



# Protein Degradation in the Gastrointestinal Tract of Broilers

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# Aim of the Project

- Develop a proteomics platform to study protein dynamics in digesta samples from broilers
- Discovery project for targeted feed enzyme development

# Sample origin

Digesta samples from a broiler *in vivo* trial

- **Birds:** Ross PM3 birds (10 cages x 8 birds per cage)
- **Model diets:** Maize-soybean meal diet  $\pm$ 20% raw soy

Iso-caloric (12.6 MJ ME/kg)

Iso-nitrogenous (21.4% CP)

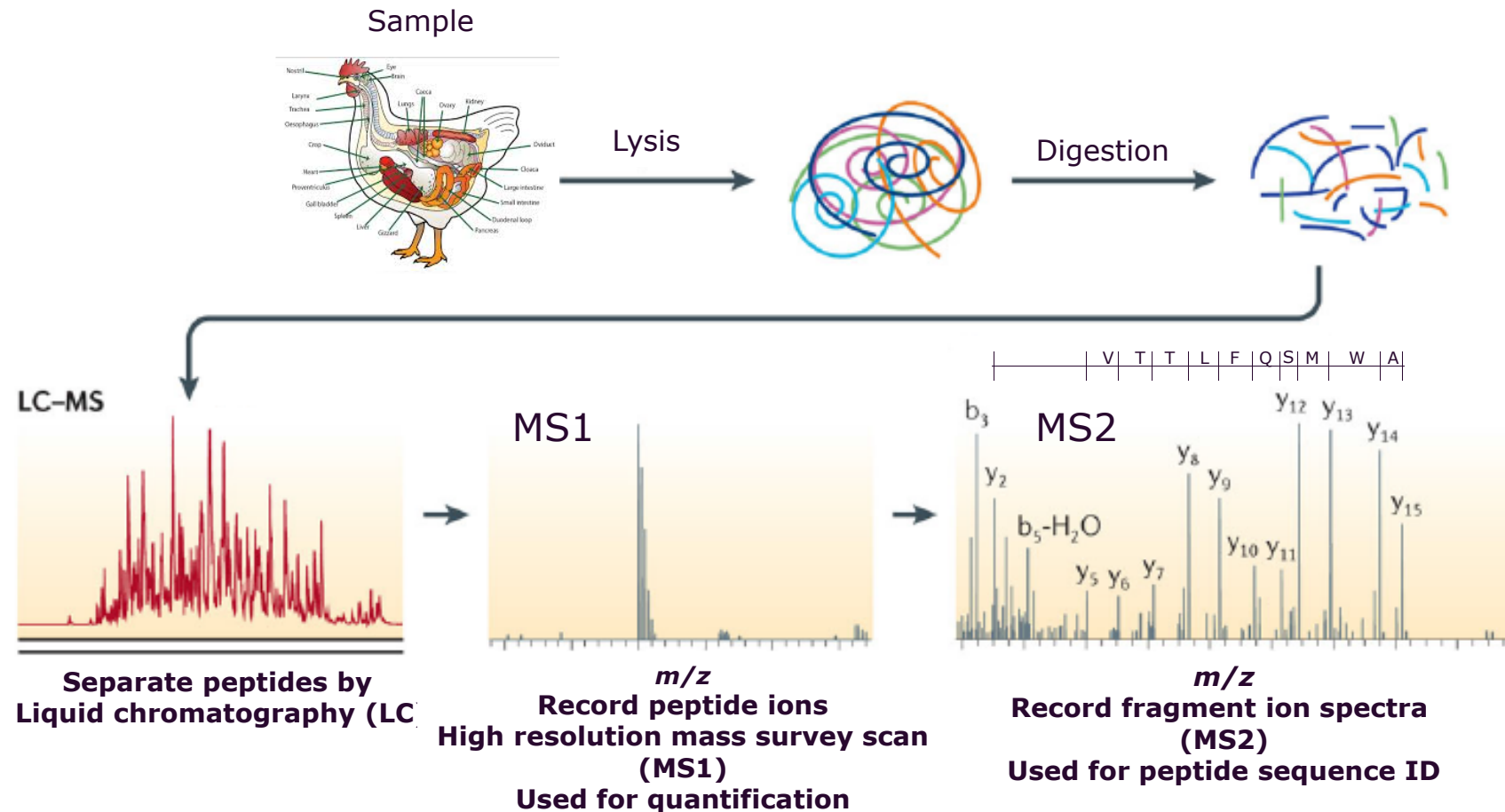
- **Trial duration:** Day 0-21 after hatch
- **Sampling site:** Terminal Ileum
- **Observed effects of raw soy inclusion *in vivo***
  - Decreased Nitrogen digestibility
  - Increased pancreas weight relative to body weight

Ingredients (%)	Normal diet (no raw soy)	Raw soy diet (20% raw soy)
Maize	53	52
SBM 48	39	24
Raw soybean		<b>20</b>
Soy oil	3,4	0,5
Other components	4,6	3,5

# Protein identification by LC/MS

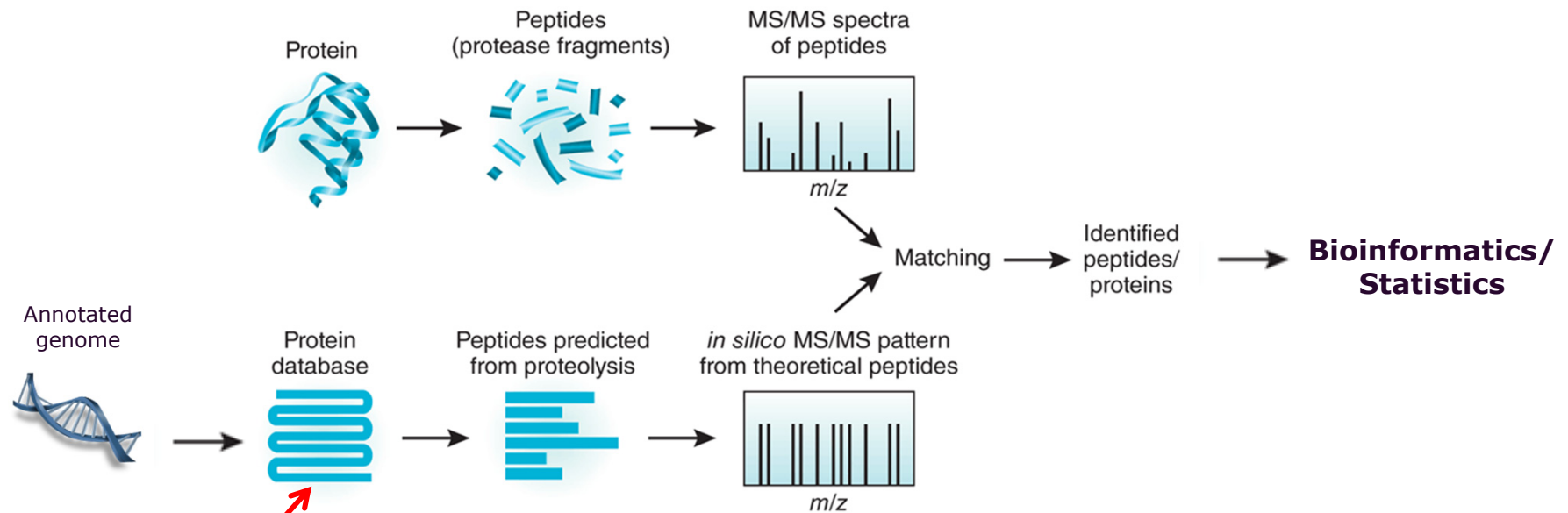
Proteomics: The large scale study of protein structure, function and dynamics

Generalized mass-spectrometry-based proteomics workflow:  
Shotgun mass spectrometry or bottom-up proteomics



# Peptide sequence ID

MS2 spectra are used for peptide sequence ID using specialized database search engines – Mascot (Matrix Science)



Uniprot database: *Gallus gallus*, *Glycine max* and *Zea mays* (drawn Feb 4<sup>th</sup>, 2014, [www.uniprot.org](http://www.uniprot.org))

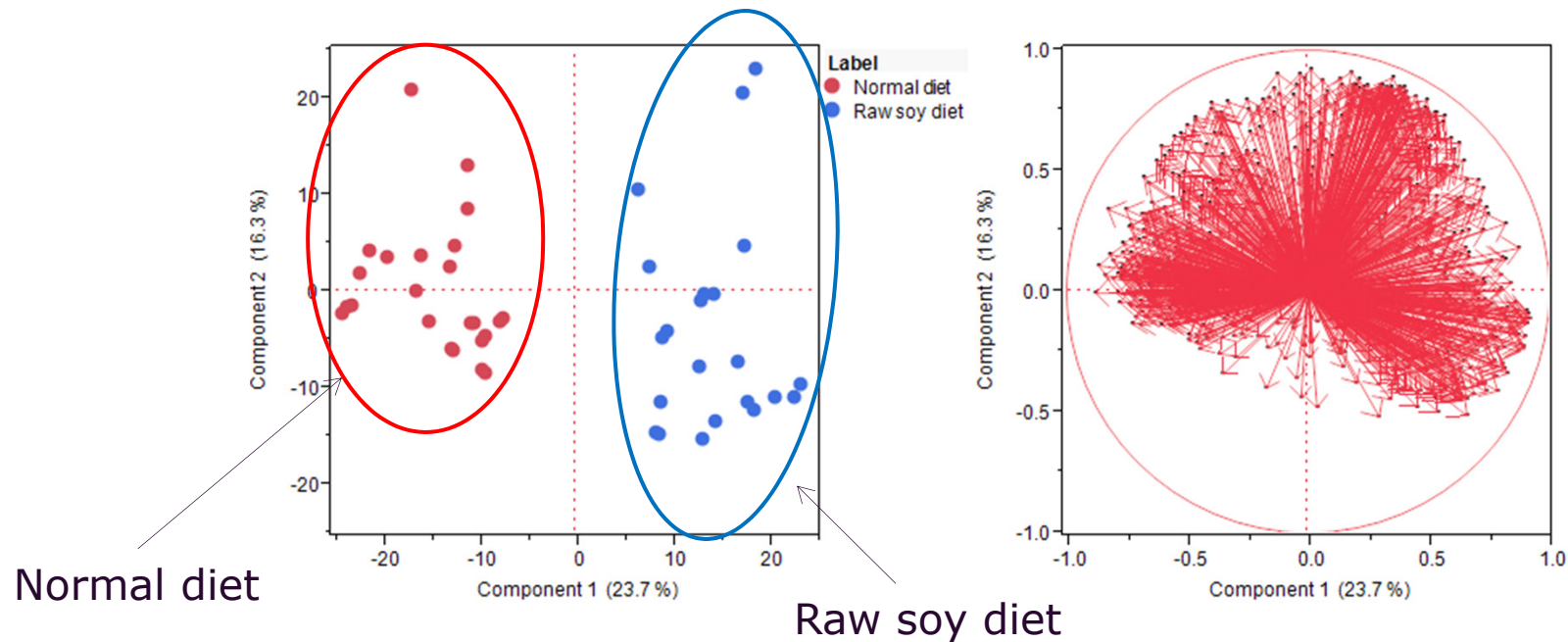
# Proteomics: Data analysis

2511 peptides ID – 1666 unique peptides = 999 proteins

2 diets x 8 birds x 3

Close to 48.000 data points in the full data set.

PCA on all proteins identified across all replicates



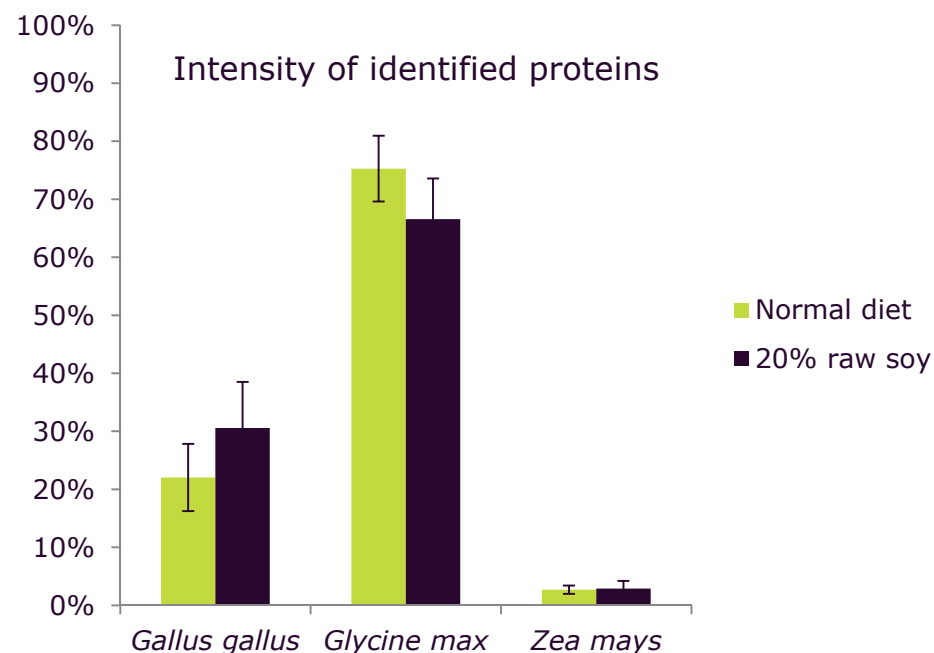
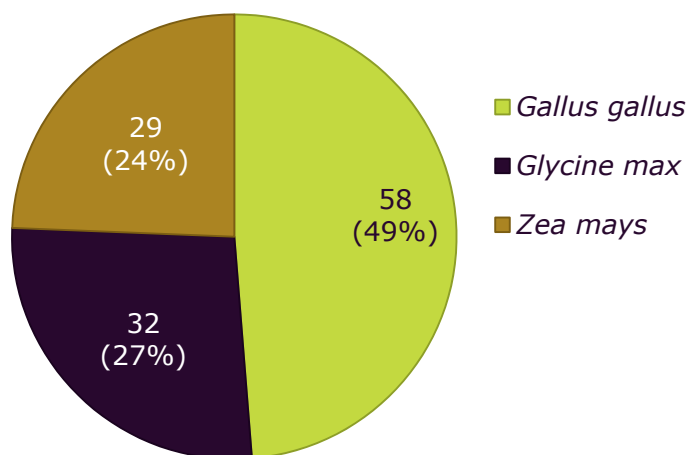
Clear effect of 20% raw soy in the model diet

## Terminal ileum – the numbers

999 proteins were identified from chicken, soy and corn in both normal and a raw soy diet

119 proteins were quantified with minimum of 2 unique peptides

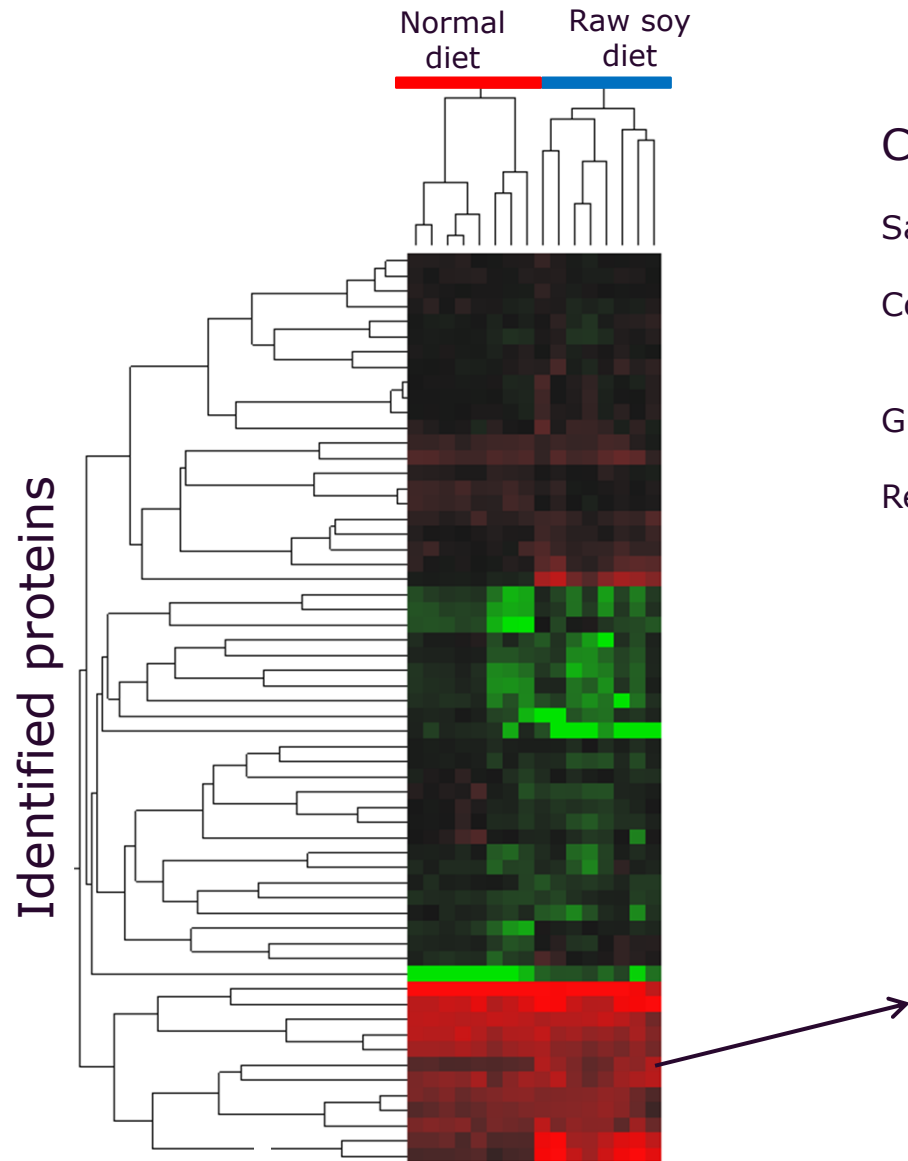
Number of identified proteins  
(normal & raw soy diet)



**Soy protein is 27% in numbers but up to 75% in intensity**

**Chicken is 50% in numbers but only 20-30% in intensity**

# Feed proteins



## Cluster analysis on soy and maize protein

Samples cluster nicely between the two diets

Color scale: Green (low) to black to red (High)  
range 6 orders of magnitude in abundance

Green: Low abundant protein – missing values

Red: High abundant protein

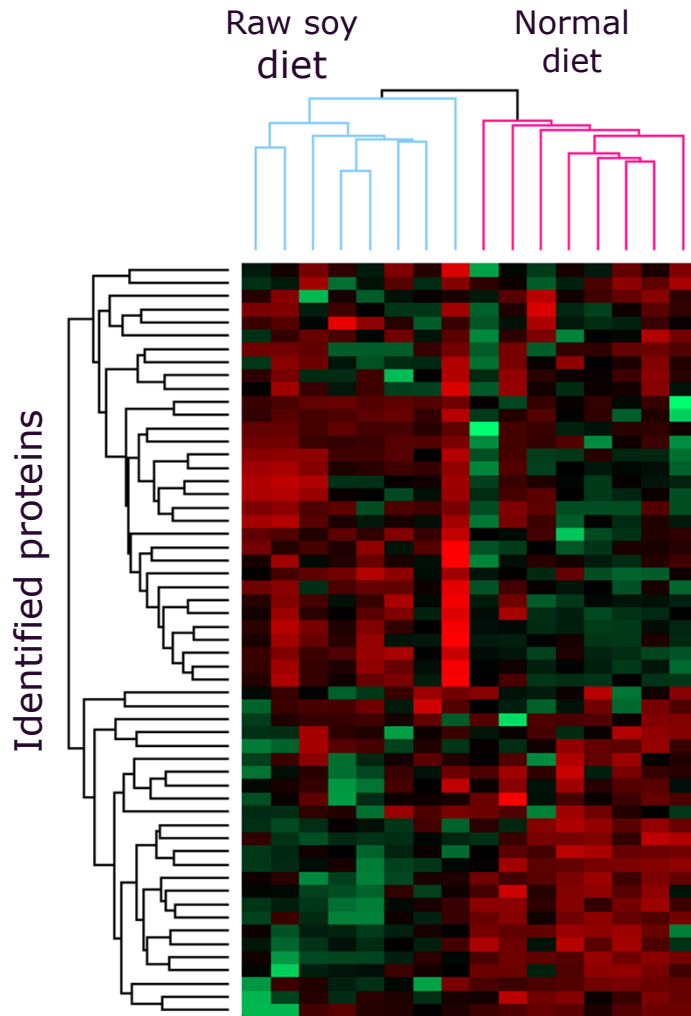
Organism	Description
Glycine max	Glycinin G1
	Beta-conglycinin
	Glycinin
	Basic 7S globulin
	Cupin 1
	Basic 7S globulin-2
	Lectin
	Glycinin G2
Zea mays	Kunitz inhibitor
	Beta-conglycinin alpha subunit
Zea mays	Glutelin-2

Primarily storage proteins



# Chicken proteins

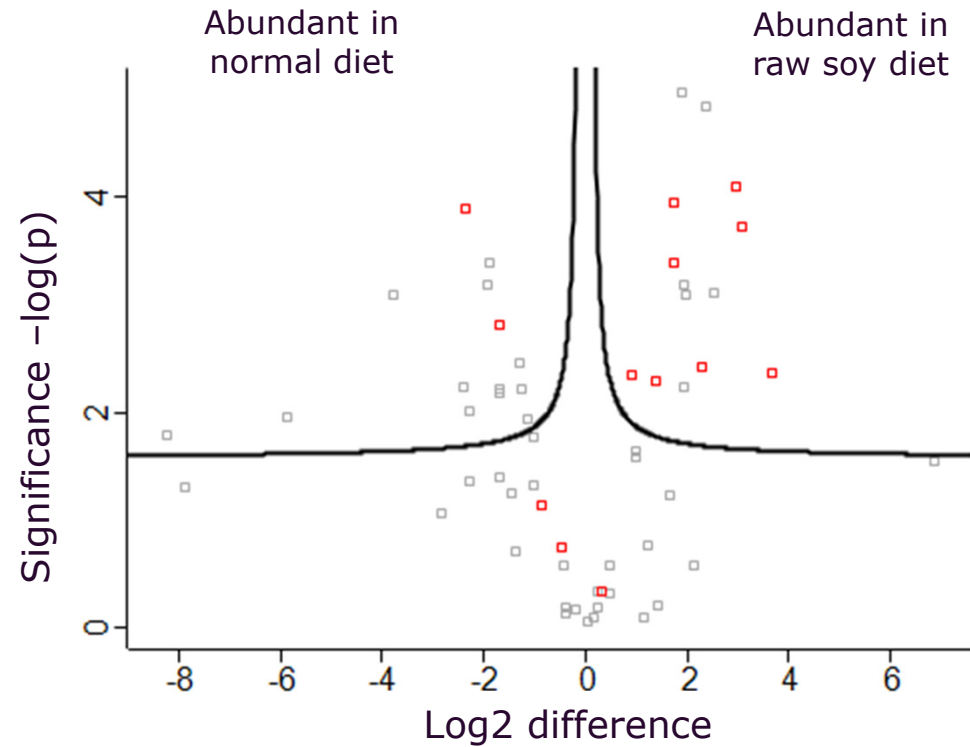
Proteolytic response



## Vulcano plot

Scatterplot of fold change vs. significance

Line indicates 1% FDR and  $\alpha=0.05$



**Red squares = Proteases**

# Chicken proteases

Effect of raw soy in the diet

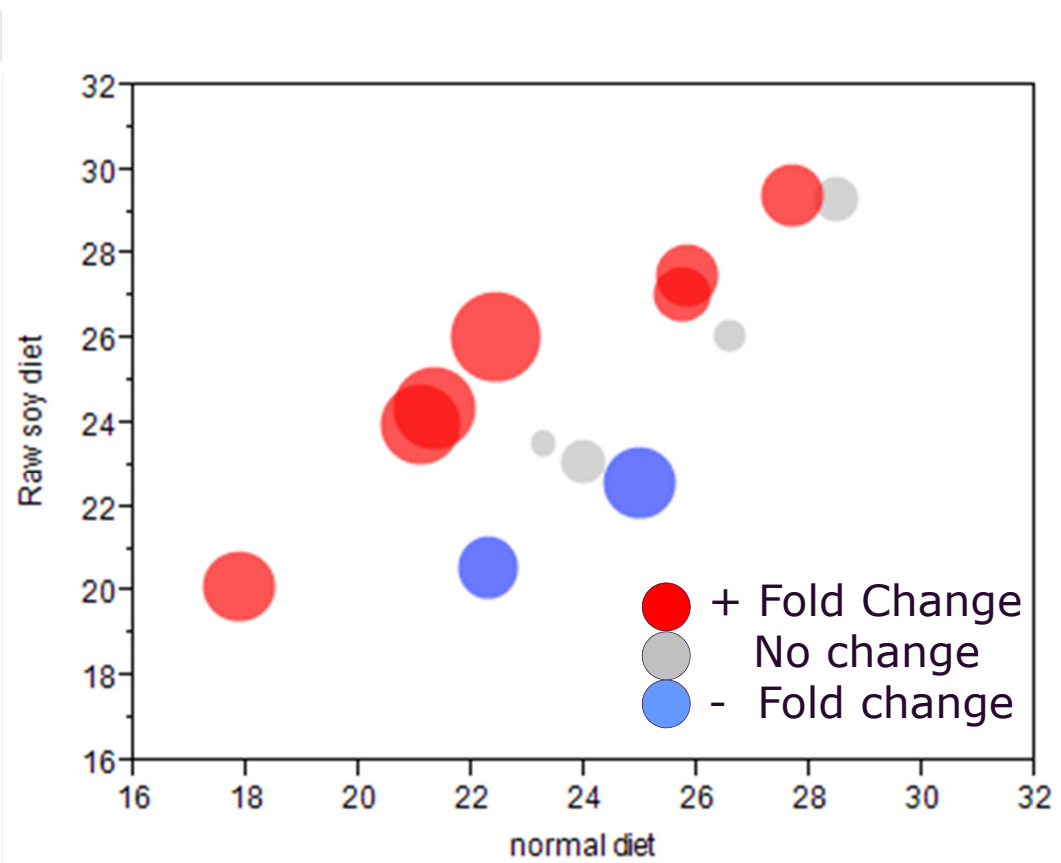
13 endogenous chicken proteases were identified

7 out 13 proteases show a significant >2-fold increase

Bubble plot

Relative size of the bubbles indicates fold change

5 S1A 'tryptic' endoproteases  
2 metallo exo-peptidases



## Conclusions

- Observed nitrogen digestibility and pancreas response to diet change translates to the protein level
- Provenance of residual protein in the terminal ileum of birds fed the normal corn-SBM diet were primarily soybean storage proteins
- A clear endogenous response to inclusion of raw soy in the diet was observed as a change in protease pool composition
- Future perspective
  - Better understanding of chicken digestive response to variations in the diet at the protein level

Technical support and guidance from the Protein Research Group at the University of Southern Denmark



Thank you for your attention!

