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Recent advances in exogenous enzyme application for monogastric nutrition

Prof. Aaron J. Cowieson
DSM Nutritional Products

HEALTH · NUTRITION · MATERIALS

Presentation overview

- History and scene setting
- Importance of benchmarking
- Obscurity and opportunity
- Future direction
- Conclusions

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History and scene setting

Feed Enzymes

- **Kirchoff (1815) demonstrated the presence of hydrolytic enzymes in plant material**

- **Ellenberger (1881) was the first to demonstrate the potential of endogenous enzymes in plant material in nutritional terms**

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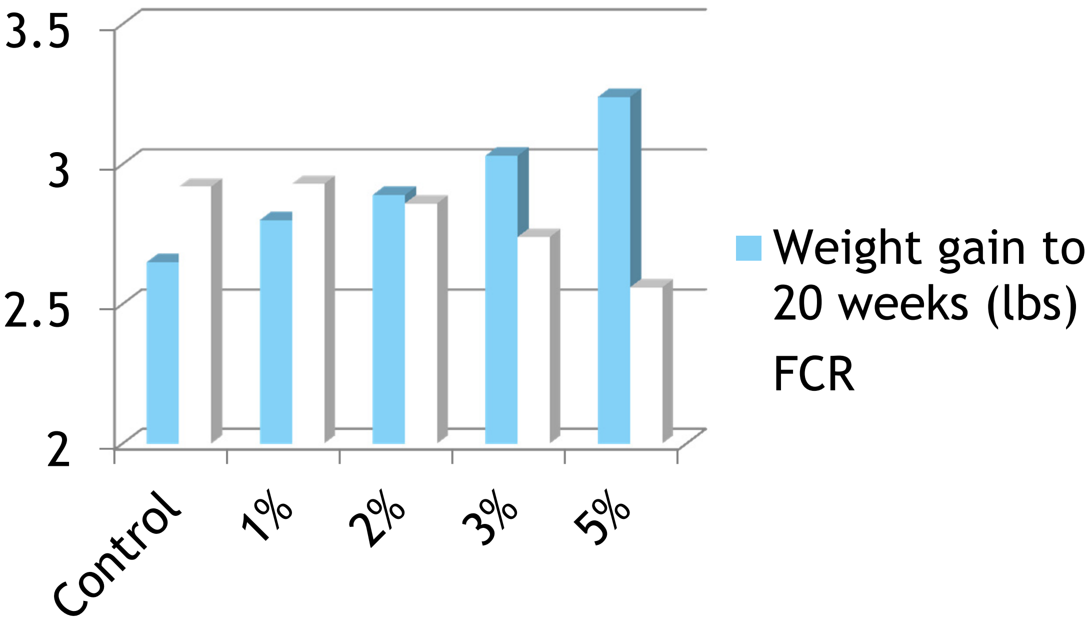
1925

Dr. George W. Hervey (Rutgers, NJAES) becomes the first* to publish a paper on the potential of feed enzymes to improve performance and digestibility in poultry!



SPECIAL ARTICLES

A NUTRITIONAL STUDY UPON A FUNGUS ENZYME[1]

Possibilities in a comparatively uninvestigated field of nutrition have been suggested by the feeding of a vegetable fungus enzymic material called protozyme to growing chicks in a preliminary test at the New Jersey Agricultural Experiment Station. Over a thousand Leghorn chicks of both sexes were used in the investigation for a period of seven weeks, after which the males were eliminated. The test has now continued for a period of twenty weeks. The birds in question are divided into five groups. All receive a normal scratch mixture daily of cracked cereal grains and a ground mash mixture of bran, middlings and corn meal. All have free access to liquid skim milk. The enzymic material is incorporated in the mash mixture as follows: 5 per cent. of the weight of the mash in Group 1, 3 per cent. in Group 2, 2 per cent. in Group 3, 1 per cent. in Group 4 and none in Group 5. Group 5, consuming no accessory enzymic material, acts as a check upon the other four groups.

During the twenty-weeks' feeding in every instance the chicks consuming the enzymic material have manifested a more rapid growth than those not consuming it. The following table indicates the feed consumed and growth attained.

Summary of Growth and Feed Consumption upon Leghorn Females Consuming Different Amounts of Fungus Enzymic Material—End of Twentieth Week of Age

Amount of fungus enzymic material in mash	Number of chicks	Mean weight lbs.	Mean total grain consumption lbs	Mean total mash consumption lbs.
5 per cent.	100	3.24	7.65	8.31
3 per cent.	100	3.03	7.67	8.31
2 per cent.	100	2.89	7.68	8.27
1 per cent.	100	2.80	7.70	8.21
None	95	2.65	7.77	7.73

Duplicate qualitative tests upon material taken from the crop and gizzard at the end of the sixth week of age indicated increased starch and protein digestion in individuals consuming the enzymic material over those not consuming it.

George W. Hervey

New Jersey Agricultural Experiment Station

[1] Paper No. 244 of the journal series New Jersey Agricultural Experiment Stations, Department of Poultry Husbandry.

GASTRIC TRANSPLANTATION

The study of gastric motility as ordinarily carried out is attended with many technical difficulties. It was conceived that it might be possible materially to reduce these difficulties by transplanting the stomach to a subcutaneous locus so that it could be viewed directly from the outside. Whether the animal could survive such an operation, maintain normal nutrition and gastric movements was the crux of the problem.

The problem was attacked in the following way, using young albino rats. The animals were given no food for twenty-four hours before the operation and none for twenty-four hours after. A median longitudinal incision of 2 cm was made through the abdominal wall along the linea alba. The stomach was lifted through this opening and the abdominal muscles sutured together beneath it, but leaving openings sufficient to transmit the esophagus and the pylorus. These structures were anchored to the abdominal wall by two stay sutures each. Then the integument was dissected back from the muscle laterally on each side to form a pouch for the stomach. The cut edges of the skin were brought together and held by continuous sutures closing the incision. Thus the stomach was covered on the outside by skin and lay between the skin and the abdominal muscles. In two weeks the incision was healed and the animal ready to be observed. Of ten animals first subjected to the operation five lived. Later five more animals were operated on and all lived for considerable periods. Three are alive and apparently normal at the end of nine months. These have been studied with care. They show a normal weight graph, normal appetite and apparently normal peristalsis. They have at no time shown signs of esophageal or pyloric stasis.

The contour of the stomach and its contraction waves can readily be seen through the skin. To permit study of these contractions the rat is placed in a small glass-bottomed cage. This cage is suspended above a bench upon which the observer reclines.

At present the writer is working on the question of the relation between gastric contractions and muscular activity. The observation cage is suspended from one end of a Fitz pneumograph (Porter type) which is connected through rubber tubing with a tambour (Durrant method).[1] This is a highly sensitive method of detecting movements of the animal. For example, the rat's nibbling a grain of corn sets the system into marked oscillations. The tambour is adjusted to write on a revolving extension kymograph drum. Below the tambour lever is adjusted a signal magnet with simple key in circuit and a chronograph.

[1] Durrant, *Am. J. Physiol.* (Balt.), 1924, *70*, 344.

Hervey, G.W. (1925) A nutritional study upon a fungus enzyme. *Science*, **62**: 247.
*Clickner, F.H. & Follwell, E.H. (1926) Application of Protozyme (Aspergillus orizae) to poultry feeding. *Poultry Science*, **5**: 241-247.

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Holst (1926)

Artifical enzymes and poultry feeding. Poultry Science 5:261-265.

"…extension workers should be cautious in advocating the use of artificial enzymes in poultry feeding."

- Considered that residence time in the GI tract of poultry was insufficient to allow enzymes to act successfully
- Lack of evidence and no understanding of mechanism
- At the time only 2 papers had been published on this topic

(1894) and Ellenberger's (1906) work shows that such is not the case. Bergmann's different results (1906) have been explained quite apart from the action of enzymes. To destroy the feed enzymes he autoclaved the food, thereby coagulating the proteins and causing a decrease of their digestibility. Ellenberger, on the basis of experiments with stomach extirpations, showed that the intestinal digestion is able to compensate for any lack of enzymes in the feed. Grimmer (1906) states that the feeds remain long enough in the animal body that, provided a normal digestive tract with a normal secretion of body enzymes, it may be assumed that the food utilization, which really is governed by the morphology of the animal, is as complete as possible without the assistance of any enzymes from the outside.

In the light of these facts the results of Hannas (1926) and Hervey (1925) are extremely interesting, because they are so contrary to the results to be expected from the chemical and physiological considerations, and the results of other investigators who have worked on the introduction of so-called artificial enzymes. If further experiments give similar results, it would introduce something new into the accepted explanation of the digestive processes. Proof thereof has not been given, however, and cannot be given until many more detailed and controlled investigations have been carried out.

In the meantime experiment station and extension workers should be very cautious in advocating the use of artificial enzymes as such, in poultry feeding.

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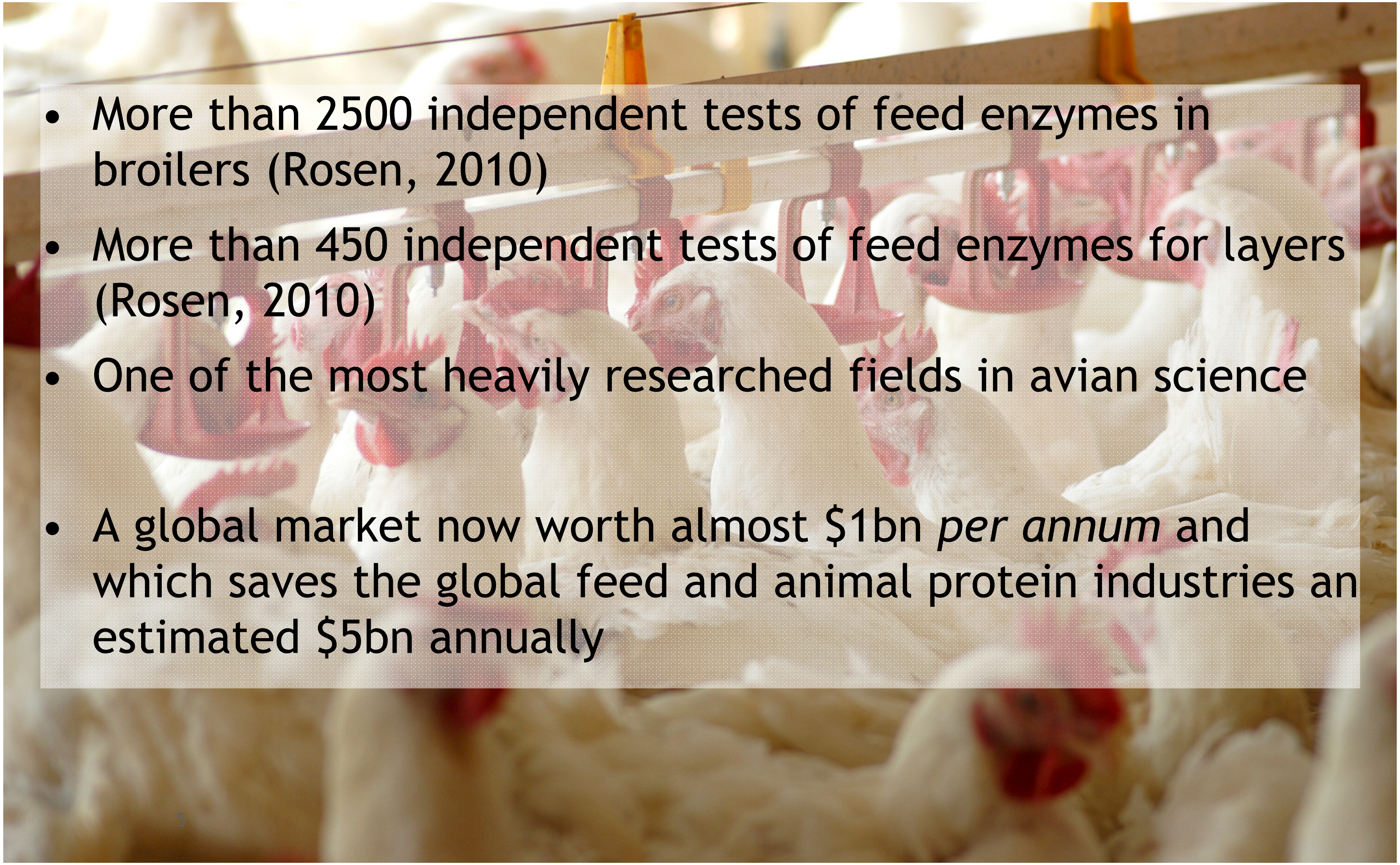
1925 - 2013



- More than 2500 independent tests of feed enzymes in broilers (Rosen, 2010)
- More than 450 independent tests of feed enzymes for layers (Rosen, 2010)
- One of the most heavily researched fields in avian science
- A global market now worth almost $1bn *per annum* and which saves the global feed and animal protein industries an estimated $5bn annually

The importance of benchmarking

A PIVOTAL QUESTION:

Which blend of enzyme products will deliver the economic optimum value for my business?

A 'POLITICIANS' ANSWER!:

This depends entirely on the current limiting factor in the profitability of the enterprise in question.

As George Harrison (*Beatles*) wrote:
"If you don't know where you're going, any road will take you there…."

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The importance of benchmarking

Some logical thought processes:

1. What is the relative performance of birds in your business relative to:
    1. Competitors
    2. Breeder recommendations
    3. Historical norms
    4. Top and bottom quartiles within your business?
2. What is the variability across different locations in bird performance within your business?
3. How much of the gap from the top locations to the bottom could be closed by altering nutrient delivery to the birds?
4. How much room is there in the various diets fed to enhance nutrient delivery?
5. What interventions would be appropriate given points 3 & 4?

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Enzymes and inherent digestibility

Phytase, xylanase and protease efficacy declines as the inherent digestibility of the focal nutrients increases

Cowieson (2010) *Journal of Poultry Science*: for xylanase/glucanase

Cowieson & Bedford (2009) *Worlds Poultry Science Journal*: for xylanase and phytase

Cowieson & Roos (2014) *Journal of Applied Animal Nutrition*

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Meta-Analysis

- Enzyme responses (digestibility and performance) vary around a mean
- Meta-analyses (recently published – see Cowieson & Bedford, 2010; Selle & Ravindran, 2008; Cowieson & Roos, 2014) are instructive
- Model development and prediction tools
- Generic matrix values may become obsolete as prediction tools allow optimisation based on diet, animal and environmental conditions

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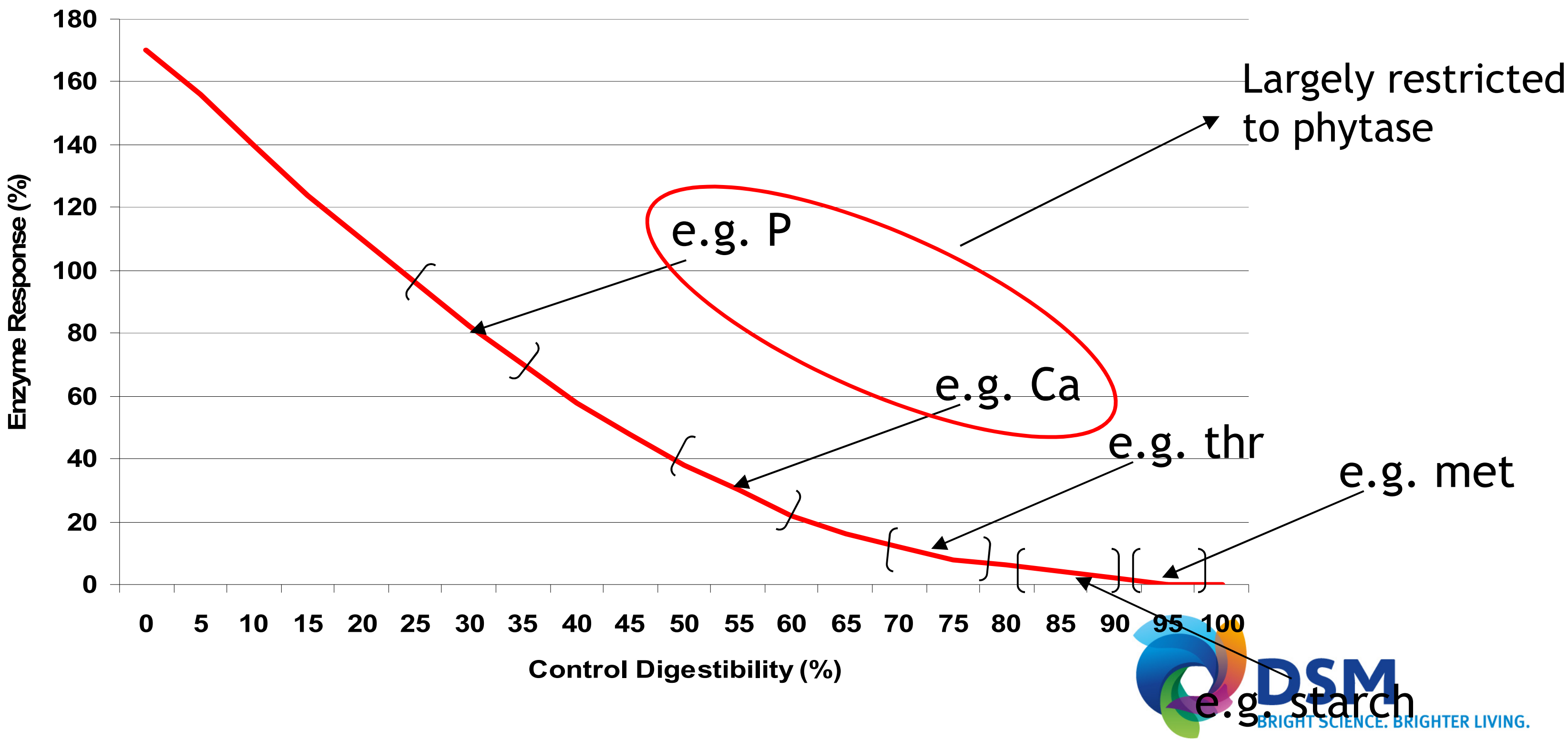
Establishing the limits

- Enzyme effect follows a distinct law of diminishing return, well correlated with control



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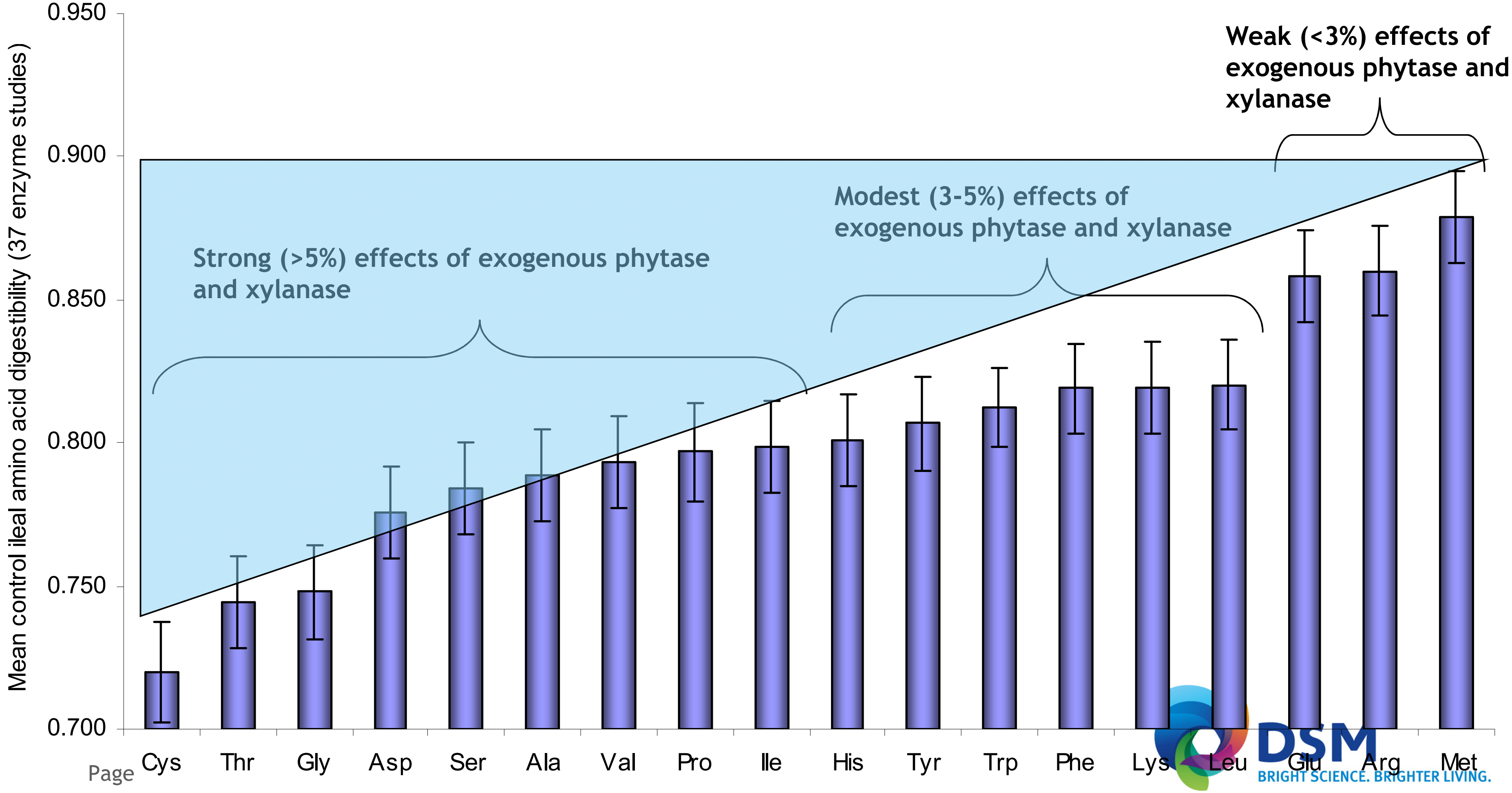
Ileal amino acid digestibility



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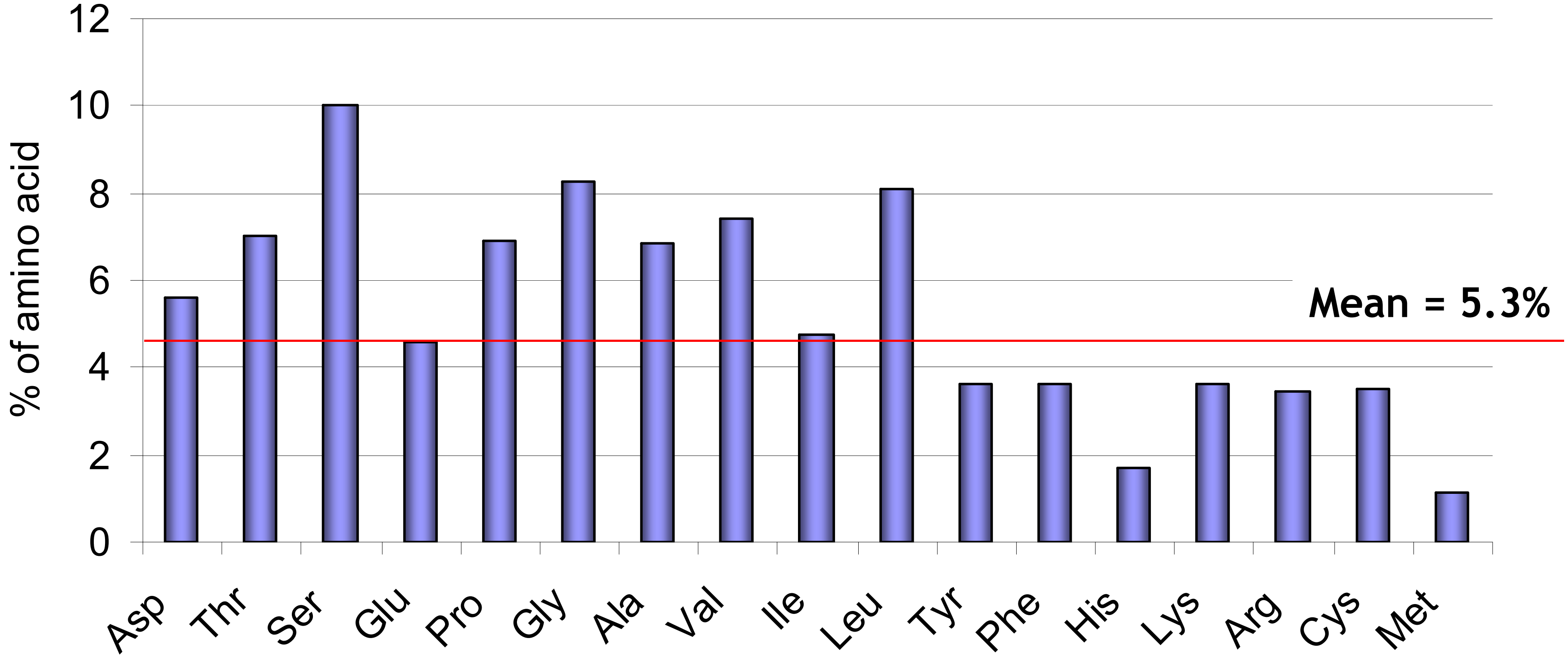
Amino acid profile of endogenous proteins

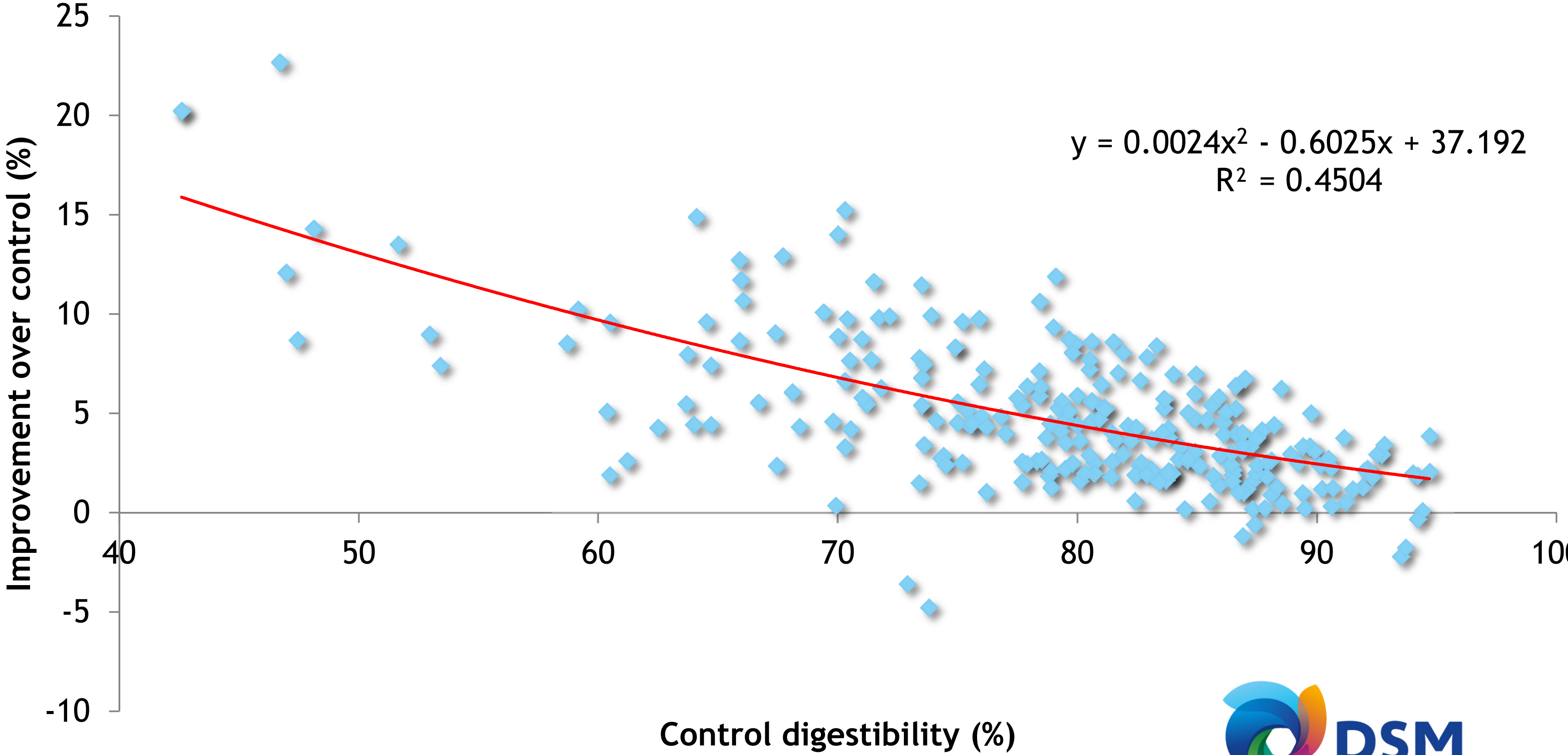
- mean amino acid profile of 8 sources of endogenous protein



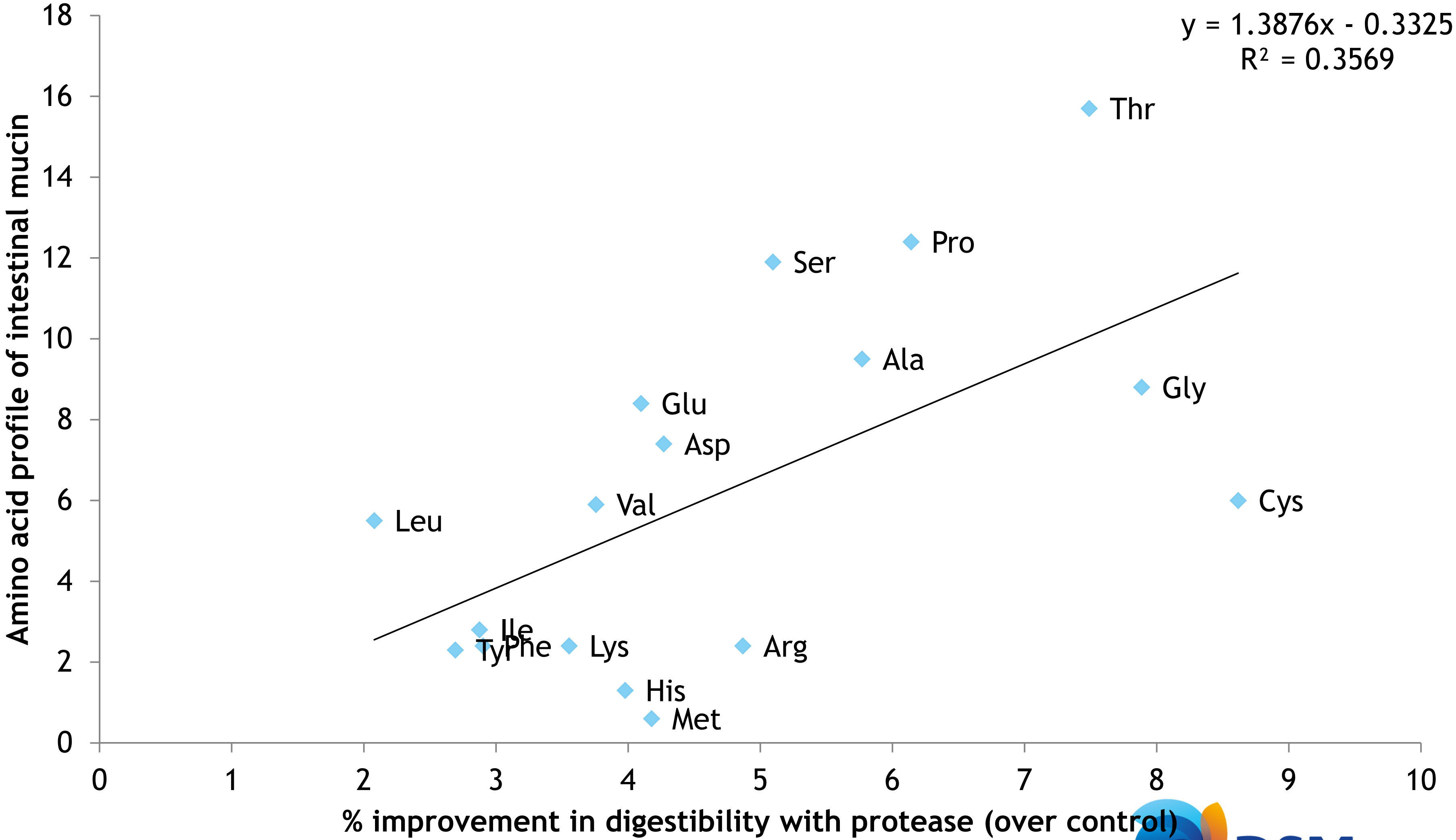
- amino acids of most significance, overall, are ser, gly, leu, pro, val, thr, as
- of least significance are met and his

Inherent digestibility is key - published papers bacterial protease

- Angel et al. (2010b); Iwaniuk et al. (2010); Angel et al. (2010b); Angel et al. (2011); Olukosi et al. (2013); Gugenbuhl et al. (2013); Viera et al. (2011); Messias et al. (2011); Carvalho et al. (2011a); Bertechini et al. (2011a); Carvalho et al. (2011b); Bertechini et al. (2011b)
- Total of 255 data points, diets and single ingredients, mostly broilers (piglets, turkeys, layers)



Significant correlation between mucin and protease effect



Enzymes and inherent digestibility

- So, any intervention that increases inherent digestibility will reduce the magnitude and consistency of response of feed enzymes

- Highest risk are proteases and carbohydrases as other than phytase there are few interventions that improve phytate-P digestibility

- Factors to consider include:
  - Feed form (pelleting conditions etc)
  - Bird age
  - Environmental conditions (including climate, stocking, housing, disease)
  - Quality of the diet fed and balance of nutrients
  - Water quality
  - Presence of other growth/digestibility promoting additives
    - AGP, other enzymes, acids, POM, eubiotics etc

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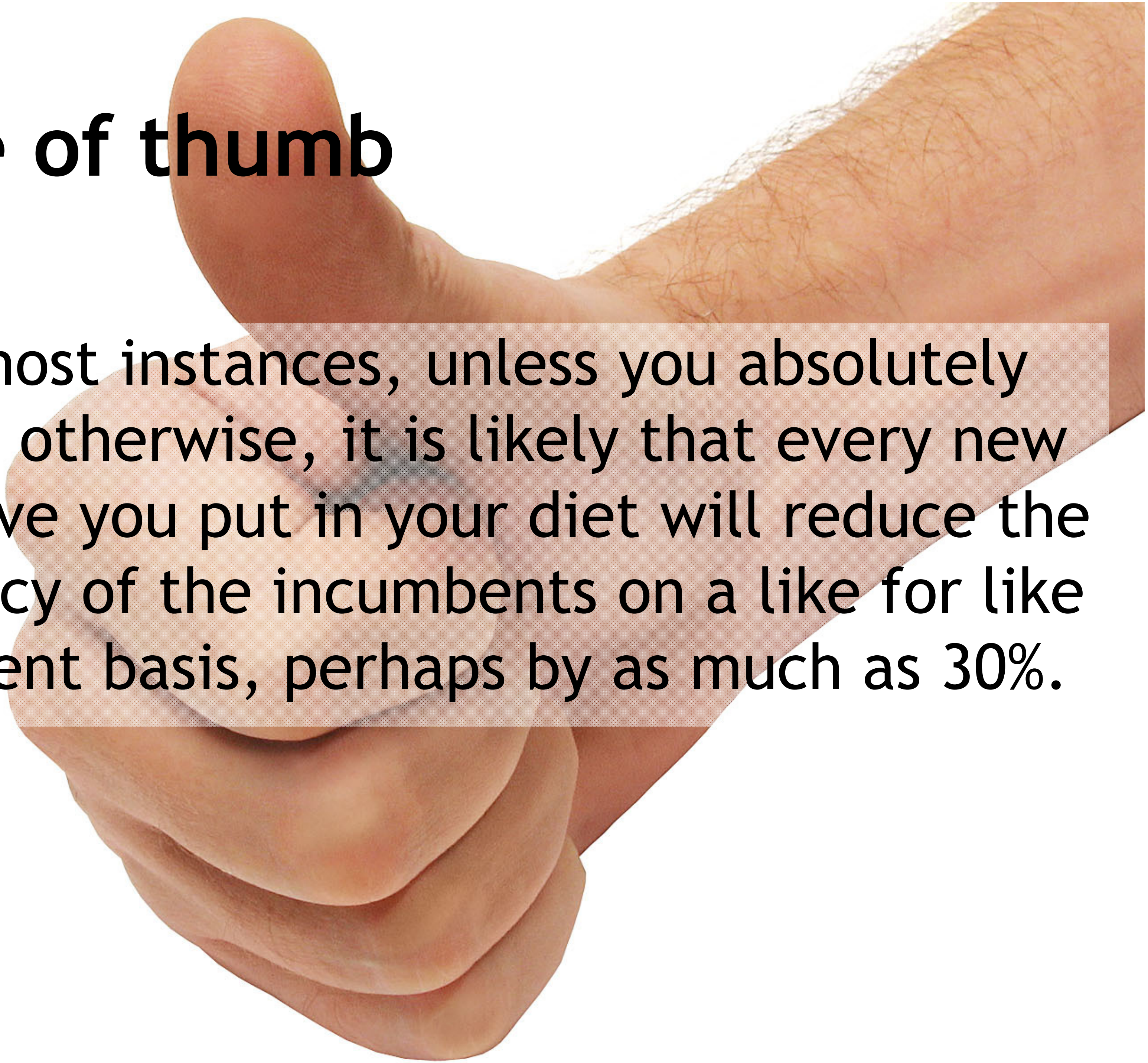
Rule of thumb



In most instances, unless you absolutely know otherwise, it is likely that every new additive you put in your diet will reduce the efficacy of the incumbents on a like for like nutrient basis, perhaps by as much as 30%.

Bird age - ontogeny of enzyme value

- If enzymes work best in diets with poor focal nutrient digestibility then how may this change with bird age?

- Intuitively nutrient digestibility increases as birds get older

- This is not universally true:

  - Corring (1980) - adaptation of digestive approach to diet modification
  - Huang et al. (2005) - age*cereal interactive effects on AA digestibility

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Adaptation to new diets – Corring (1980)

- GIT physiology is fluid and adapts readily to changing diet composition.

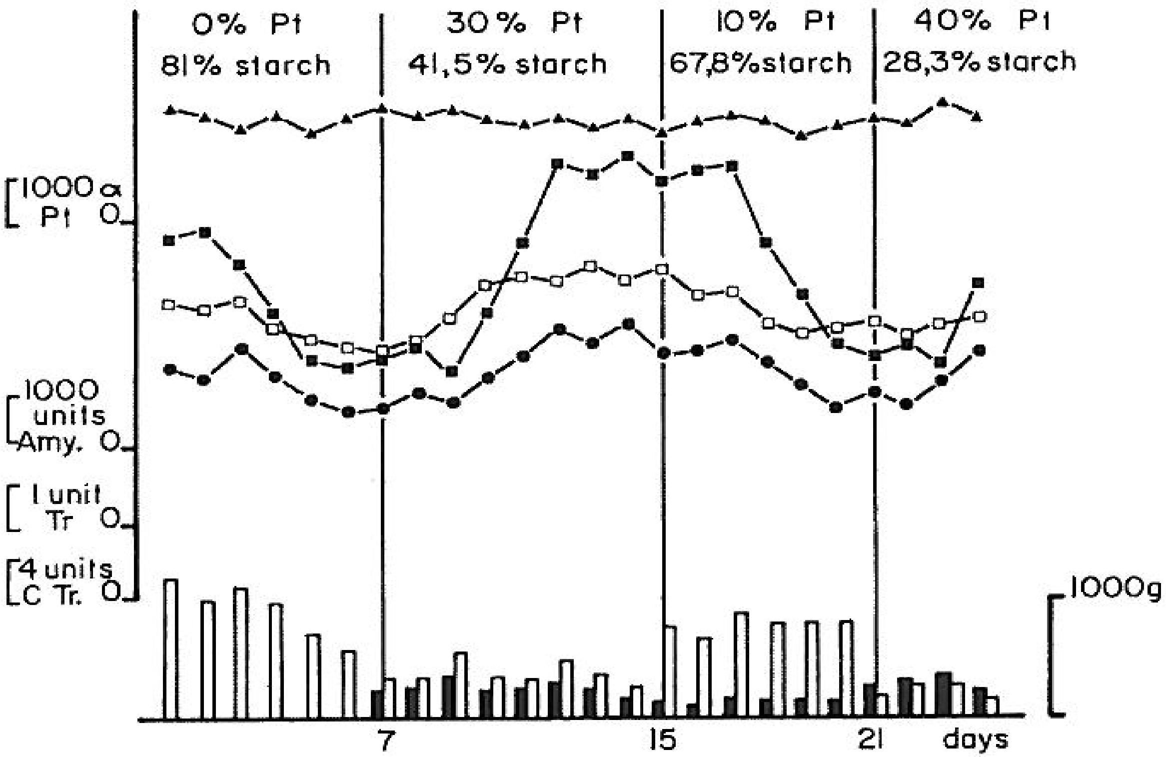
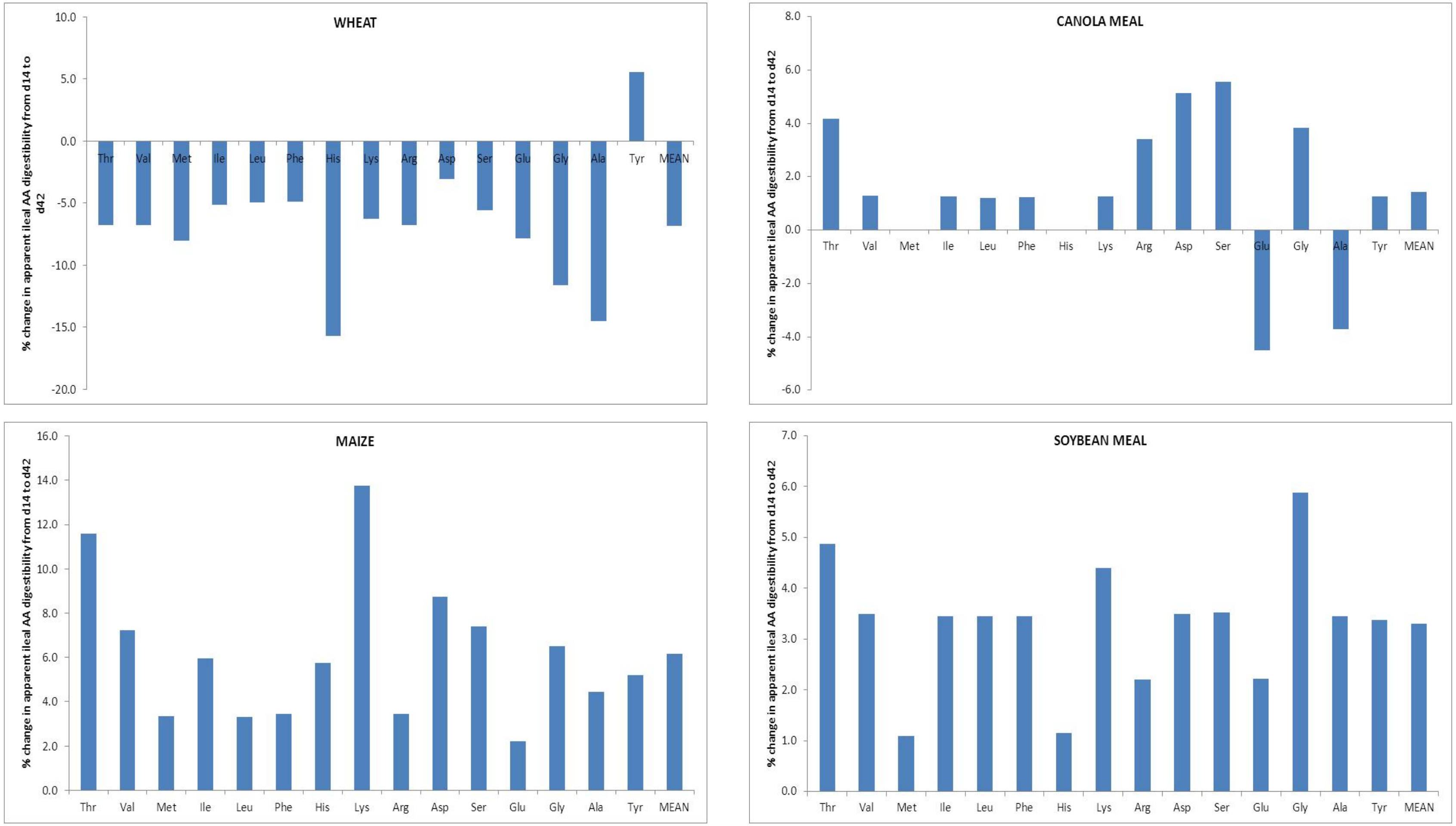


FIG. 2. — *Exocrine pancreatic adaptation to dietary protein in the pig.* Changes in specific enzyme activities and in total protein per ml of pancreatic juice. (Corring and Saucier, 1972). ■—■ : chymotrypsin ; □—□ : trypsin ; ●—● : amylase ; ▲—▲ : total protein ; ⊓ : starch intake ; ■ : protein intake.

Huang et al. (2005) British Poultry Science



- Wheat/Canola – overall a <u>decrease</u> in AA digestibility d14-42
- Corn/Soy – overall an <u>increase</u> in AA digestibility d14-42

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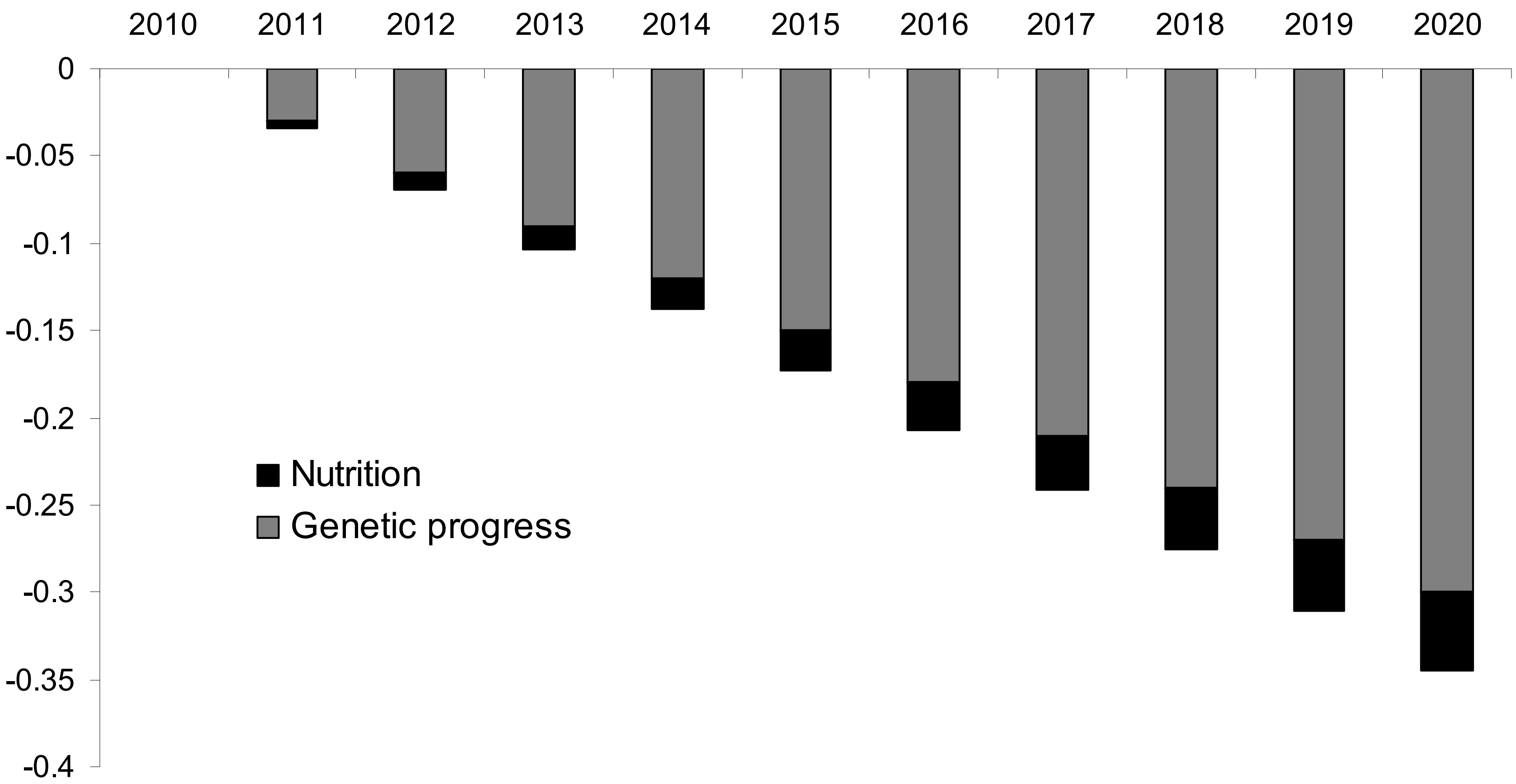
Chronological effects

Enzyme (especially non-phytase) efficacy declined from 1980-2010?

- Rosen (2002) suggests that non-phytase bioefficacy declined over time from the 1980s to early 2000s predicting that if the trend continued ZERO effect would be reached in 2004
- This did not happen
- Whats going on?

Amount of feed required to grow a bird to a 4 lb live weight
16
12
8
4
over 50% reduction!
16 lbs
6.5 lbs
1940s
Today

Projected Progress



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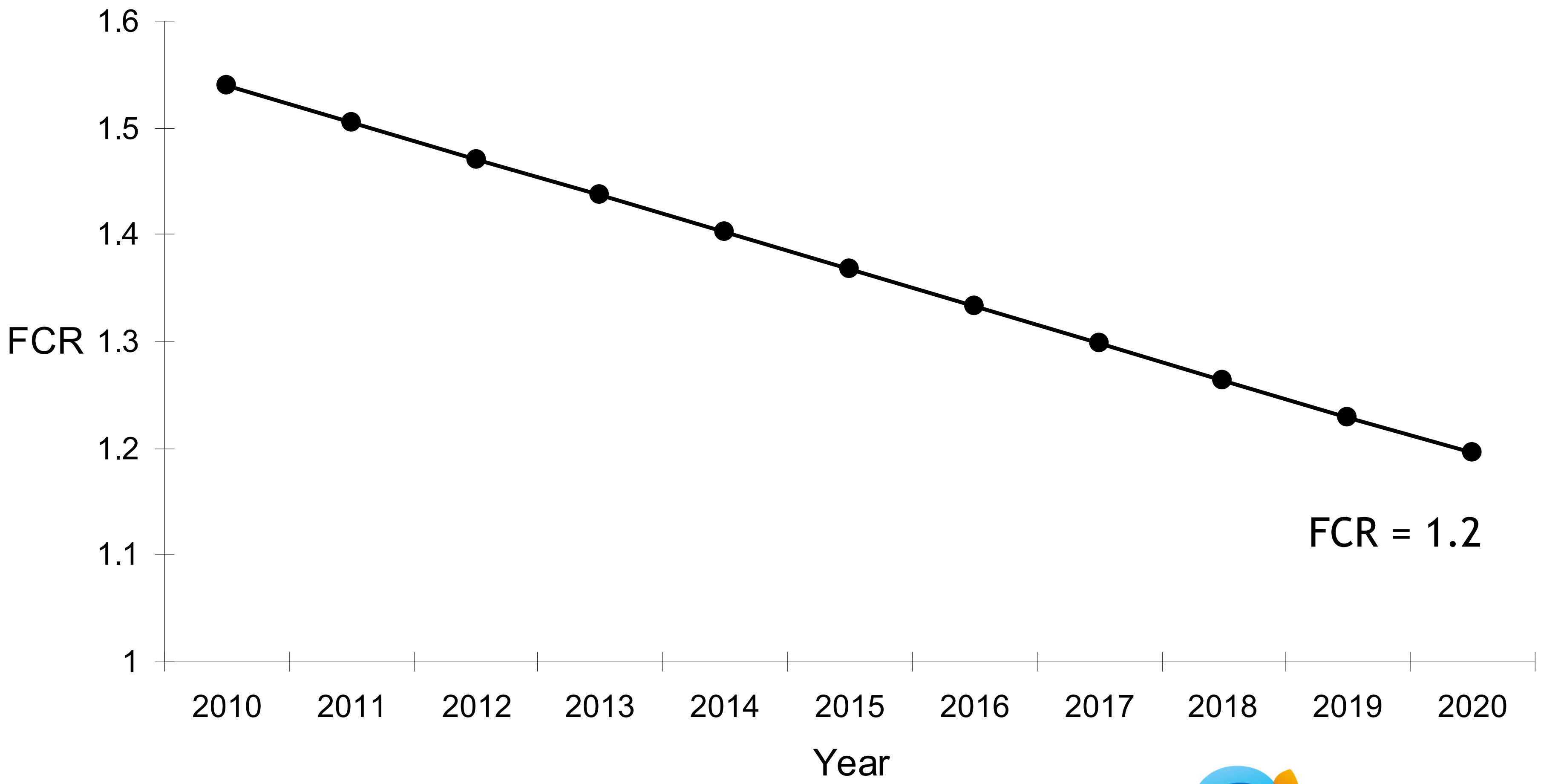
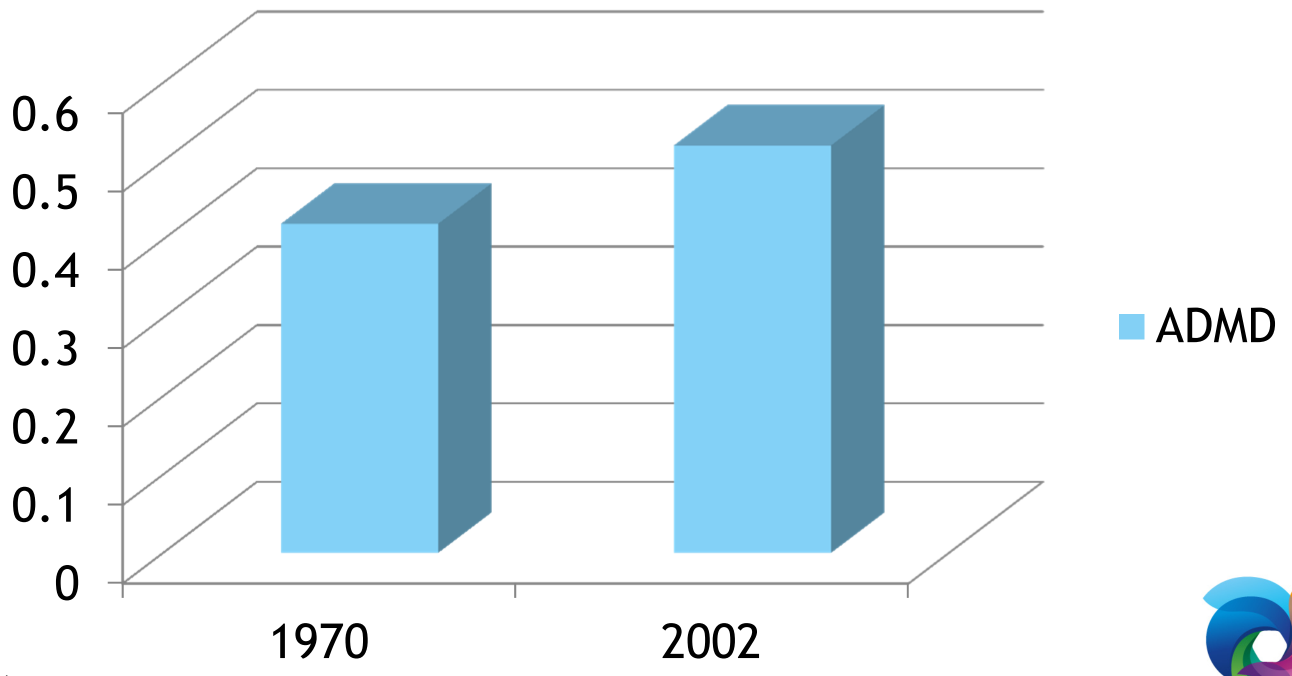
FCR in the Future



Where does the genetic improvement come from?

- Largely from increased feed intake and decrease maintenance requirements (Huang, 2012; de Beer, 2012 – personal comm.)
- HOWEVER, McDevitt et al. (2006) found that selection from 1970 to 2000 has increased ($P<0.05$) DM digestibility coefficients and AME



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So what?

- Genetics have altered the birds efficiency, partly by altering nutrient digestibility.
- Simultaneously cereal and grain legume varieties are nutritionally enhanced
- Husbandry, nutrition and biosecurity is improved since 1970s
- The closer bird performance is to the genetic potential the less opportunity there is for enzyme to elicit a beneficial effect.
- Nutrient digestibility cannot exceed 100%.
- A CONCEPTUAL FRAMEWORK TO UNDERSTAND THE LIMITS

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A caveat!

FCR is not fair......!

- Today a 2kg may eat 3kg of feed and so return an FCR of 1.5
- HOWEVER, the bird is 35% DM and the feed is 88% DM
- THUS, the reality is that on a DM basis the above is a 700g (DM) bird eats 2.65kg (DM) so the ‘true’ FCR of dry matter intake and dry matter retention is actually 3.8.
- We still have a fair way to go to reach an FCR of 1.0, even on a ‘fresh’ basis.
- The easiest way to achieve this is to drop the DM content of the live bird.

Future of phytase

- "Superdosing" (3x or more of standard) is here to stay
- Poorly defined and understood
- Dose response curves and inositol

- In the future:
  - New generation phytases (ongoing competition)
  - Combinations of phytases and phosphatases (and kinetic complementarity)
  - Extension to companion animal, aquaculture....
  - Human nutrition e.g. "MixMe" "Sprinkles" etc – huge potential for neonatal nutritional intervention
  - PHYTATE may be a 'second and third world' and production animal issue

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Phytase and protease?

- Bohn et al. (2007) phytate/protein globoids
- The protein shell makes these resistant to phytases
- Leske & Coon (1999) - phytate susceptibility

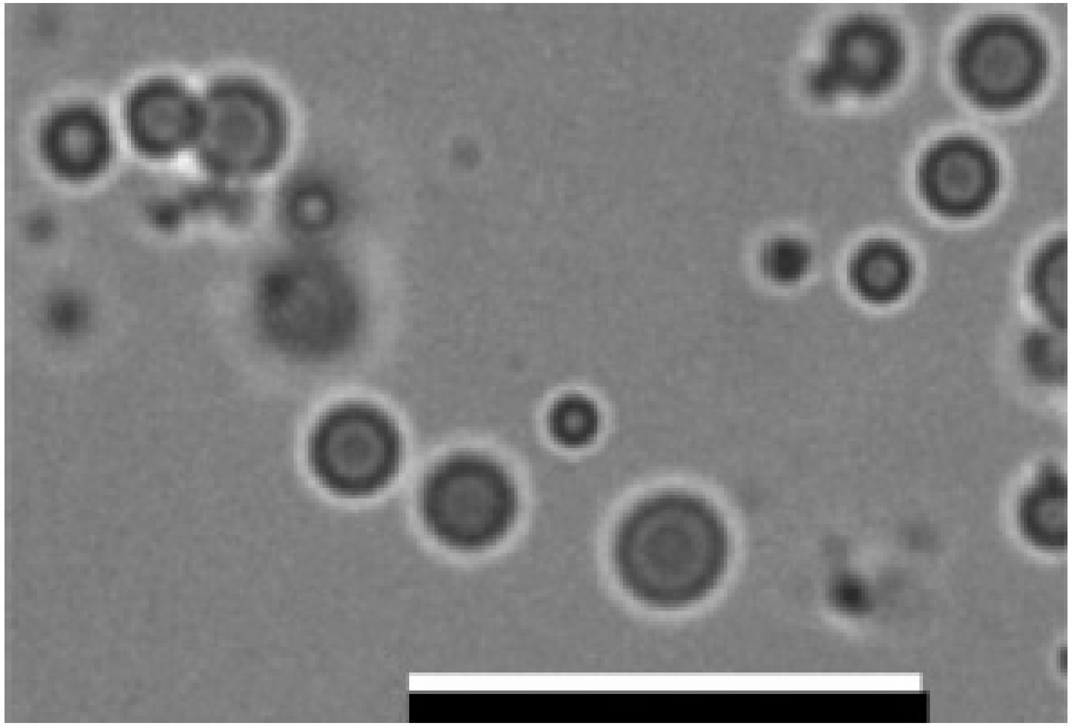

Figure 2. Phytate globoids isolated from wheat bran. Globoids are the spheres visualized by light microscopy (×100). Scale bar = 20 μm.

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Bye et al. (2013)

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Dual Effects of Sodium Phytate on the Structural Stability and Solubility of Proteins

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ABSTRACT: The interaction between sodium phytate and three proteins was studied using solubility experiments and differential scanning calorimetry (DSC) to assess structural stability. Lysozyme, which is positively charged at neutral pH, bound phytate by an electrostatic interaction. There was evidence that phytate cross-linked lysozyme molecules forcing them out of solution. Myoglobin and human serum albumin, which were neutral or negatively charged, respectively, displayed association rather than binding, and there was no complex formation. All of the proteins were structurally destabilized by the presence of phytate but were not denatured. From these findings, we predict that phytate would bind electrostatically to a wide variety of positively charged proteins in the stomach as well as to trypsin and chymotrypsin in the duodenum. Both binding reactions may compromise the digestion of the protein component in feed stuffs. Because the interaction between phytate and protein is electrostatic, the presence of anions, such as chloride, would nullify the antinutritional effect of phytate.

KEYWORDS: *Hofmeister, unfolding, precipitation, phytase, digestion*

Anions compete with phytate for the binding sites on proteins (including the digestive enzyme trypsin and the substrate proteins). It is also possible that higher salt (NaCl) diets for pigs and poultry may counter phytate binding to digestive enzymes, protein substrates, or any other positively charged material in the digesta, minimizing any antinutritional effect.

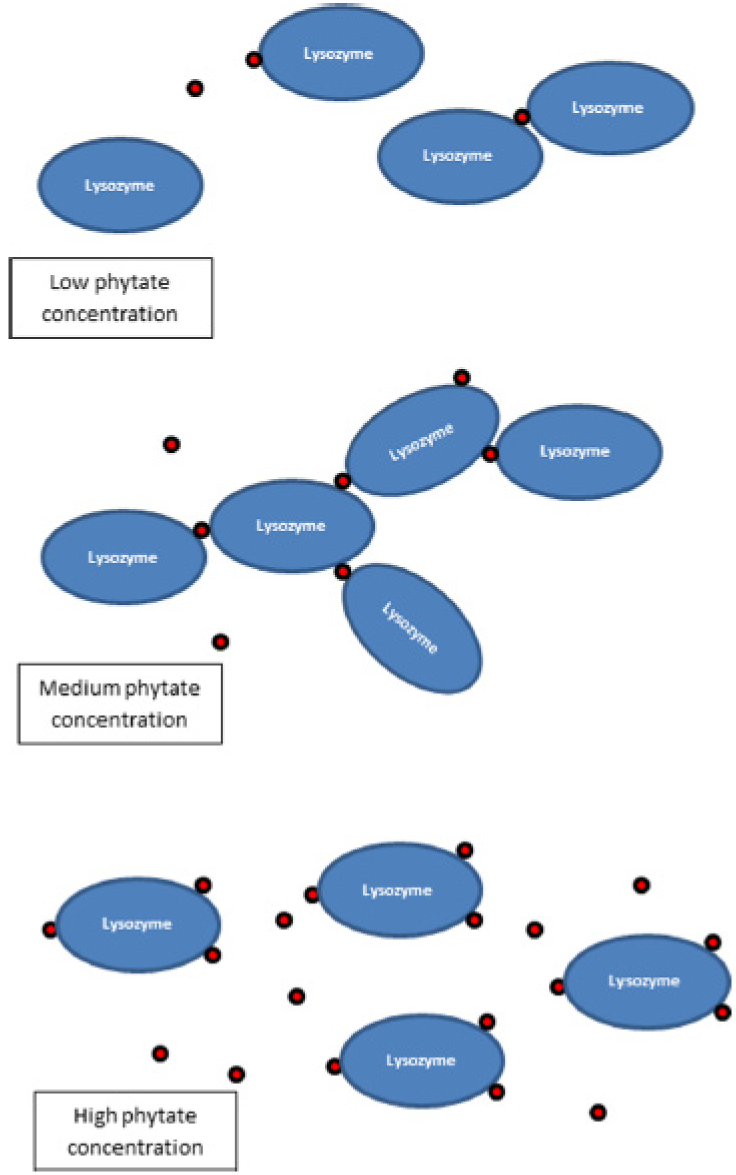

Figure 2. Interaction between proteins and phytate at pH < pI.

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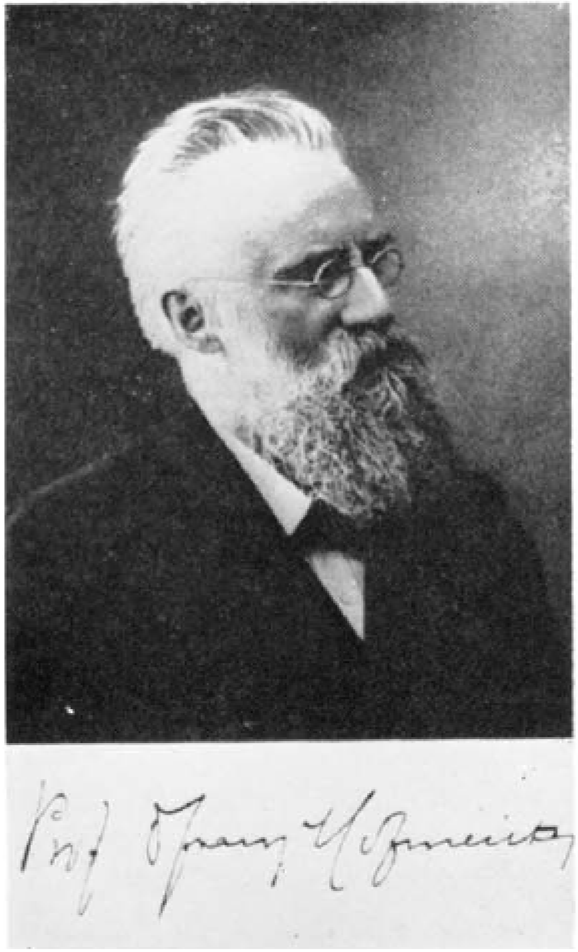
Franz Hofmeister



- Prof. Franz Hofmeister (1850-1922)
- Born in Prague, 1850
- Pharmaceutical chemistry
- Proposer of the 'Hofmeister Series' ionic grouping based on their ability to influence protein solubility

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Hofmeister Series

- **Effect of ions on protein solubility**

$CO_3^{2-} > SO_4^{2-} > HPO_4^{2-} > OH^- > F^- > HCOO^- > CH_3COO^- > Cl^- > Br^- > NO_3^- > I^- > SCN^- > ClO_4^-$

- **Fig. 1** Representation of Hofmeister anions with increasing chaotropic potency from left to right (adapted from Leontidis, 2002; Zhang & Cremer, 2006).

$Cs^+ > Rb^+ > NH_4^+ > K^+ > Na^+ > Li^+ > Mg^+ > Sr^{2+} > Ca^{2+}$

- **Fig. 2** Representation of Hofmeister cations with increasing chaotropic potency from left to right (adapted from Hess & van der Vegt, 2009)

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Ions

- Certain ions are kosmotropic and so reduce protein solubility
  - Carbonates, sulphates, chloride, potassium, phosphate, PHYTATE
- Certain ions are chaotropic and so improve protein solubility
  - Na, Ca, Mg

- Well understood in soil chemistry, colloid chemistry, surface interactions, protein chemistry etc
- Neglected in nutrition

- Phytate, from recent research appears to be a very potent kosmotropic anion

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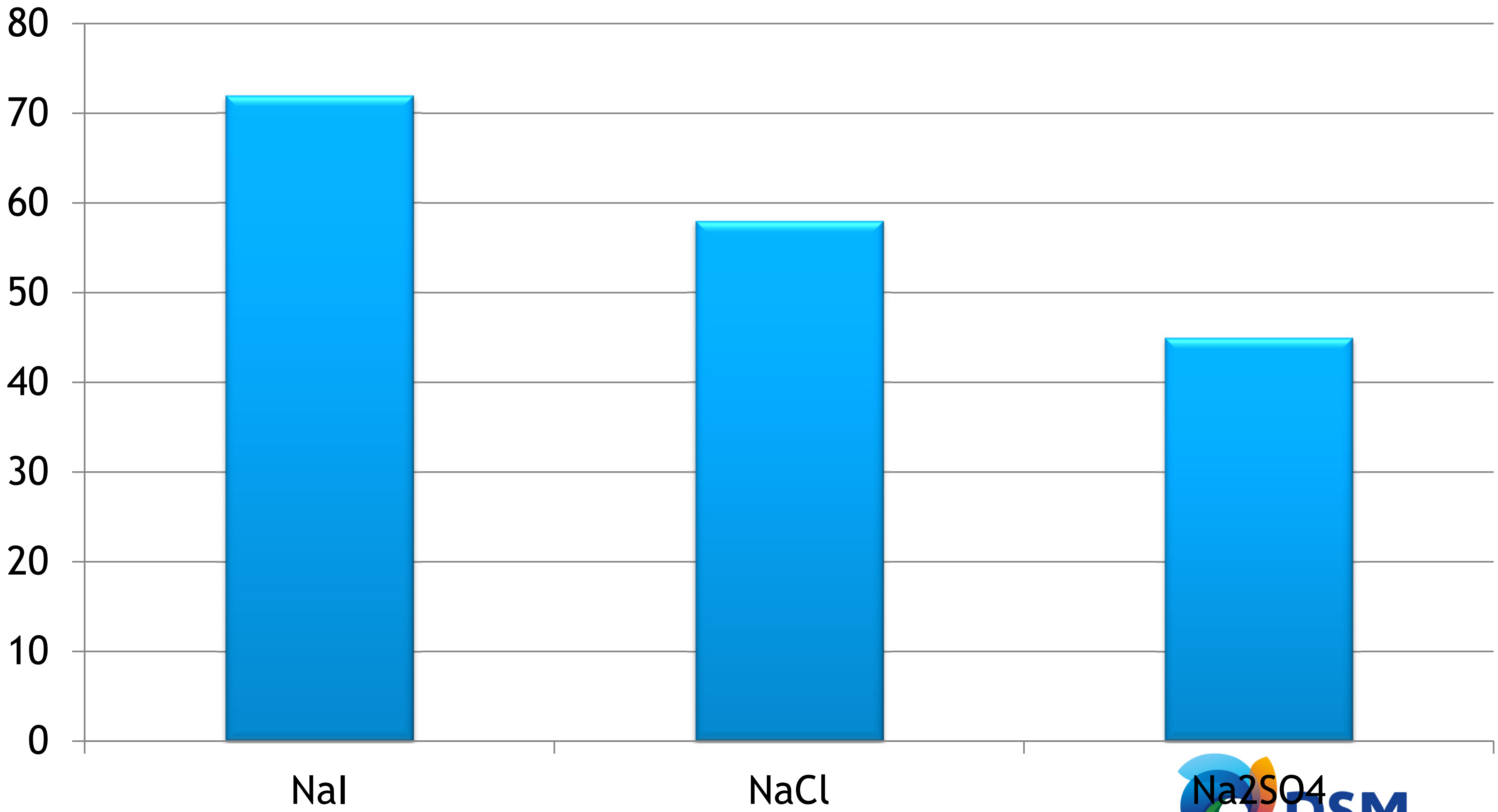
Damodaran & Kinsella (1982)

- Effect of 1M ion salts on soy protein solubility (%):



Beyond phytase.......

What is left to go for?

SOME BALLPARK FIGURES

- 0.05-0.10% digestible P
- 0.30-0.50% digestible Ca (what value does this offer?)
- 0.10-0.15% digestible SAA
- 0.15-0.20% digestible LYS
- 0.20-0.25% digestible THR
- 1200 kcal/kg digestible energy
  - 850kcal locked up in lignified cellulose, AX and pectin-type fibre
  - 350kcal locked up in undigested starch, protein and fat
    - 130kcal starch, 120kcal protein, 90kcal fat
- Where the energy comes from IS important.

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Low hanging fruit

- Little excuse for P digestibility to be substantially less than 100%
- Phytases are inexpensive so use more
- Next generation phytases can give close to 90% phytate recovery at 1500 FTU/kg

- Formulation to digestible Ca (nutritional geometry)

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Medium hanging fruit

- If the low hanging fruit is higher phytase doses and/or combinations of different phytase at conventional doses what is next?

- Xylanase OR glucanase (Cowieson et al., 2010)
  - Enhanced protein digestibility (gives some energy release)
  - Variable improvements in fat and starch digestibility
  - Marginal P and Ca digestibility improvements

- New mechanisms for xylanase emerging (Masey-O'Neill et al., 2012; Cowieson & Masey-O'Neill, 2012) that explain the 'generic effects' (Cowieson & Bedford, 2009) on all nutrients

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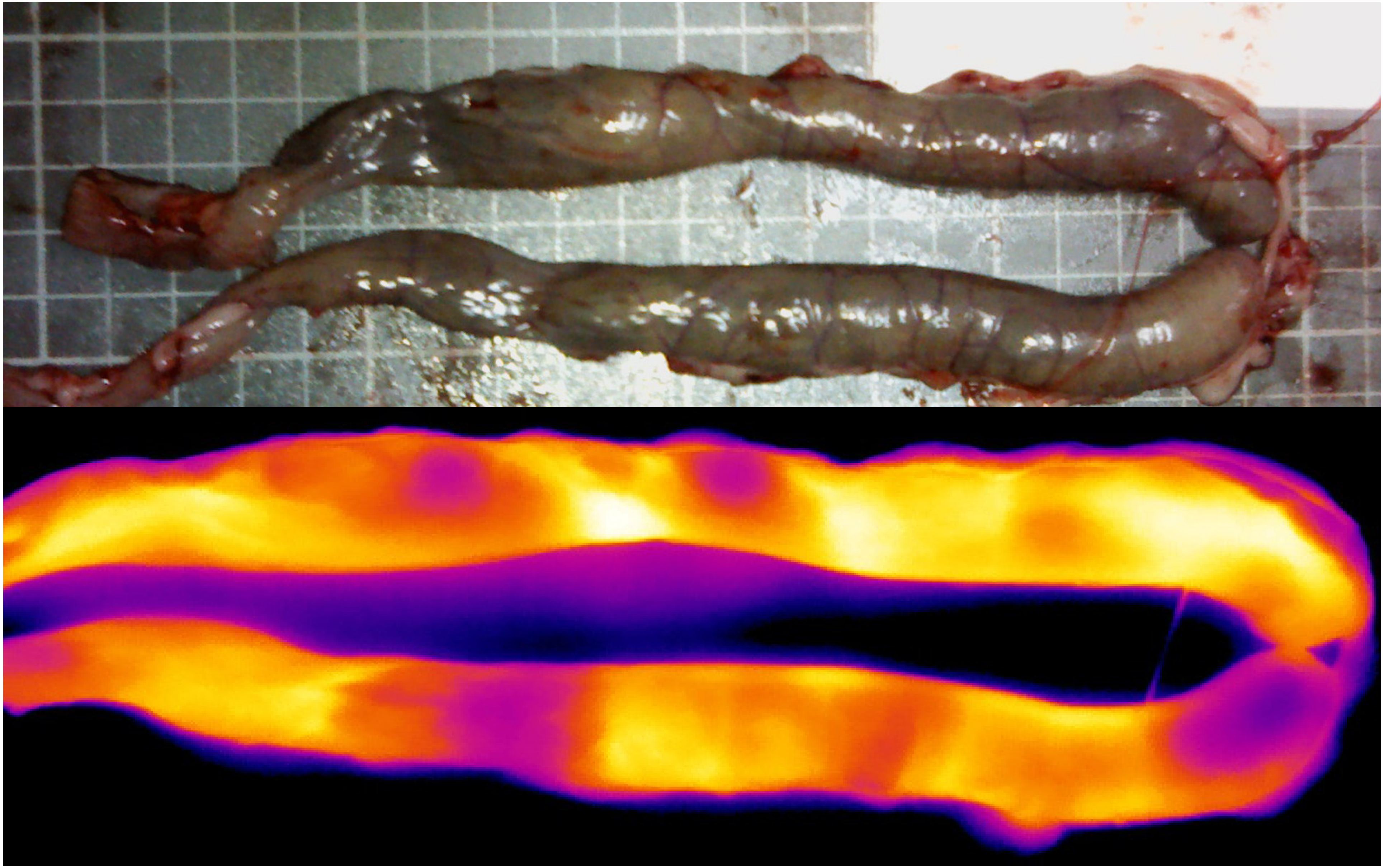
Caecal thermogenesis

Cowieson & Masey-O'Neil (2013)



Ok, high phytase doses, plus xylanase, what else?

- Realistically the use of 2000-3000FYT/kg phytase, protease and an aggressive xylanase leaves little ‘meaningful’ undigested nutrients behind
- Some room to capture additional Lys, Thr and potential for Val, Ile, Gly, Ser, Arg (may be able to drop SBM inclusion if done carefully)
- Little additional energy or P potential
- “high hanging fruit”
  - Pectin, amorphous cellulose degradation.
  - New proteases (more data today than ever before but mechanisms remain unclear)
    - Driven by high SBM prices, definitely worth exploration
    - Acidic cystine peptidases and aspartic acid peptidases for gastric regions in addition to serine peptidases for SI functionality?

Myo-Inositol

- The core of phytic acid
- Has not historically been considered as a product of phytase effect
- New evidence of up-concentration of MYO in plasma of broilers and pigs (Guggenbuhl et al., 2013; Cowieson et al., 2014)

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Myo-Inositol

- MYO is an INSULIN MIMETIC (Dang et al., 2010; Yamashita et al., 2013)
- Translocates GLUT4 in mammals (and a yet to be identified glucose transporter in avian species) – (Tokushima et al., 2005; Sweazea & Braun, 2006)
- Orally administered MYO improves FCR in broilers (Cowieson et al., 2013; Zyla et al., 2013)

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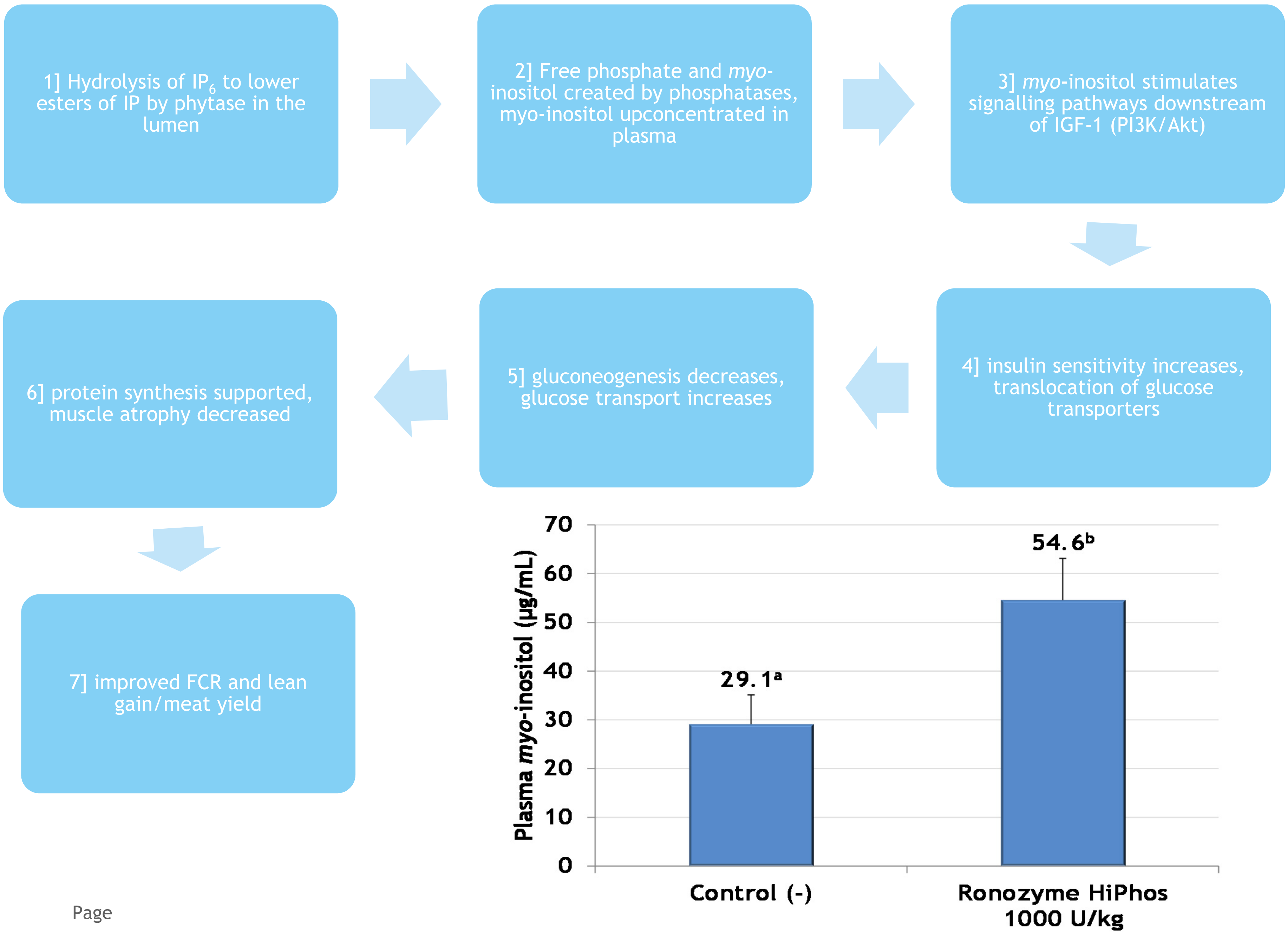
1] Hydrolysis of IP6 to lower esters of IP by phytase in the lumen
2] Free phosphate and myo-inositol created by phosphatases, myo-inositol upconcentrated in plasma
3] myo-inositol stimulates signalling pathways downstream of IGF-1 (PI3K/Akt)
4] insulin sensitivity increases, translocation of glucose transporters
5] gluconeogenesis decreases, glucose transport increases
6] protein synthesis supported, muscle atrophy decreased
7] improved FCR and lean gain/meat yield
Plasma myo-inositol (µg/mL)
70
60
50
40
30
20
10
0
29.1a
54.6b
Control (-)
Ronozyme HiPhos 1000 U/kg

Future

- Genetic improvements likely to drop FCR for a 2kg bird to 1.20 by 2020-2025
- More microingredient launches and so more competition

- Though digestibility coefficients approach 100% there is room for intervention in NUTRIENT REQUIREMENT and MAINTENANCE

- Meta-analyses will become increasingly important to explain variance in enzyme effect and allow integration of optimisation tools with enzyme use

- Enzyme combinations can be assembled based on overlap in meta-analysis outcomes and through mechanistic exploration

Conclusions

- Global feed additive market to grow from $13.5bn in 2010 to $17.5bn in 2018
- Digestibility coefficients are difficult to improve beyond 0.95.
- FEED COST savings with enzymes may max out around $20/tonne
  - Many paths, but not all paths, will get you there!
- Very few genuine paradigm shifts in the feed enzyme market since phytase was launched in the early 1990s, perhaps due to law of diminishing returns.

- I wonder what Dr. George Hervey would make of his humble Leghorn experiment having led to an international market worth in excess of $800m USD! He's not even mentioned on Rutgers 'historical achievements' list!


THANK
YOU

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