Advances in Understanding Enzyme Substrates in Feed and Available Solutions

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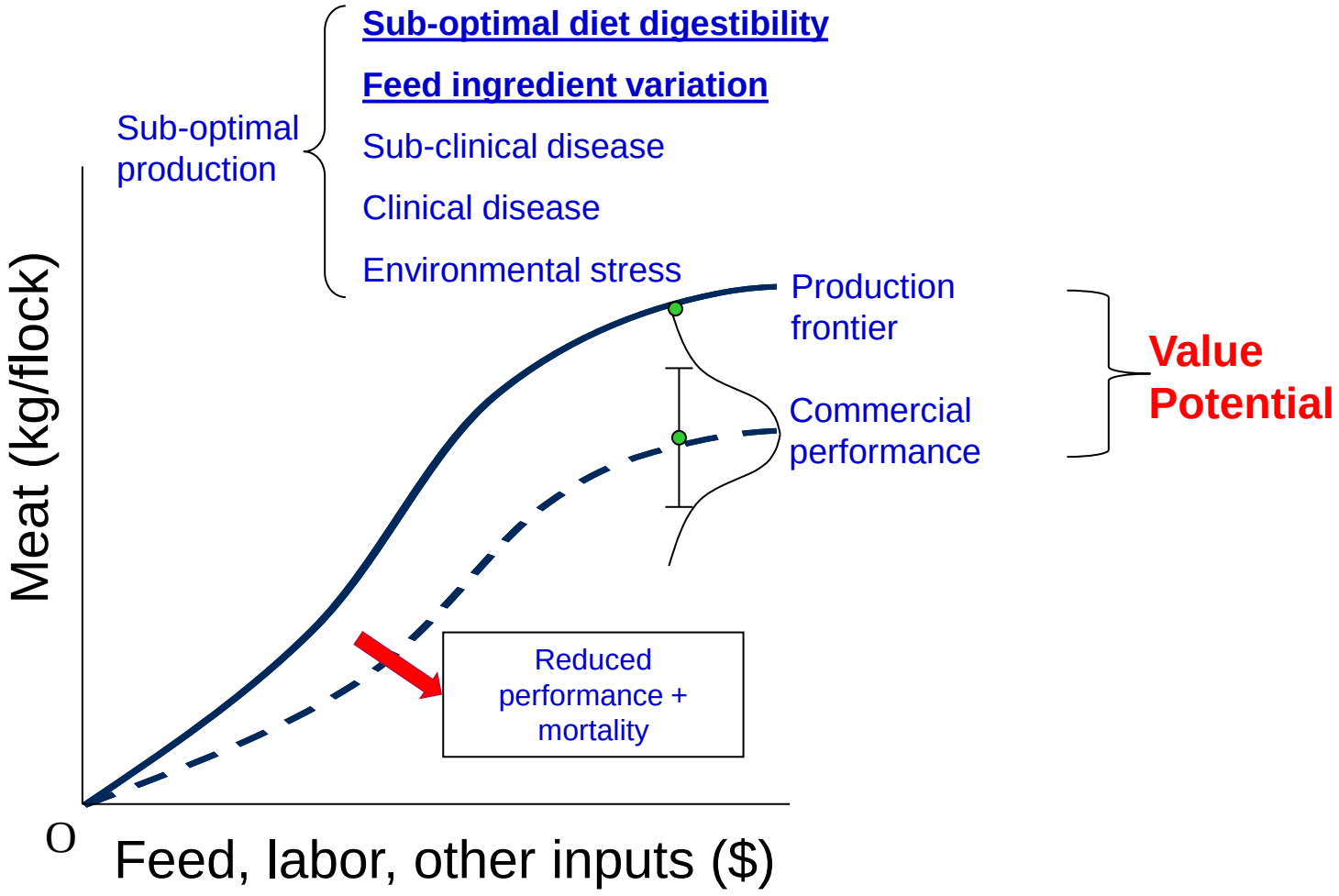
October 14th, 2015

Outline

- Introduction. Mode of action and value of enzymes
- Substrate and enzymes interactions in the gut
  - Phytase
  - Carbohydrases
  - Proteases
- The microbiota as a key player
- Capturing increased animal performance

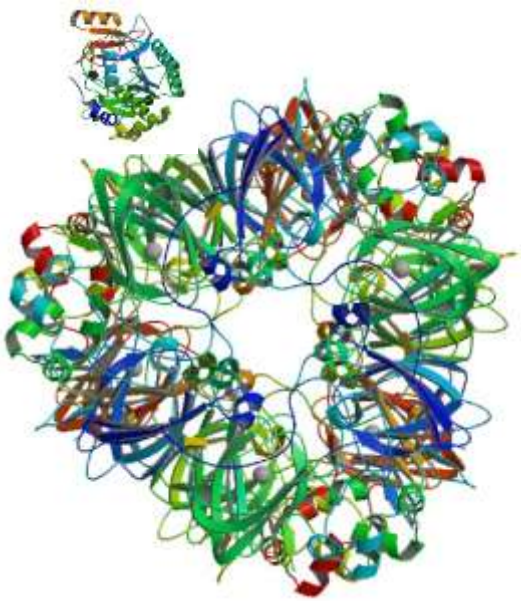
There is a gap between genetic potential and commercial performance in animal production



Romero, 2010

Mode of action of exogenous enzymes in animals is not limited to hydrolysis of one substrate

Enzyme + substrate



- Digesta
  - Physical structure
  - Solubility of nutrients
- Host
  - Feedback mechanisms
  - Endogenous enzymes
  - Gut development
  - Immune response
- Microbiome profile and function

Balance and retention of nutrients

Understanding exogenous enzymes for animal nutrition

Assay and product specifications

Screening

Disrupting technologies

Mode of action
Systems biology

Customer need

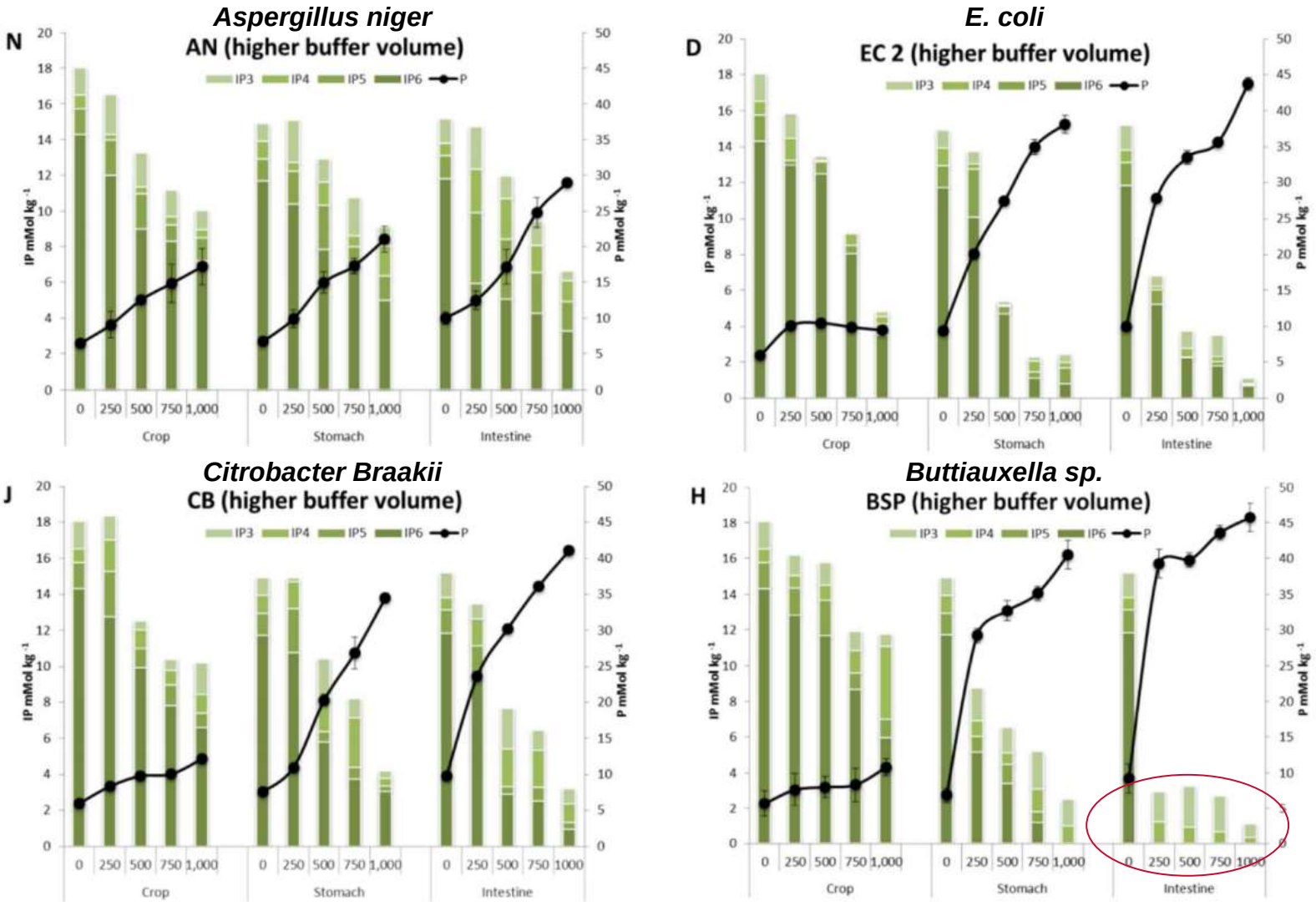
Small scale testing

Commercial application

Customer value

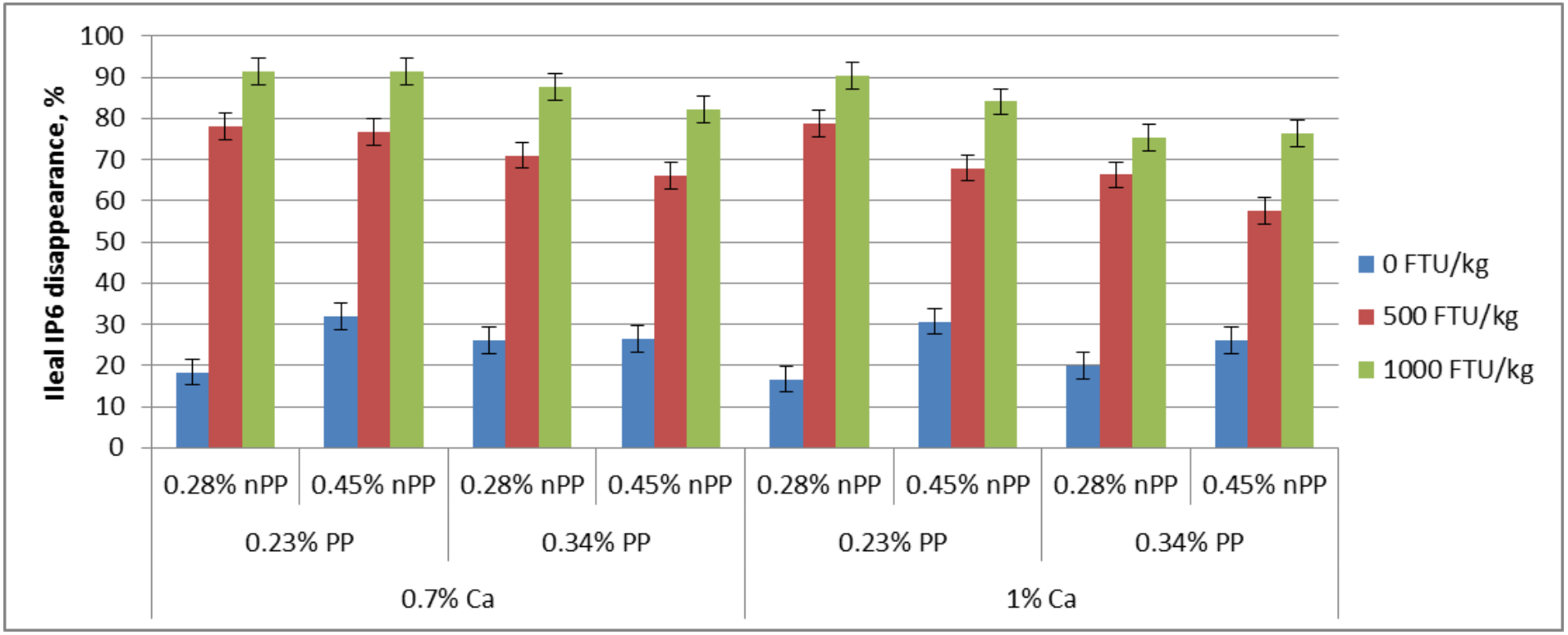
Substrate and enzymes interactions in the gut

Characterization of phytase activity in-vitro



Menezes-Blackburn et al. 2015. J. Agr. Food Chem.

Maximum IP degradation in the digestive tract is now reaching levels that are close to 90%, ***but*** multiple factors affect maximum disappearance

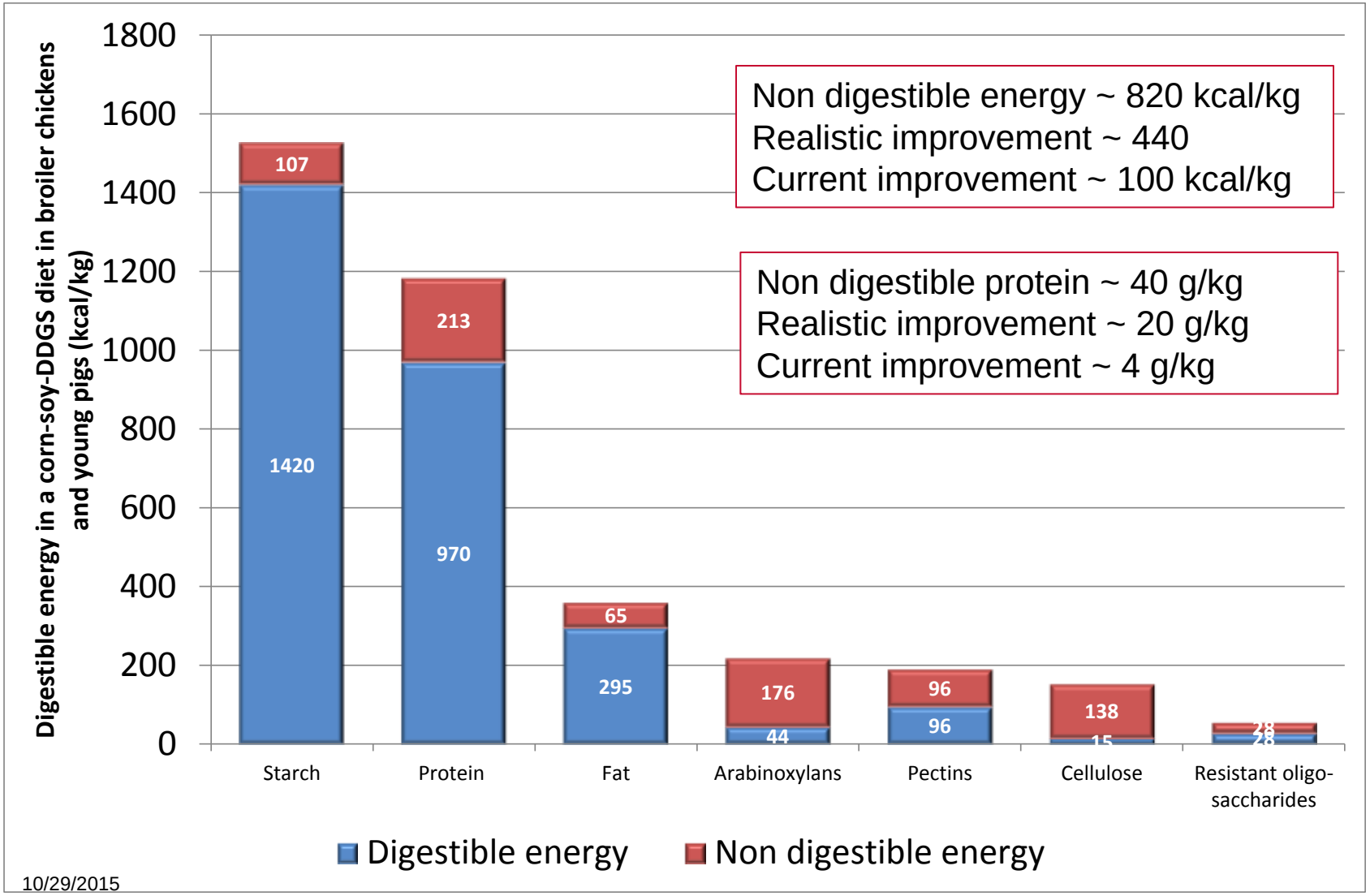


Factor	Ca	nPP	PP	Phytase	Ca×nPP	Ca×PP	Ca× Phytase	nPP×PP	nPP× Phytase	PP× Phytase	Ca×nPP× PP	Ca×nPP× Phytase	Ca×PP× Phytase	nPP×PP× Phytase	Ca×nPP× PP×Phytase
F Prob	**0.0003**	0.86	**<0.0001**	**<0.0001**	0.66	0.22	0.38	0.17	**<0.0001**	**0.0024**	0.12	0.27	0.88	0.15	0.79

* 0, 500 or 1000 FTU/kg of *Buttiauxella sp*. phytase were supplied to broilers from 11 to 13 d of age

Li, Angel, et al. Submitted for publication, 2015

Undigested energy and protein substrates



Mechanisms of action of carbohydrases and proteases in broiler diets

Xylanase, beta-glucanase

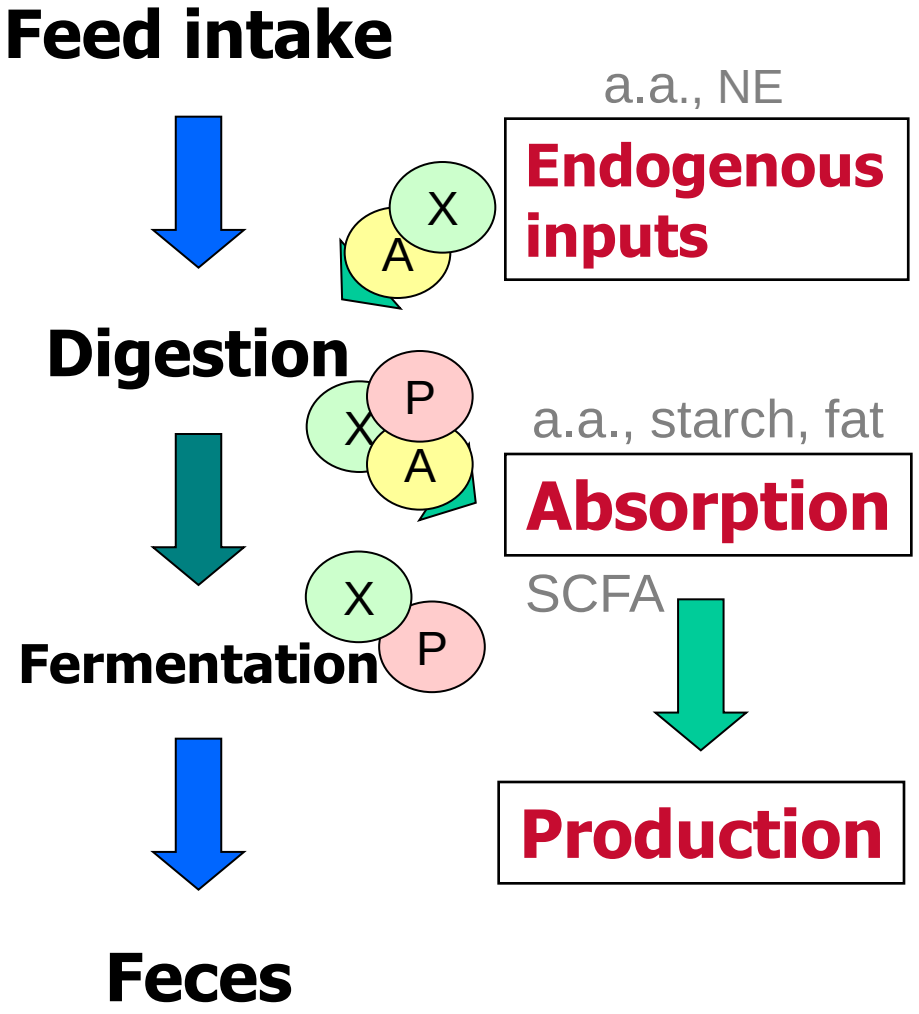
- Reduced viscosity (Choct, 1999)
- Improved access to cell contents (Cowieson, 2005)
- Prebiotic effects (Fernandez et al., 2000)
- Possible reduction of endogenous inputs (Satchithanandam et al., 1990)

Amylase

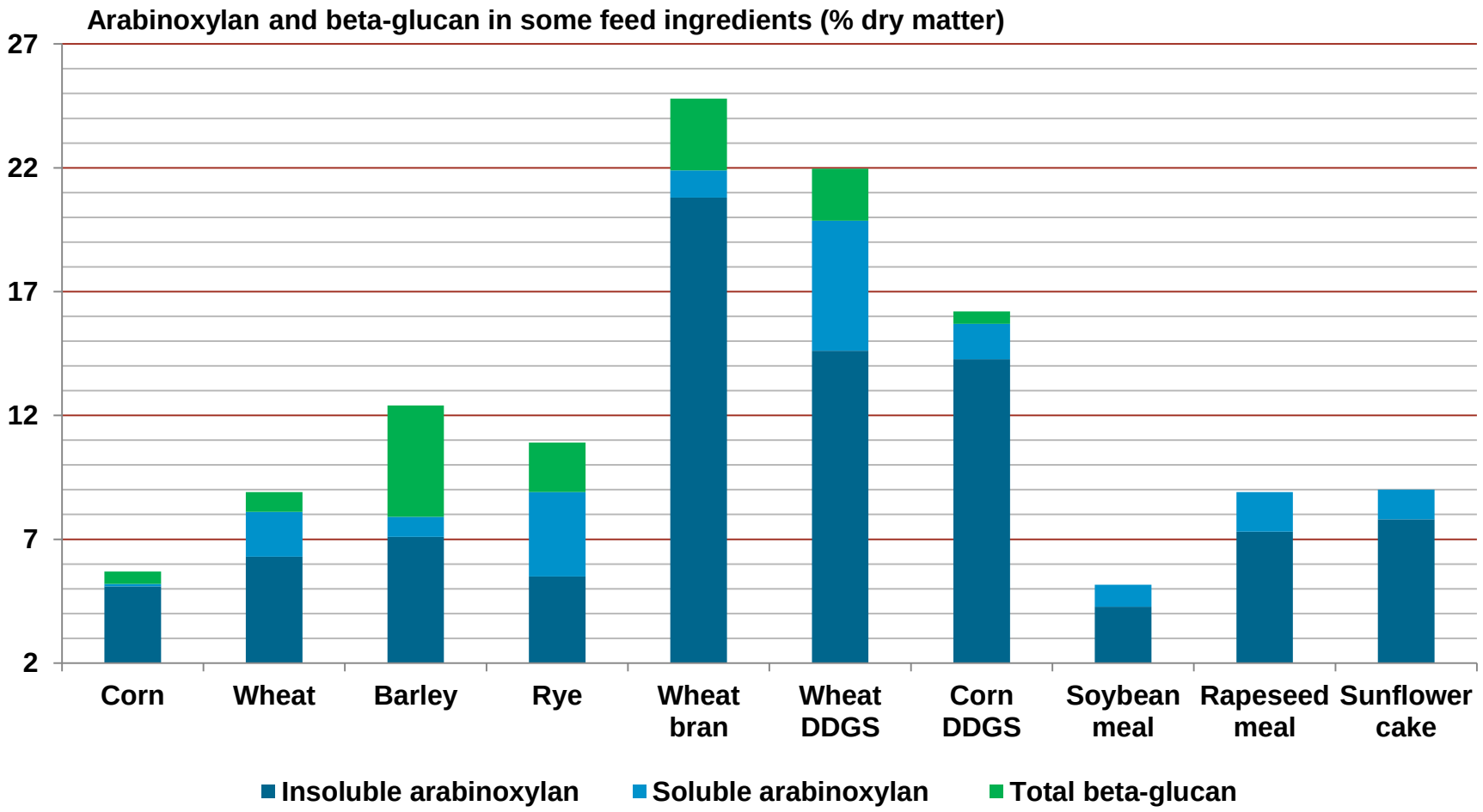
- Down regulation of pancreatic amylase (Jiang et al., 2008)
- Augmentation of pancreatic amylase activity in young animals (Gracia et al., 2003)
- Improvement of digestion of resistant starch in corn and corn by products (Sharma et al., 2010)

Protease

- Hydrolysis of dietary protein and increased protein solubility (Caine et al., 1998)
- Disruption of protein-starch interactions in corn (Mc Allister et al., 1993; Belles et al., 2000)
- Disruption of protein-fiber interactions (Pedersen, 2015)



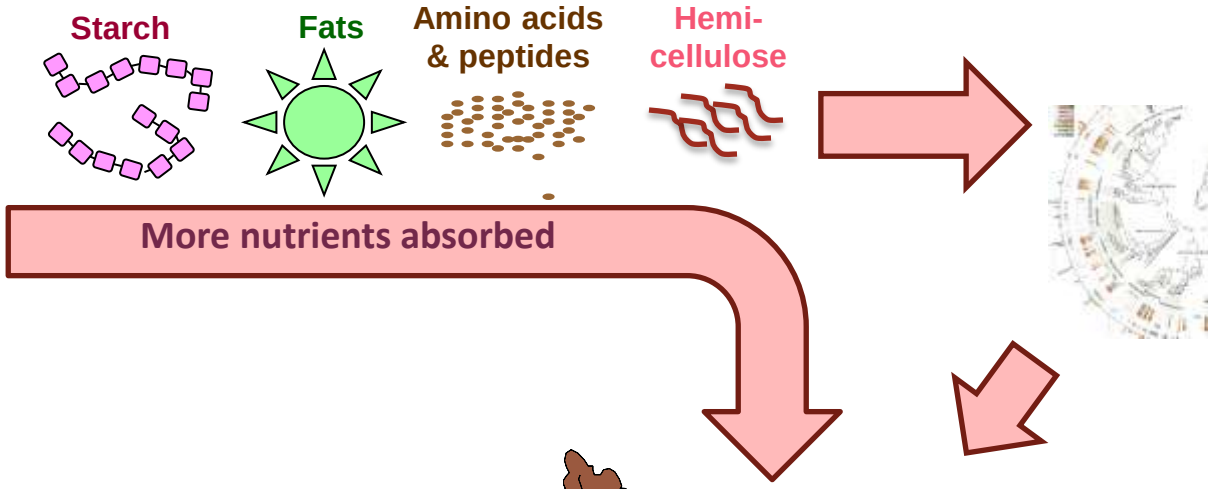
Xylanases and beta-glucanases. Shifting the target from the soluble to the insoluble fractions



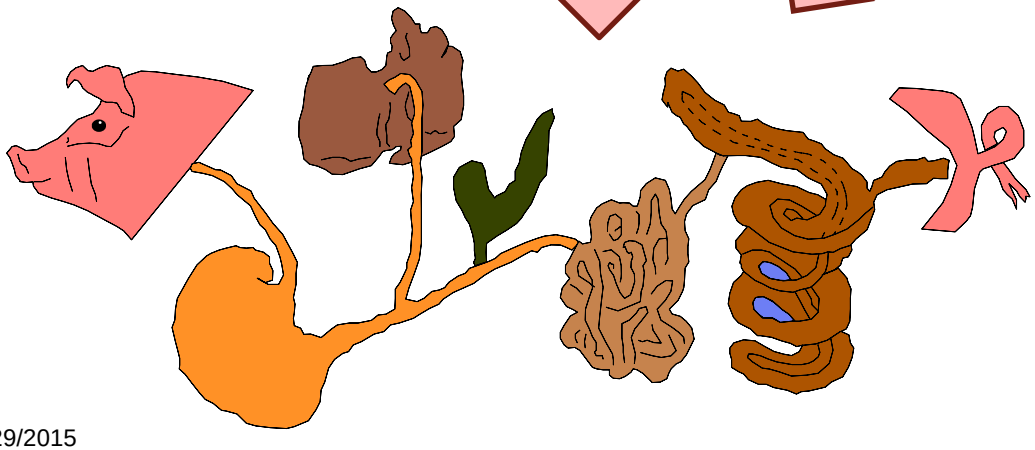
Source: Choct (2006); Danisco Non Starch Polysaccharide (NSP) database (2012)

It is not just about solubilisation of fibre; it is about net energy for the animal

Enzymes:
Solubilize fiber and release nutrients



Microbial fermentation



Availability of energy from fiber via SCFA production:

- Butyrate (2185 kj/mol)
- Propionate(1528 kj/mol)
- Acetate(874kj/mol)

There is considerable potential to increase protein digestibility in commercial diets

Ingredient *	Crude protein		ME (Kcal/kg)		Main proteins	Key amino acids
	%	App. dig**	Poultry	Swine		
Corn	**8**	**0.82**	3390	3350	Zein	Leu, asp, glu
Wheat	**11**	**0.81**	3210	3415	Glutenin	Glu
Soybean meal	**48**	**0.85**	2458	3140	Glycinin, beta-conglycinin	Phe, tyr, leu
Corn DDGS	**27**	**0.65-0.85**	2800	3300	Globulin, glutelin, zein	Leu, asp, glu
Sorghum	**11**	**0.68**	3310	3230	Kafirin	Pro, glu
Canola meal	**38**	**0.77**	2110	2600	Cruciferin, napin	Glu, asp
Sunflower meal	**41**	**0.83**	2310	2740	Helianthinin	Glu, asp
Feather meal	**85**	**0.50-0.75**	2880	2270	Keratin	Ser, pro, gly
Meat and bone meal	**50**	**0.65-0.80**	2530	2435	Collagen	Gly, ala, pro

Exogenous proteases

Serine proteases (3.4.21.xx)

- Catalytic mechanism = His-Ser-Asp
- Alkaline pH optimum

Trypsin subfamily proteases (3.4.21.4)

- Higher specificity to negatively charged substrates

Subtilisin proteases (3.4.21.62)

- Higher specificity to hydrophobic substrates

Most commercially available proteases fall into this category

Metallo-protease

Cysteine proteases

Aspartic acid proteases

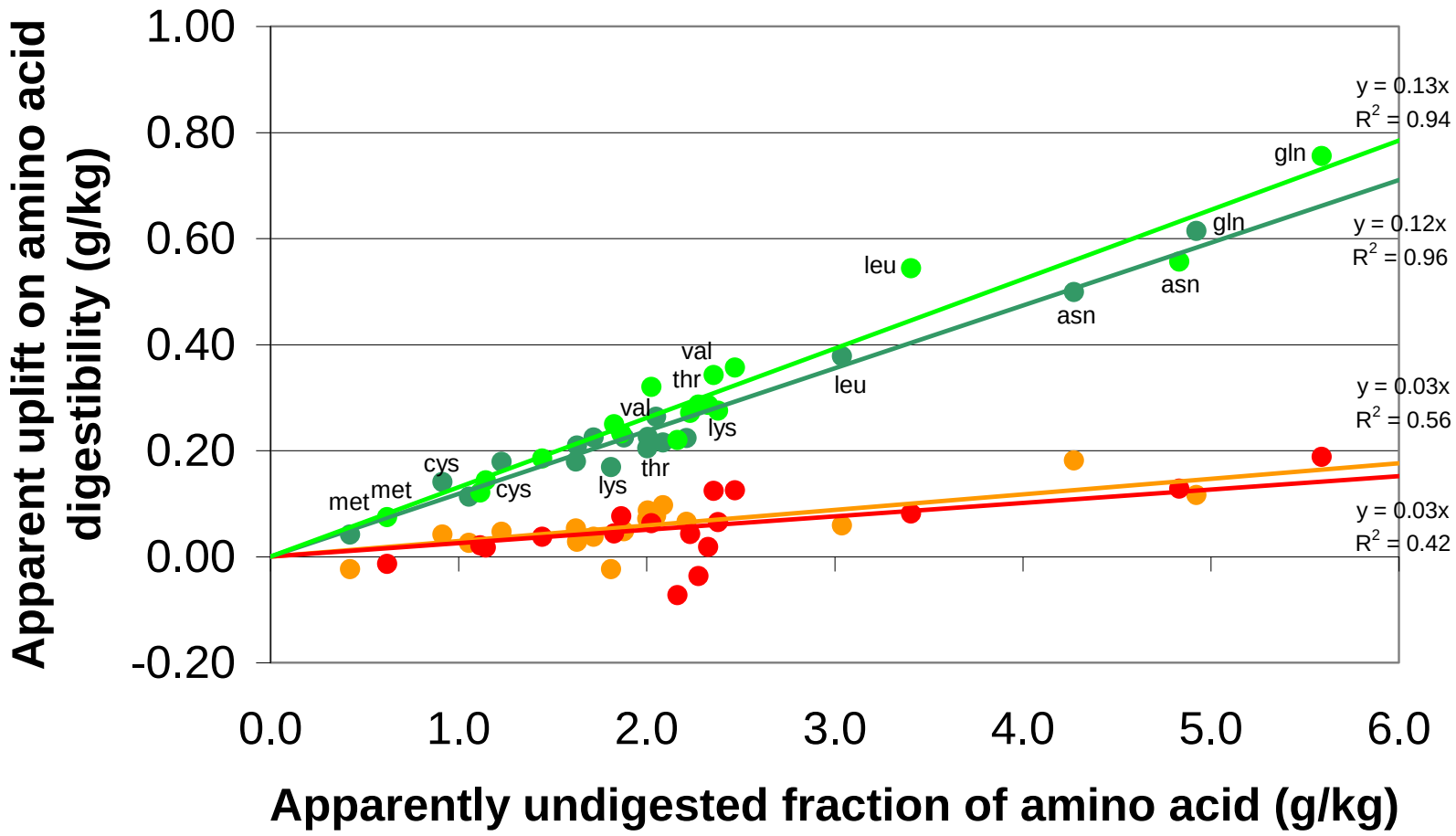
Proteomics - a new tool

Proteomics is the large-scale experimental analysis of proteins present in a biological sample. It usually relies on the extraction of the proteins from the sample, their separation, protein digestion, followed by mass spectrometry analysis

Proteomics has been applied to the study of food or feed protein digestion, and the terminology “protein digestomics” has been put forward by Picariello et al, 2013

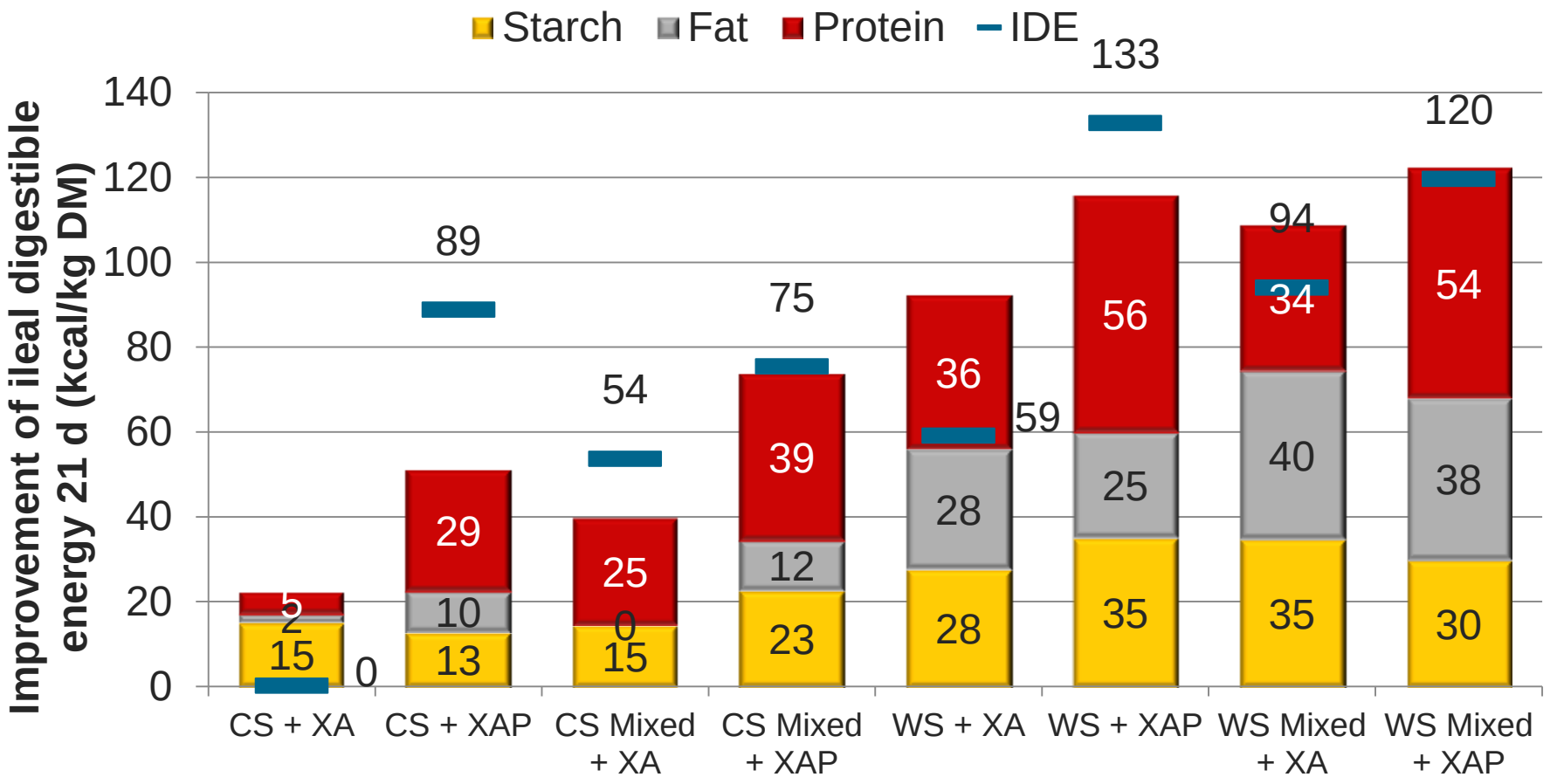
- Le Gall et al. (2005)
    - Identified two proteins in peas, lectin and albumin PA1b, that were totally resistant to gastric and small intestinal digestion in pigs
- Fisher et al. (2007)
    - Identified aggregated peptides of partly degraded β-conglycinin alpha subunits in the undigested ileal residue of pigs fed soybean meal

Improvements on amino acid digestibility due to enzymes are proportional to ileal undigested amino acids in control diets



Romero et al., 2013

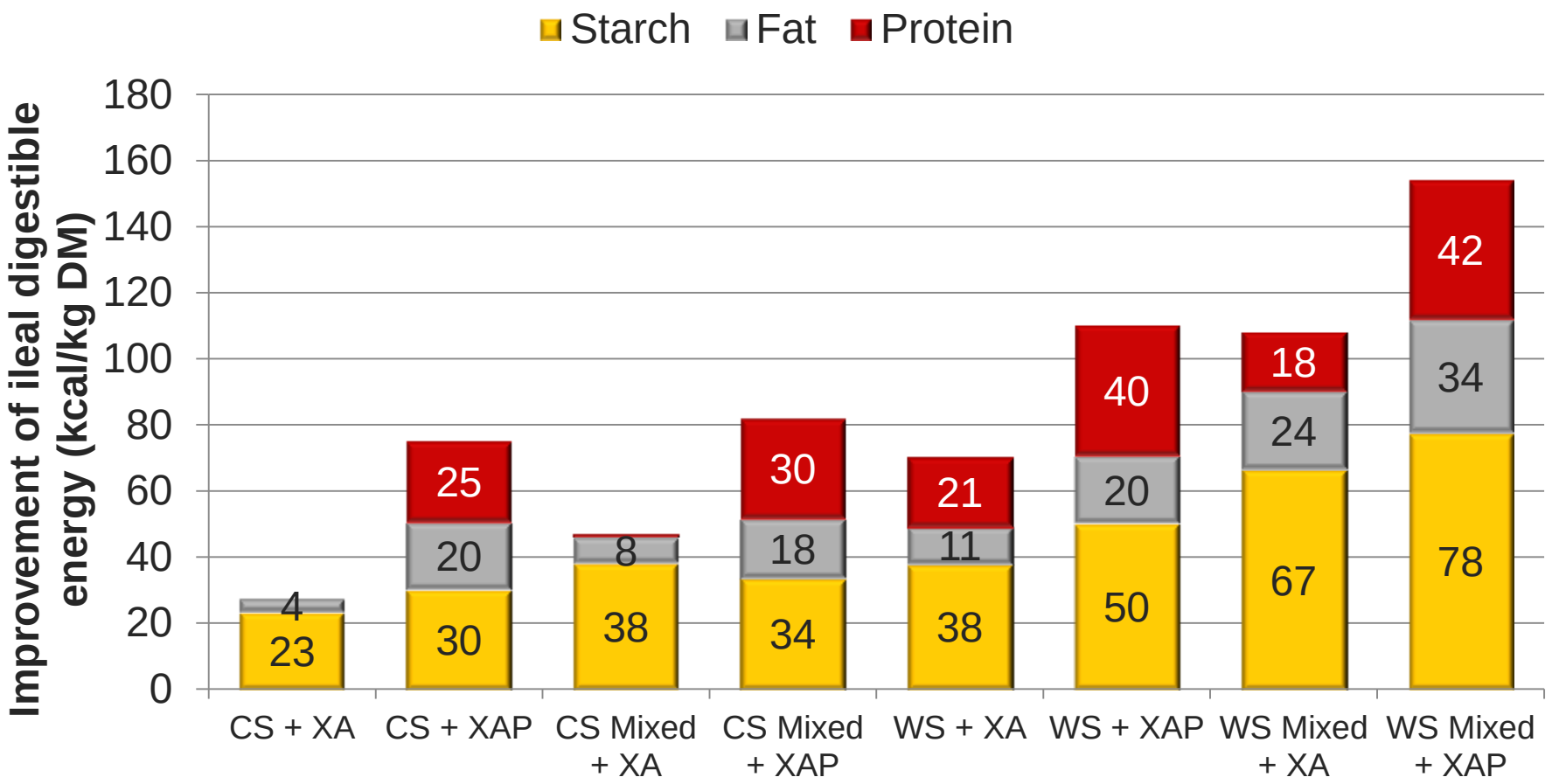
Protein contributed a significant amount of energy in response to enzymes, particularly protease, in 21-d broilers



IDE = ileal digestible energy
CS=Corn/Soy; WS=Wheat/Soy
XA = xylanase and amylase; XAP = XA plus protease
Mixed diets contained 10% corn DDGS and 5% canola meal

Romero et al., 2015

At 42 days, starch contribution was relatively greater, and carbohydrase had smaller effects on protein contributions



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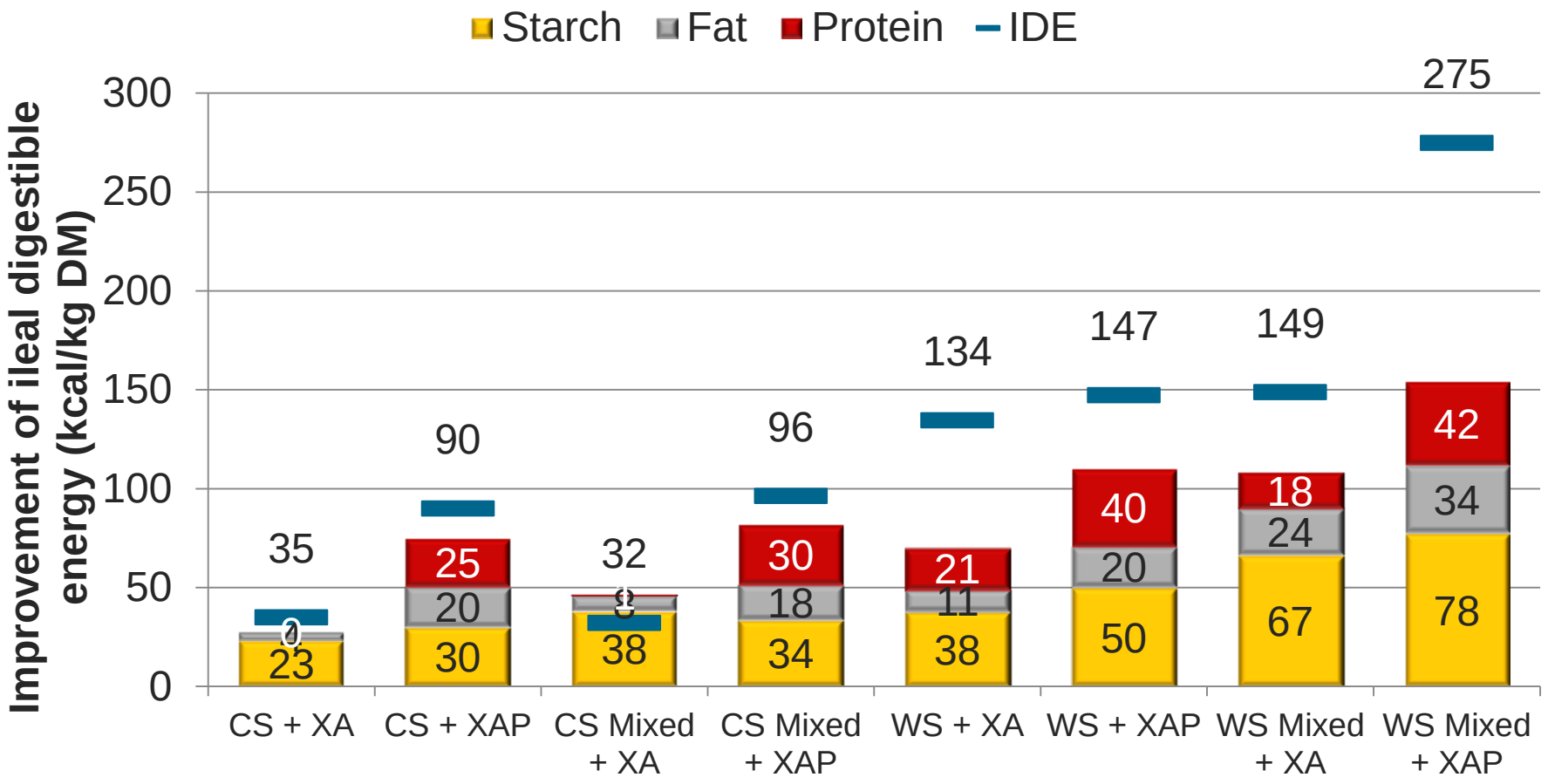
Romero et al., 2015

Contributions of starch, fat and protein did not explain ileal digestible energy in wheat based diets at day 42 d



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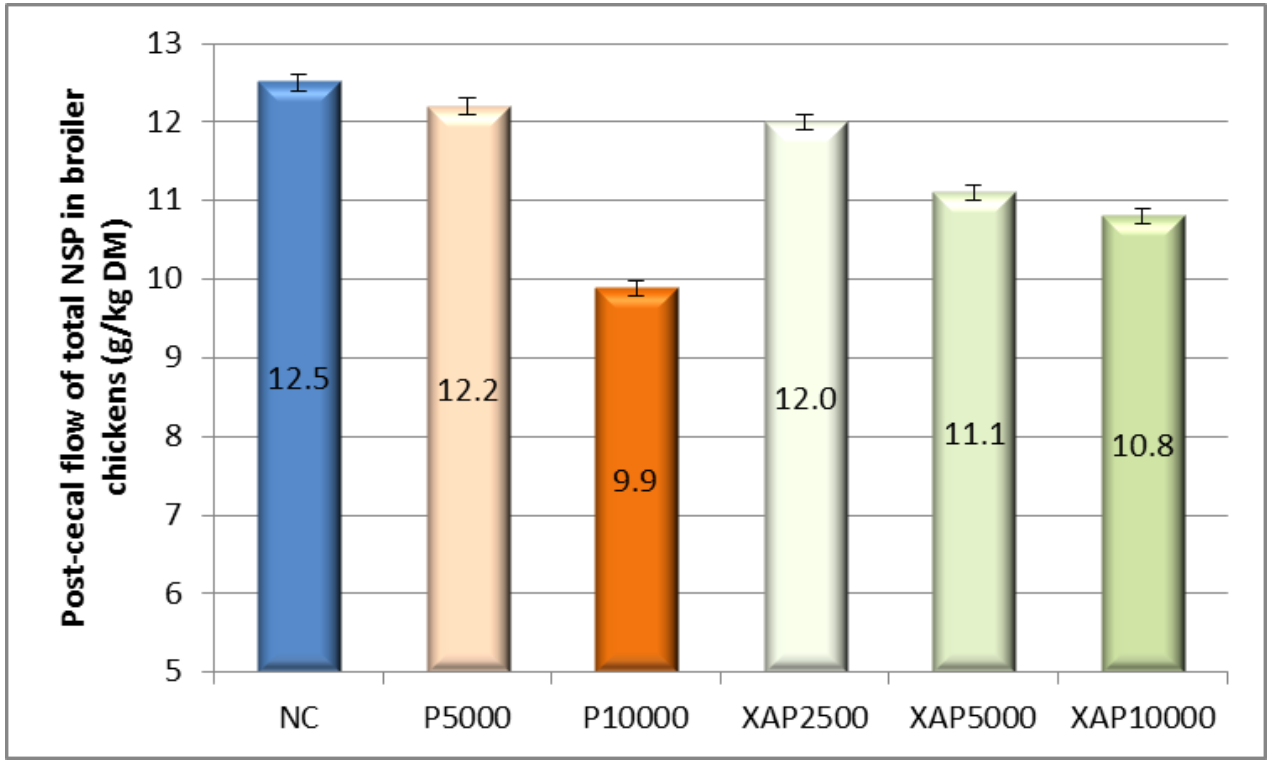
Romero et al., 2015

Proteases can have significant effects on total tract NSP disappearance in broiler chickens



* A subtilisin protease at two doses (P5000 and P10000) and a combination of xylanase, amylase and protease at three doses (XAP2500, XAP5000, XAP10000) were supplemented in a corn-based diet from 14 to 21 d to broiler chickens

Olukosi et al, 2015

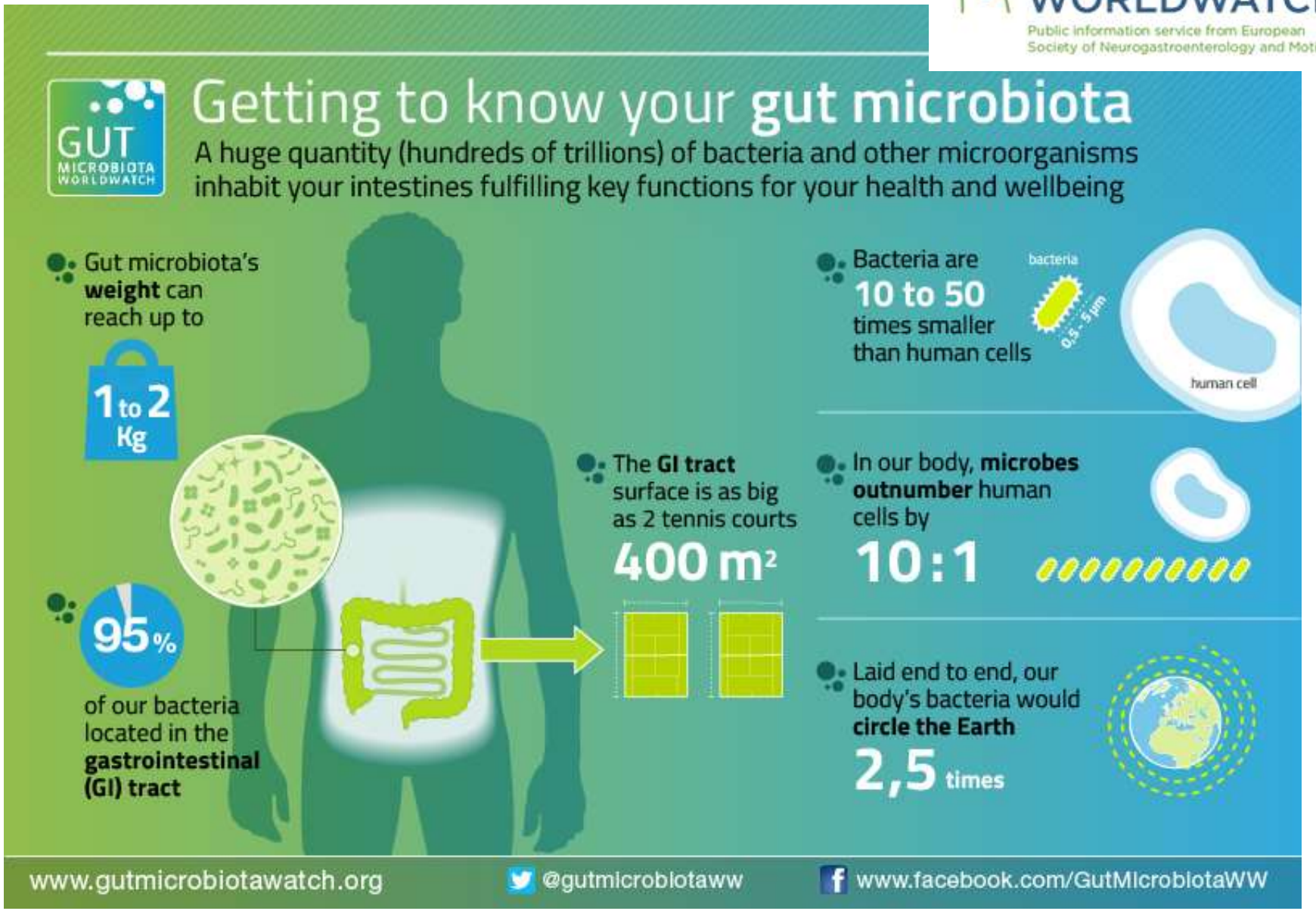
Some practical implications

- Net effects of enzymes on performance are greater in diets with greater undigested substrates, which normally correspond to diets with more fibrous ingredients.
- Effects of exogenous enzymes on the digestibility of different energy substrates overlap. Matrices are not additive.
- Therefore, it is always better to work with enzymes combinations with enough knowledge of mode of action and robust matrices.

The microbiota as a key player

You are bacterial and so are your birds and pigs!

GUT MICROBIOTA WORLDWATCH
Public information service from European Society of Neurogastroenterology and Motility



Avian gut microbiota

Total bacterial pop. ~10^{14} cells (10x more than host cells")

The avian gastrointestinal tract consists of **many small ecological niches**; either mucosal or luminal

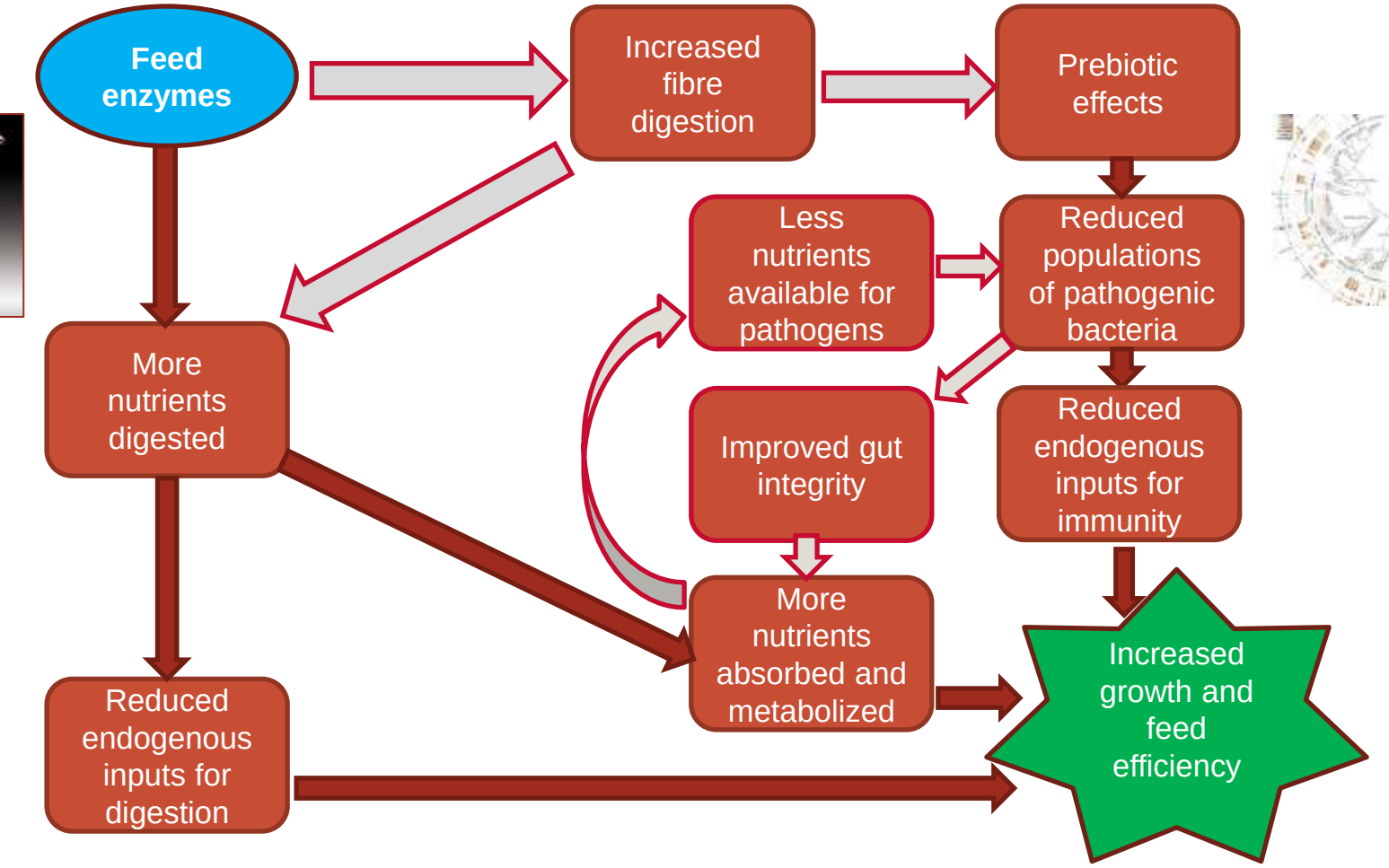
Gut bacteria are in the mucus layer at the epithelium and on digesta forming **biofilms**

Essentially **sterile at hatch**, the gut flora of an adult bird is relatively **stable** and difficult to change by feed additives

Microbiota influences:

- Development and function of immune system
- Metabolism, appetite
- Disease
- Behavior

Critical interactions of exogenous enzymes and the gut microbiome

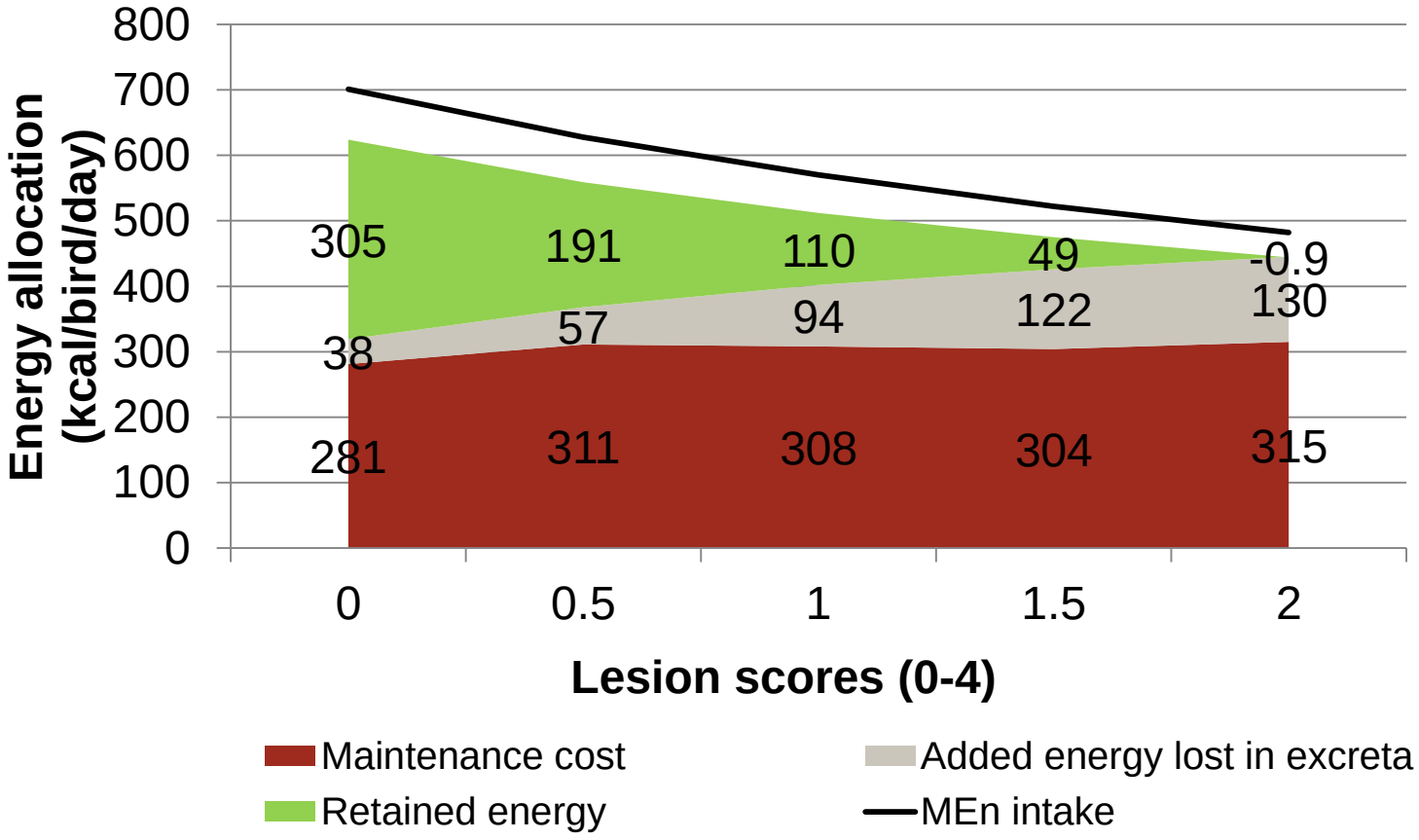


Enzymes might affect gut health through changes in the available substrate and direct effect in the mucosa

- Xylanases have been shown to have pre-biotic effects in poultry (Fernandez, 2000) and other species through selective stimulation of beneficial bacteria and production of short-chain fatty acids (SCFA) (Broekaert et al., 2011)
- Increased undigested protein appears to be a predisposing factor for dysbacteriosis related to necrotic enteritis (Dahiya et al., 2007)
- Protease has been shown to improve performance of chickens challenged with *Eimeria spp.* (Peek et al, 2009)

Enteric disease is a limiting factor to the efficacy of exogenous enzymes due to mal-absoption

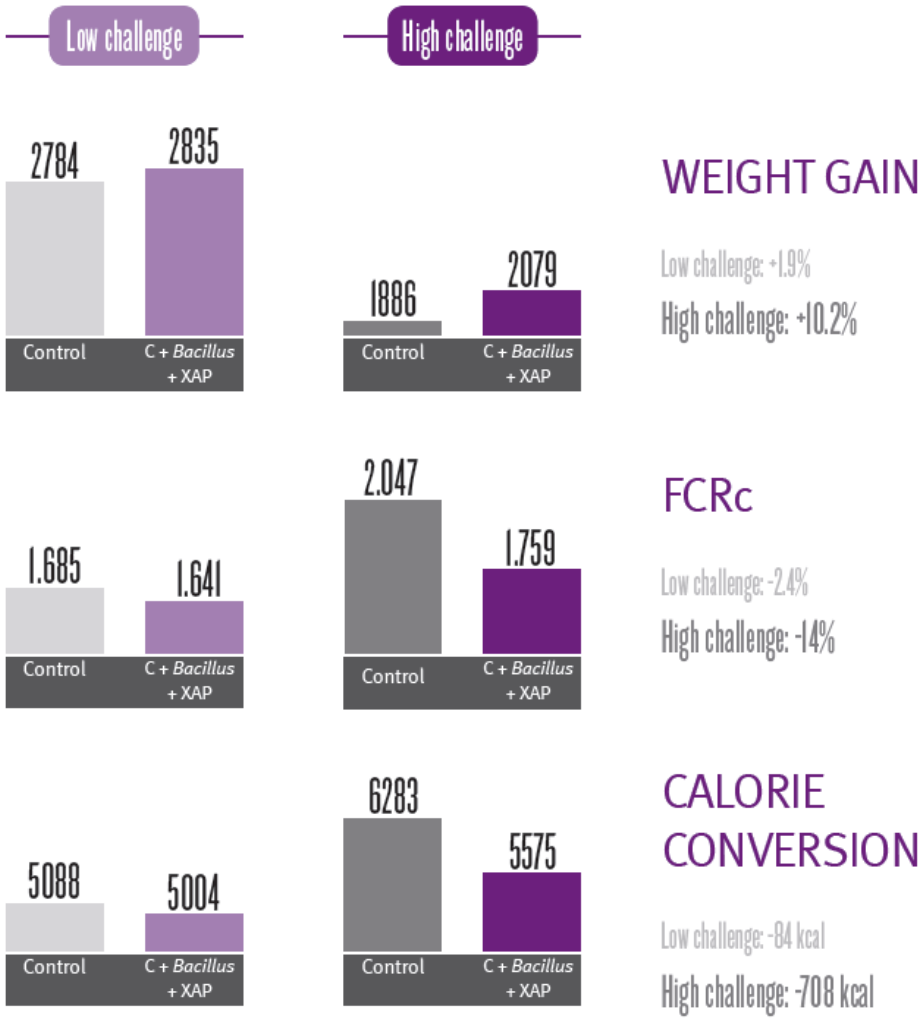
Energy partitioning of 42-48 d old broilers challenged with oocysts of three Eimeria species



Teeter et al. 2011; Broussard et al., 2008

Combinations of enzymes and DFMs increase the consistency of response in diverse levels of challenge

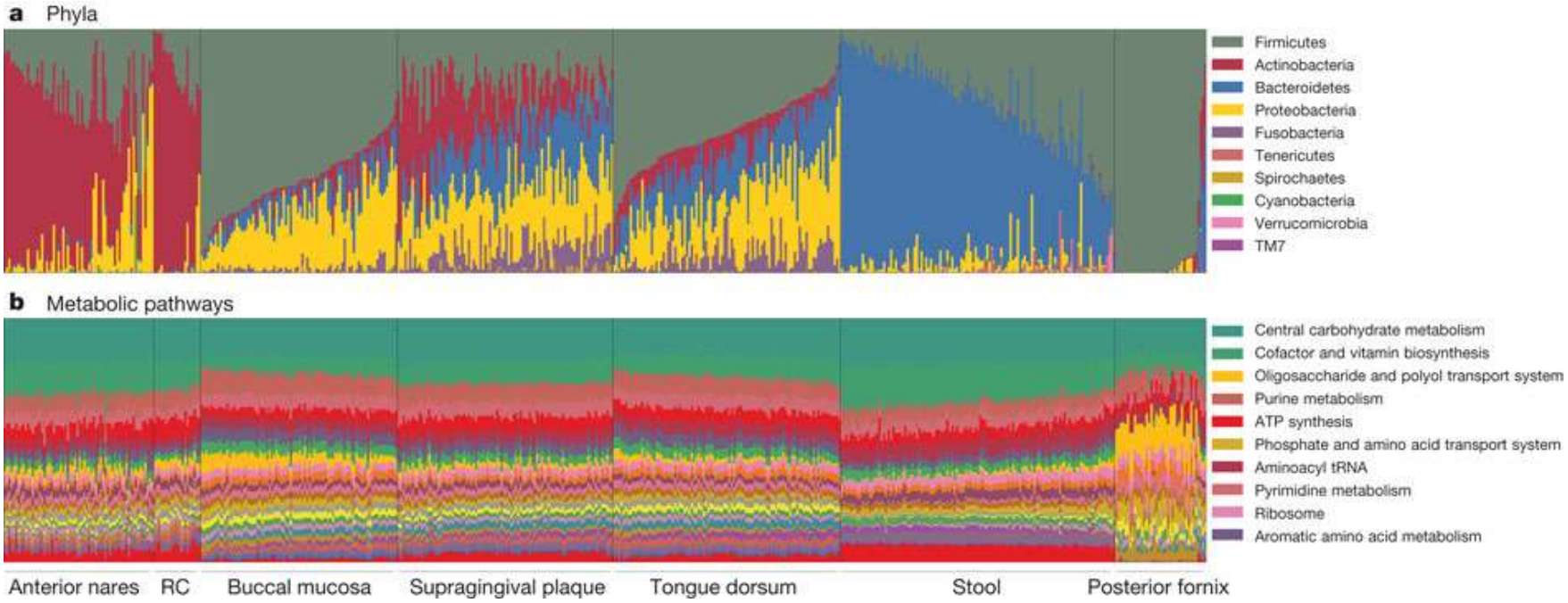
Response of broilers to the feed additive under low and high challenge over 42 days



Dersjant-Li et al., 2014

The development of deep sequencing techniques offers completely new insights into the role of the gut microbiome

Carriage of microbial taxa varies while metabolic pathways remain stable within a healthy population



C Huttenhower *et al. Nature* **486**, 207-214 (2012) doi:10.1038/nature11234

Fraher et al., 2012

Capturing increased animal performance

Capturing value in commercial conditions

- Selection of enzymes should be based on the effects on undigestible substrates of base diets
- Enzymes combinations with significant, measurable and reliable activity levels are preferable:
  - Wide and consistent range of functionalities
  - Higher chance of reliable net benefits in variable commercial conditions
- Proactive management of nutrient interactions are necessary:
  - Effect of Ca on phytase activity
  - Estimation of AME and protein quality
- Respond to seasonal or supply driven changes in ingredient quality
- Optimization of gut health in critical

Exogenous enzymes - Future R&D directions

Increase research on application knowledge

- Ingredient, animal and additive interactions
- Improvements on in-vitro simulations

Application of omics tools

- Proteomics. Increased digestion of undigested proteins
- Metagenomics. Increased energy from fibre; optimization of gut health
- Metabolomics and transcriptomics. Intestinal and systemic mechanisms

New applications of enzymes beyond digestion of nutrients

Thank you

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