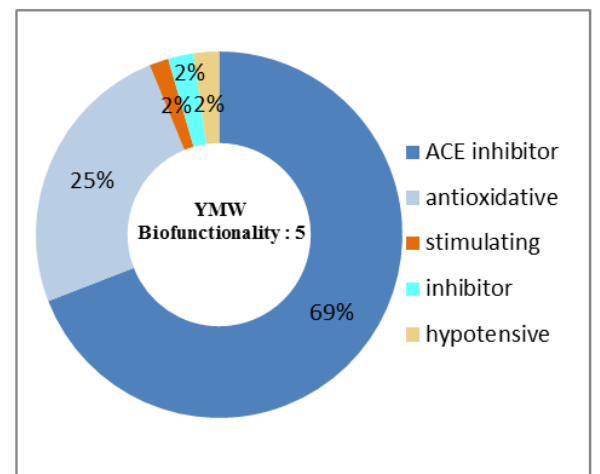
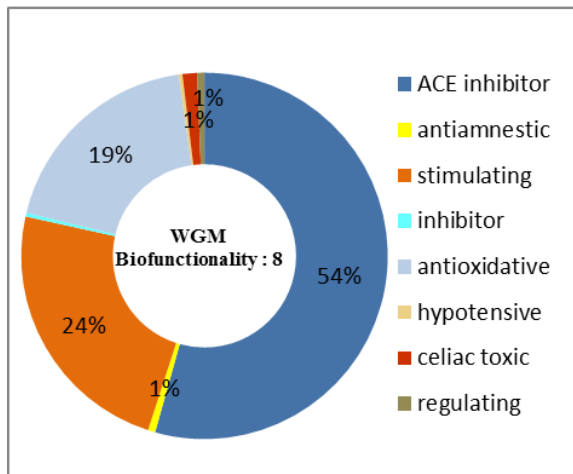
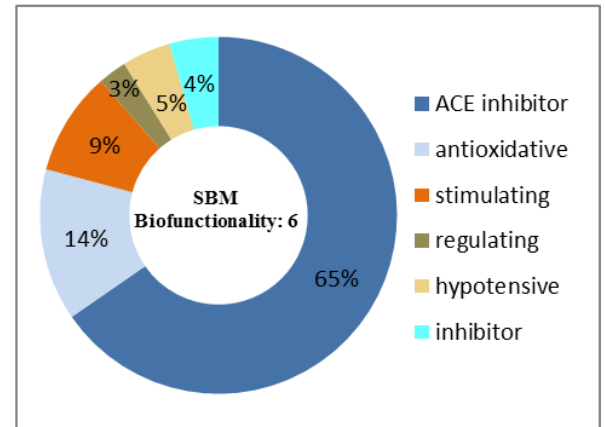
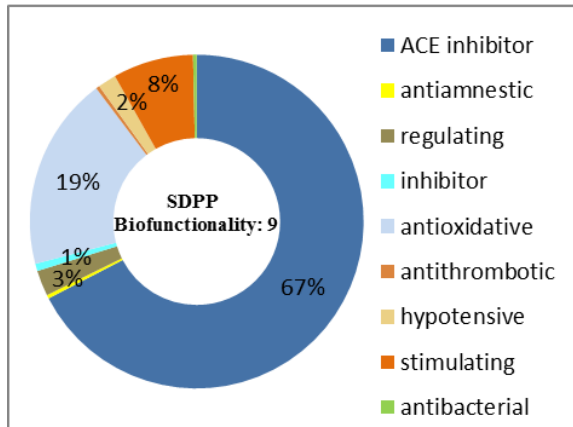
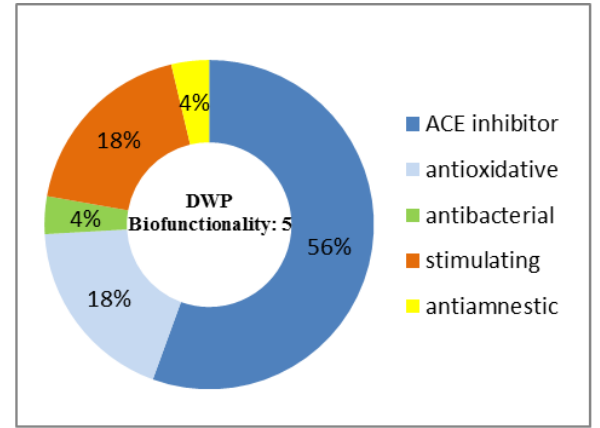
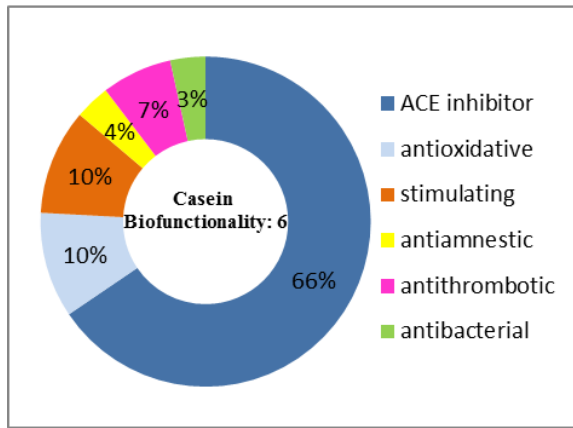
r: 0.87



--- MS-based predicted ACC  
--- Chemically defined ACC

# Results: bioactivity

## Proportion of bio-functional properties of proteins



# Proteomics/bioinformatics analysis: conclusions

- MS-based analysis provides more detailed information on the composition of complex protein sources compared to conventional (nutritional) analytical approaches.
- MS-based analysis allows the detection of individual proteins in complex matrices at very high resolution.
- MS-based approach was effective in predicting the amino acid composition of protein sources.
- MS-based analysis allows the prediction of bio-functional properties of protein.



# Layout of mice experiment

Animals: C57B

Chemical composition of the experimental diet

was replaced by the “protein

Sex of Animal : Male



derived from new sources”. The diets were identical with respect to all other nutrients compared to AIN 93-G

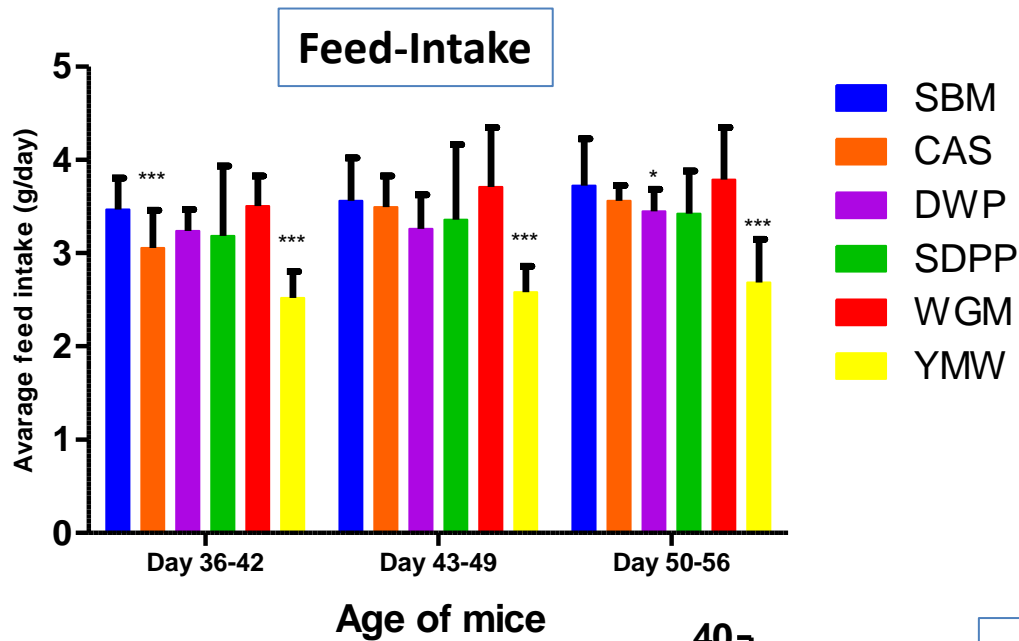
Item	Diet					
	SBM	CAS	DWP	SDPP	WGM	YMW
Dry matter, g/kg	928 (914)	931 (957)	944 (930)	933 (924)	936 (917)	942 (929)
Crude protein, g/kg	150 (152.9)	263 (267.8)	79 (79.6)	246 (247.9)	246 (252.5)	148.3 (148.3)
Ash, g/kg	50 (43)	40 (29)	83 (77)	49 (28)	52 (47)	41 (35)
Crude fibre, g/kg	55	44	44	44	46	69
Crude fat, g/kg	78 (75.5)	73 (65.3)	86 (73.7)	77 (86.6)	87 (70.3)	174 (160.1)
Starch, g/kg	251	249	249	249	268	261
Sugar, g/kg	295	263	403	263	271	263
NSP, g/kg	71	8	17	14.9	2	18
Gross energy, KJ/g	(17.1)	(18.6)	(16.3)	(18.4)	(18)	(19.8)
Ca, g/kg	5.9	5.5	10.0	5.2	5.2	6.1
P, g/kg	3.7	3.3	6.2	1.9	2.3	4.0
K, g/kg	4.4	4.0	16.5	4.5	4.0	3.6
Na, g/kg	1.1	1.2	5.9	8.2	1.3	1.0
Cl, g/kg	1.7	3.1	4.4	12.7	1.9	1.6
Linoleic acid, g/kg	37.7	35.8	35.8	35.8	35.8	35.8
Electrolyte balance, Meq	266	94	388	115.2	106	92

Timeline

Experimental days  
Age of mice (days)

- ▲ Daily feed intake measurement
- ◆ Weekly body weight measurement
- ▲ Sampling of ileal tissue and content
- Sampling of blood and urine

# Performance parameters

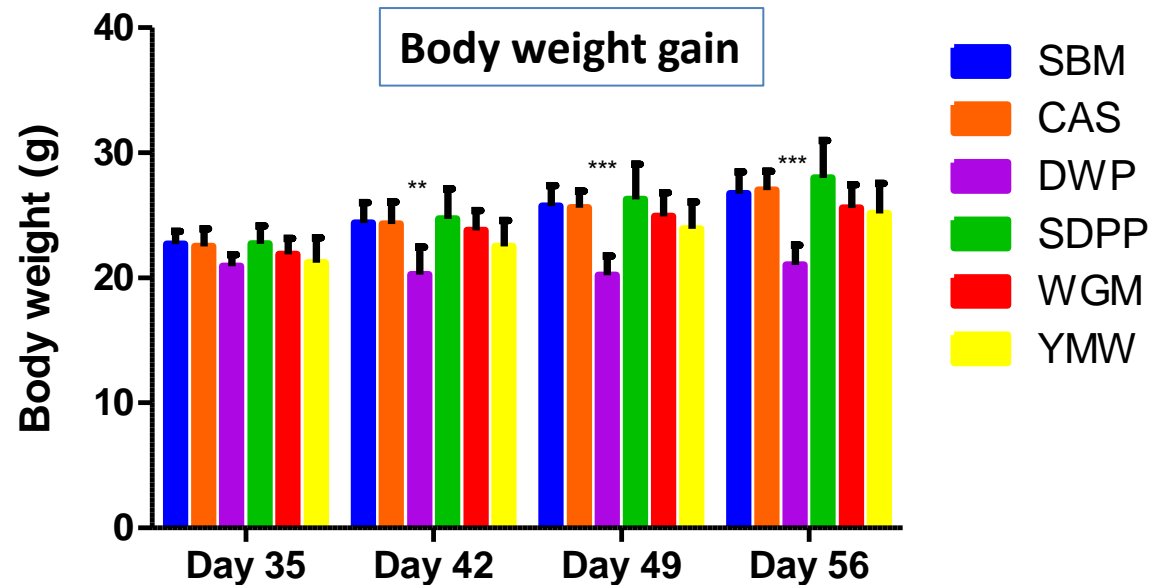


Bars and whiskers represents means values  $\pm$  SD (n = 6).

\*P < 0.05

\*\* P < 0.01

\*\*\* P < 0.001

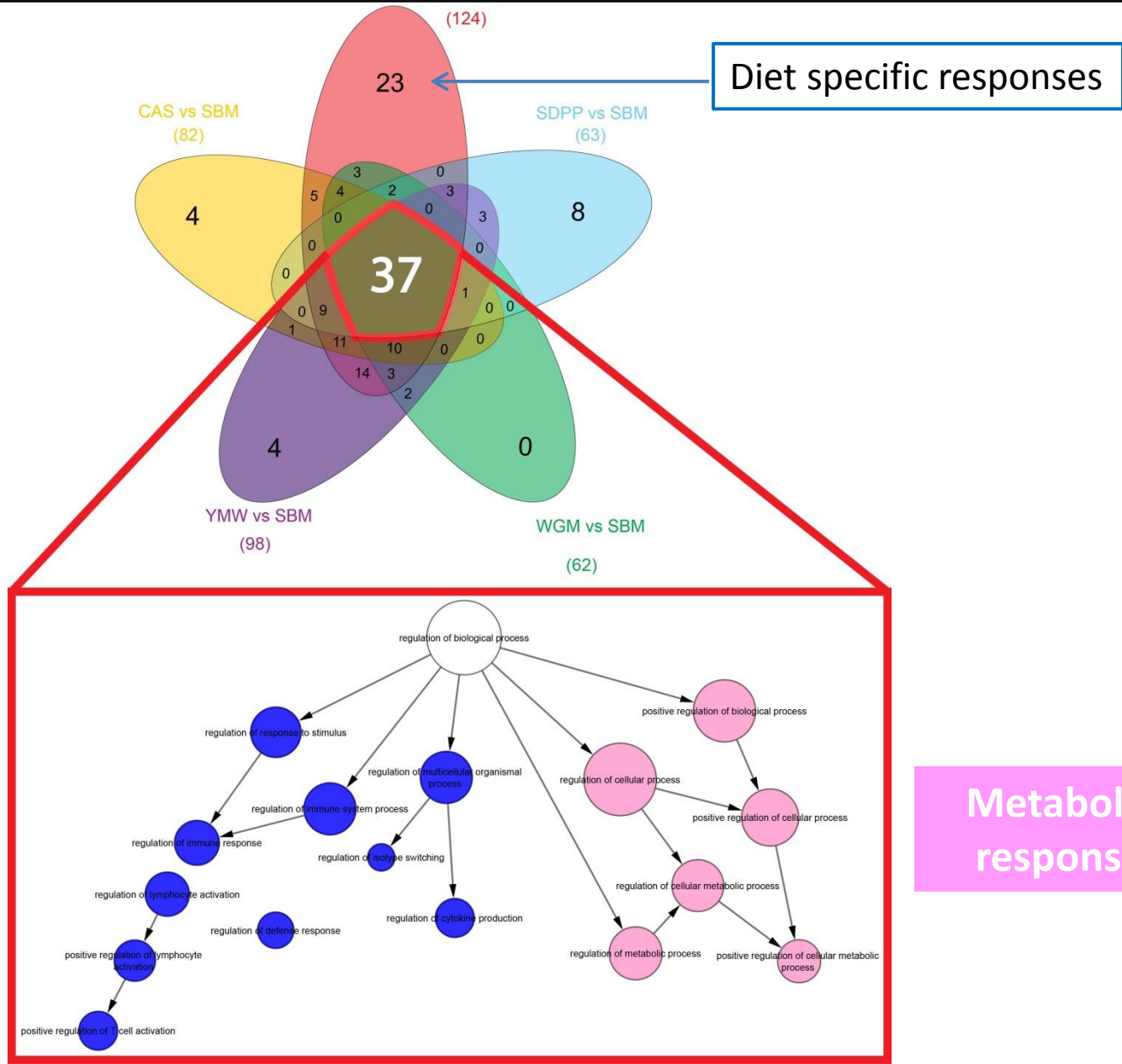


# Local response: Ileal gene expression profile (microarray)

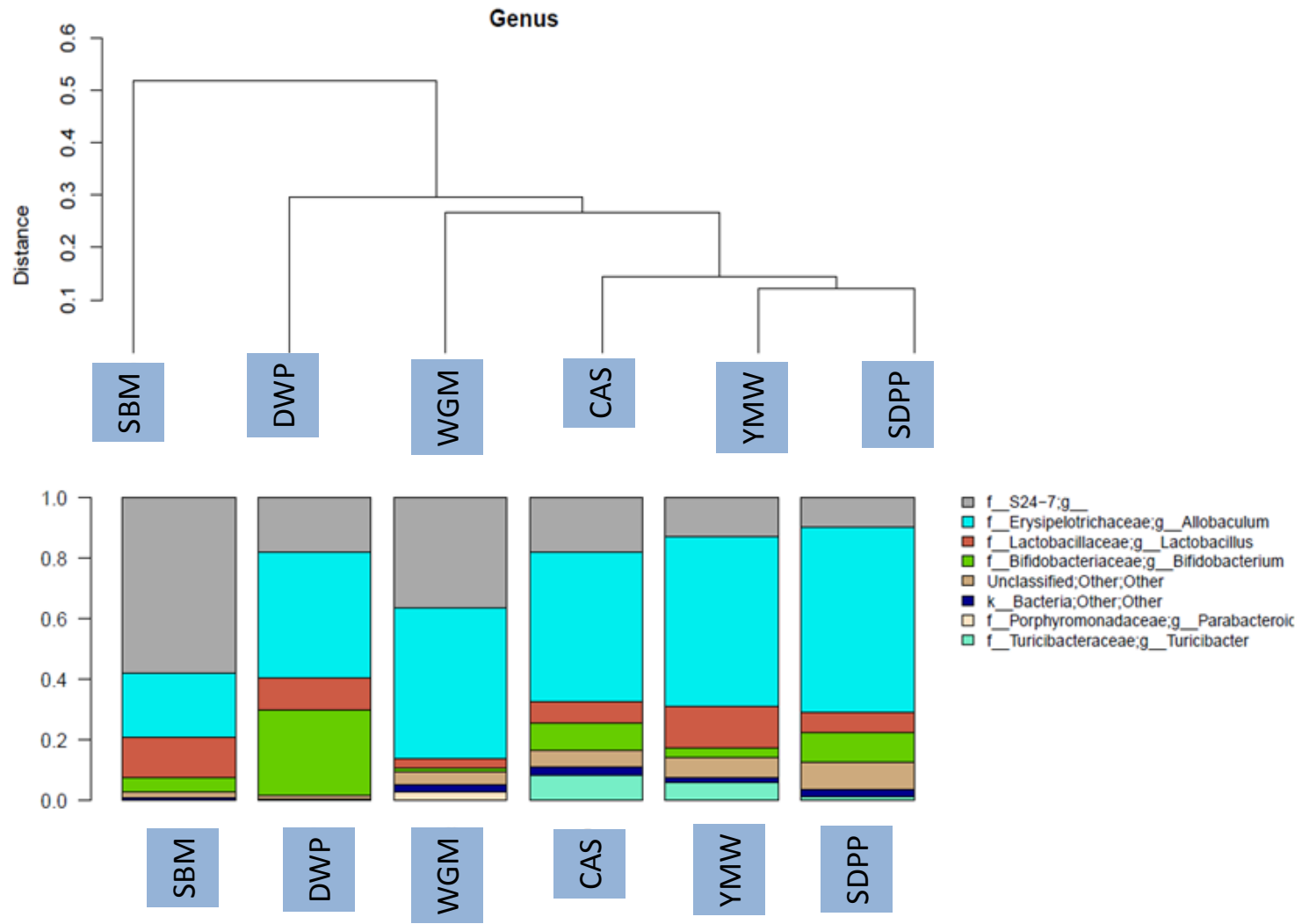
Comparison of experimental diet vs SBM	Number upregulated gene-sets	Number down regulated gene-sets	Number of significantly enriched gene-sets (FDR < 0.05)
<b>CAS</b>	559	0	<b>82</b>
<b>DWP</b>	561	0	<b>124</b>
<b>SDPP</b>	591	0	<b>63</b>
<b>WGM</b>	516	0	<b>62</b>
<b>YMW</b>	559	0	<b>98</b>



# Local response: Functional analysis of gene expression data at ileal tissue



# Local response: ileal microbiota



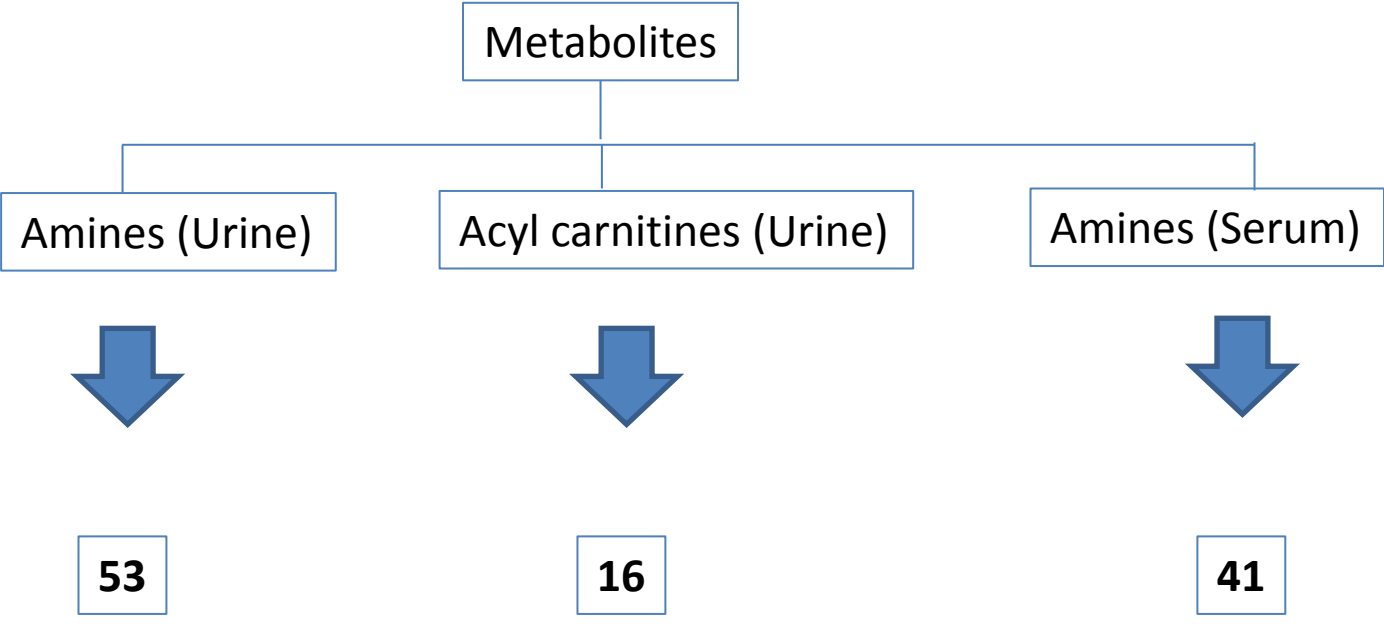


# Systemic response: Cytokines and Chemokines in blood

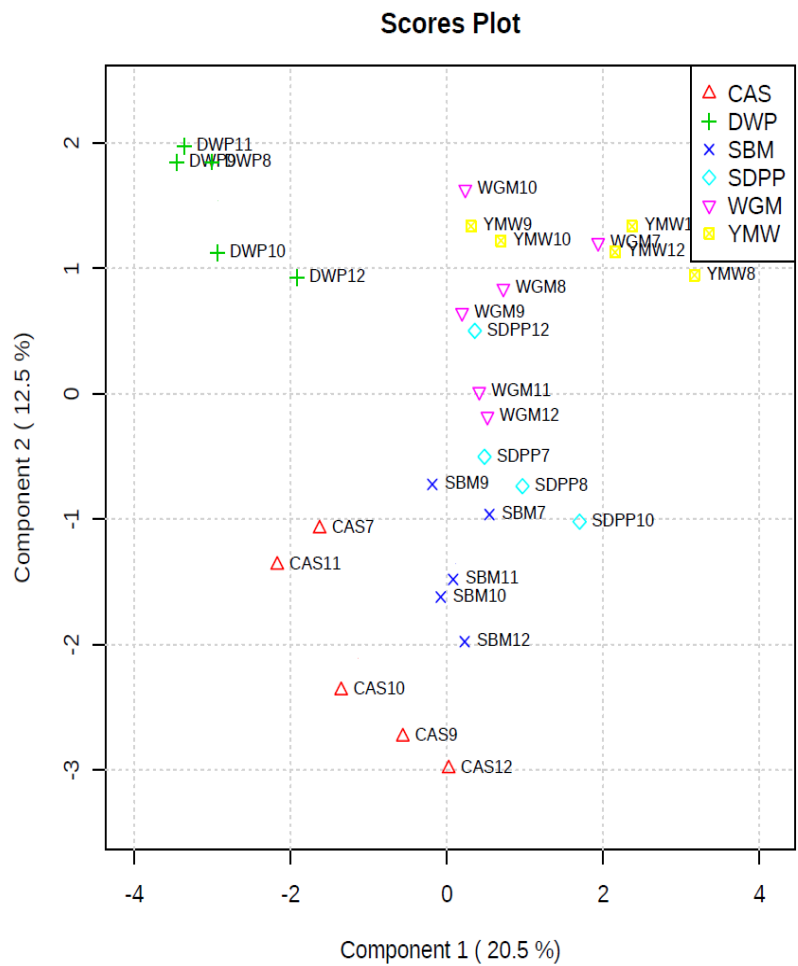
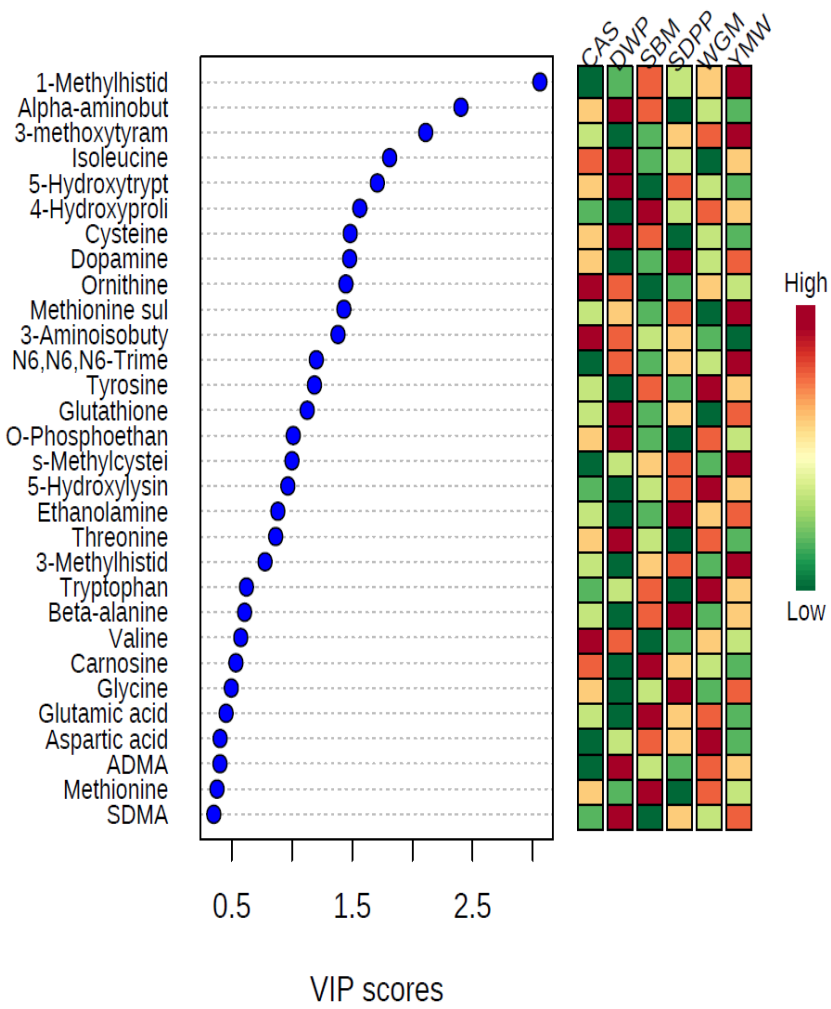
- Panel of 23 biomarkers were analysed
- Increase of granulocyte colony stimulating factor (G-CSF) in SBM fed mice
- Increase of Eotaxin for DWP- and YMW-fed mice
- Increase of IL-12p70 in DWP-fed mice
- Decrease of G-CSF, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-5, IL-6, IL-13 and monocyte chemotactic protein (MCP) in WGM-fed mice compared to SBM-fed mice



# Systemic response: Metabolites



# Systemic response: Amines, urine



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# Mice experiment: conclusions and discussion

- Diets based on different protein sources affect host responses:
  - local (ileal microbiota, expression of (immune-related) genes)
  - systemic (serum cytokines/chemokines and urine metabolites)
- SBM differs clearly from the other experimental diets
- Diet specific effects identified (not shown here)
- DWP and YMW responses more similar to each other
- Knowledge may help to formulate monogastric diets



# Acknowledgement



Host-Microbe  
Interactomics



LIVESTOCK RESEARCH  
WAGENINGEN UR

Netherlands  
Metabolomics Centre

*nutreco*

**DARLING**  
INGREDIENTS  
INTERNATIONAL

IPOP CN team of



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