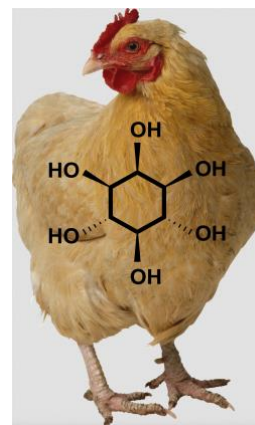


Institution: University of East Anglia		
Unit of Assessment: 5 – Biological Sciences		
Title of case study: "Superdosing" with phytase: improving animal feed with a new understanding of enzyme action		
Period when the underpinning research was undertaken: 2003 - 2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Professor Charles Brearley	Professor of Biochemistry	2001- present
Period when the claimed impact occurred: August 2013 – December 2020		
Is this case study continued from a case study submitted in 2014? No		
1. Summary of the impact <p>Phytate in animal feed impairs the growth and sustainable production of poultry. Consequently, phytases - enzymes that degrade phytate - are added to 95% of commercial poultry feeds across the globe. Brearley's research on how phytases affect phytates has impacted the animal feed industry on a global scale. This has been achieved by direct intervention and via knowledge transfer to AB Vista, a UK animal nutrition technology company. Brearley's research has changed AB Vista's R&D strategy, know-how, marketing behaviour and technology adoption, enabling AB Vista to become one of the three largest animal feed enzyme suppliers in the world. Overall, this impact and its knock-on effects have benefited an industry sector producing more than 10 billion chickens per year - approximately 17% of global production – in a multibillion-dollar enzyme market.</p>		
2. Underpinning research <p>Phytate, a component of animal feed, is a potent anti-nutrient that is estimated to cost the global poultry industry USD2,000,000,000 (05-2014) per year in lost growth performance. Feed supplements that contain phytases aid digestive degradation of phytate, improve mineral, protein and amino acid digestibility, and release phosphate, which is essential for bone development. They therefore strongly improve animal growth.</p> <p>Since the first commercial phytase was released to market in the late 1990s, phytases have become essential components of commercial poultry and pig feeds. However, until 2014, the benefits of phytase to poultry growth performance were thought to lie predominantly with degradation of phytate, also known as inositol hexakisphosphate, to lower inositol phosphates. This interpretation came from animal feed trials in which growth performance was enhanced by phytase. But, at that point, few studies had considered the detailed mechanism of phytate degradation or its interaction with animal performance.</p> <p><i>Elucidating the mechanism of phytase (Quantum Blue) action in partnership with AB Vista:</i> In 2012, AB Vista's Research Director and team approached Brearley to contract him to conduct consultancy/contract research with the purpose of providing scientific guidance and a mechanistic explanation for the company's dosing strategy when including phytase in poultry feeds. They also invited him to give a Plenary Talk at the AB Vista-sponsored 2nd Phytase Summit. AB Vista sought to access Brearley's recognised expertise in inositol phosphate and phytase research [R1, R2] because AB Vista lacked the expertise to undertake analysis of inositol phosphates themselves. The specific questions for which answers were sought were: in what part of the digestive tract does phytate digestion occur, how is this affected by phytase dose, and how does growth performance of monogastric animals (poultry and pig) correlate with individual inositol phosphates</p>		



produced in different parts of the gut? AB Vista's approach was timely because the company had just launched its new phytase product Quantum Blue, and **Breareley** had developed 2d-HPLC (High Performance Liquid Chromatography) pulsed amperometry methods suitable for measurement of inositol, the ultimate product of degradation of phytate (research later published as [R3]). At the time, the industry had noticed that incremental increases in animal performance could be obtained by increasing doses of phytase. These increases surpassed those attainable by supplementation of diet with phosphate. These so-called "extra-phosphoric" effects became known as "Superdosing", a term coined by AB Vista (Walk CL et al. 2013. *Poultry Science* 92: 719-725. DOI: 10.3382/ps.2012-02727), even if the explanation for how exactly they worked eluded the industry.

The role of inositol: Since 2013, **Breareley** has worked with AB Vista on its Quantum Blue product to determine the optimal dosing regimen of phytase for animal production. **Breareley** recognised and reported to AB Vista that the real value of Superdosing was likely to stem from the release of inositol, historically considered a vitamin. He confirmed this, providing AB Vista with analytical data that were published by the company as their first report of the effect of Superdosing with phytase (Quantum Blue) on measured inositol release and its correlation with growth performance in poultry [S2]. **Breareley** also led the development of robust, sensitive methods of measurement of inositol phosphates (isomers) and inositol. These have been applied to feed trials of standard and Superdoses of phytase, using samples of feed and gut contents from different parts of the gastrointestinal tract. The work has been funded directly by AB Vista [Grant A] and by a joint BBSRC grant with AB Vista [Grant B].

Global developments of the Breareley/AB Vista partnership: The shift in attention from inositol phosphates and phosphate release to inositol as an explanation of Superdosing efficacy has been transformative for AB Vista. For example, because **Breareley** further refined his methods, making them suitable for measurement of inositol in plasma [R3] and animal tissue [R4, R5], AB Vista commissioned additional feed trials conducted by other contractors on four continents. These trials collected plasma and tissue samples from poultry and pigs (beside feed and digesta) for inositol analysis by **Breareley** under contract to AB Vista [R3, R5; S1].

Breareley also partnered the wider AB Vista group (AB Enzymes, Germany, and Roal Oy, Finland) with BBSRC in pursuit of next-generation phytases [Grant C]. This project arose directly from **Breareley**'s earlier discovery of a new class of phytase [R2] and led to a PhD studentship funded by AB Vista, one of six PhD studentships supervised by **Breareley** with funding solely from AB Vista or from AB Vista jointly with BBSRC or NERC [S1]. Overall, **Breareley**'s research is reported in more than twenty research outputs with AB Vista authors or co-authors (including [R4-R6]; see also [S1-S3]). This body of work has further provided valuable information to AB Vista and the wider poultry industry on the relationship of inositol to the causes of two muscle diseases of chicken, "Woody Breast" and "White Striping" [R5]. These pathologies are responsible for losses of more than USD200,000,000 (10-2017) per year to the poultry industry in the USA. The role of phytase and inositol release in the amelioration of these pathologies are, as a result of **Breareley**'s research, subjects of intense current interest within AB Vista [S1] and other feed companies.

Image: Broiler chicken and inositol molecule; *Credit:* chicken: Creative Commons 4.0 BY-NC, <http://pngimg.com/imgs/animals/chicken/>; molecule – Charles Breareley.

3. References to the research

Underpinning research: The underpinning research is published in competitive, international, peer-reviewed multidisciplinary or discipline-specific journals (citation numbers are from Google Scholar; UEA author names are in bold; **industry co-author):

- R1 Lemtiri-Chlieh F, MacRobbie EAC, Webb AAR, Manison NF, Brownlee C, Skepper JN, Chen J, Prestwich GD, **Breareley CA** (2003) Inositol hexakisphosphate mobilizes and endomembrane store of calcium in guard cells. *Proceedings of the National Academy of Sciences* 100: 10091-10095. DOI: 10.1073/pnas.1133289100 [280 citations]

- R2 Stentz R, Osborne S, Horn, N, **Li AWH**, Hautefort I, Bongaerts R, Rouyer M, Bailey P, Shears SB, **Hemmings AM**, **Brearley CA**, **Carding SR (2014)** A bacterial homolog of a eukaryotic inositol phosphate signalling enzyme mediates cross-kingdom dialog in the mammalian gut. *Cell Reports* 6: 646-656. DOI: 10.1016/j.celrep.2014.01.021 [52 citations]
- R3 Pirgozliev, V, **Brearley CA**, Rose SP, Mansbridge SC (2019) Manipulation of plasma *myo*-inositol in broiler chickens: effect on growth performance, dietary energy, nutrient availability, and hepatic function. *Poultry Science* 98: 260–268. DOI: 10.3382/ps/pey341 [5 citations]
- R4 Lu H, Kühn I**, Bedford M**, **Whitfield H**, **Brearley C**, Adeola O, Ajuwon KM (2019) Effect of phytase on intestinal phytate breakdown, plasma inositol concentrations and glucose transporter type 4 abundance in muscle membranes of weanling pigs. *Journal of Animal Science* 97: 3907-3919. DOI: 10.1093/jas/skz234 [6 citations]
- R5 Greene ES, Flees J, Dadgar S, Mallmann B, Orlowski S, Rajaram N, Rochell SJ, Kidd M, **Laurendon C**, **Whitfield H**, **Brearley C**, Walk C**, Dridi S (2019) Quantum blue reduces the severity of Woody Breast myopathy via modulation of oxygen homeostasis-related genes in broiler chickens. *Frontiers in Physiology*. 10: 1251. DOI: 10.3389/fphys.2019.01251 [10 citations]
- R6 **Acquistapace IM**, **Ziętek MA**, **Li AWH**, **Salmon M**, Kühn I**, Bedford MR**, **Brearley CA**, **Hemmings AM (2020)** Snapshots during the catalytic cycle of a histidine acid phytase reveal an induced fit structural mechanism. *Journal of Biological Chemistry* (published online ahead of print) DOI: 10.1074/jbc.RA120.015925

Funding: Funding for the underpinning research was obtained from industrial and UKRI Research Council sources. Direct funding was provided by AB Vista [Grant A]. AB Vista also contributed financially to the competitive, peer-reviewed grants obtained from BBSRC, namely a LINK Award [Grant B] and an Industrial Partnership Award [Grant C]: **Grant A:** PI: **CA Brearley**. Title: *Phytate Analysis*. Funder: AB Vista. Dates: January 2013 – December 2020. Value: GBP814,100. **Grant B:** PI: **CA Brearley**. Title: *Phytases beyond phosphate, how inositol improves feed conversion ratio in poultry*. Funder: BBSRC LINK Award. Dates: 27 June 2016 – 26 November 2018. Value: GBP188,506. AB Vista contribution GBP90,000. **Grant C:** PI: A Hemmings (UEA); Co-I: **CA Brearley**. Title: *Mapping the catalytic landscape of a novel phytase*. Funder: BBSRC Industrial Partnership Award. Dates: 30 September 2015 – 29 June 2019. Value: GBP459,551. AB Enzymes/AB Vista contribution GBP96,000.

4. Details of the impact

Brearley's research has had impacts on commerce, the economy and production, both directly and via knowledge transfer. The beneficiaries are commercial animal feed businesses on a global scale, specifically AB Vista and its partner companies, AB Enzymes, Germany, and Roal Oy, Finland, who produce the Quantum Blue enzyme. His research has also benefited other enzyme companies and the global poultry industry (both producers and nutritionists). As illustration, because of **Brearley's** work with AB Vista, the term "Superdosing" has come to convey the benefits arising from provision of inositol through adding phytase to animal feed. As such, it has been accepted as a standard term in academia and industry, with more than 570 citations in Google Scholar since 2013.

Change in industry R&D strategy: **Brearley's** research [R3-R6] and his collaboration with AB Vista [S1] have driven a key change in AB Vista's R&D strategy. This has involved the company shifting its R&D focus towards understanding the role of inositol [S1-S3], as evidenced by: AB Vista's funding of **Brearley's** consultancy contract [Grant A]; the company's collaboration with **Brearley** in obtaining BBSRC LINK and Industrial Partnership Award funding [Grants B, C]; and the company's funding of multiple PhD studentships both at UEA and internationally [S1]. In addition, AB Vista has commissioned research feed trials in 15 countries across four continents (e.g. Australia, India, USA, Germany, UK), from which it has sent samples to **Brearley** at UEA for analysis of inositol phosphate and inositol products of phytate degradation [R3-R5; S1-S2]. Overall, the involvement of **Brearley** has been a disruptive innovation for the industry's R&D. As AB Vista's Research Director stated, "By providing us with a first set of measurements of inositol in a digestive setting, something we had not considered ourselves, Charles opened up a new

research area across the animal feed industry. This has been very significant for AB Vista and the industry as a whole" [S1].

Change in industry know-how: The metadata arising from analysis by **Brearley** of more than 10,000 samples from the AB Vista-commissioned feed trials have, by providing essential scientific underpinning, reshaped AB Vista's understanding of the Quantum Blue phytase and the benefits to be gained by Superdosing. To quote AB Vista's Research Director [S1], *"Charles' work on inositol and Superdosing has allowed us to progress from an understanding of the correlation of animal growth performance to inositol phosphate release, in different parts of the gut, to a sophisticated understanding of the extent of animal growth performance attributable to inositol"* [S1].

Change in industry behaviour: marketing: AB Vista's guidance on dosing of Quantum Blue has also been changed. The guidance to customers covers the range of poultry-raising regimes used worldwide for all the chickens (over 10,000,000,000 birds) fed AB Vista's phytase products. *"Our advertising strategy includes evidence generated by UEA showing that our products function at the intestinal level better than all other phytases, and the data generated enables us to explain why superdosing, for example, works in poultry and swine from a mechanistic viewpoint"* (AB Vista's Research Director [S1]). **Brearley's** data are also reported across AB Vista's web pages [S3].

Change in industry behaviour: technology adoption and knowledge transfer: **Brearley's** interaction with AB Vista has led to the company adopting his analytical methods. To quote AB Vista's Research Director [S1] *"His analytical expertise with inositol phosphates and inositol has provided a route to analysis that was not available to us before on a scale relevant to our needs. In order to be able to respond rapidly to industry needs, we are now investing heavily in Charles' methodologies in our analytical services division in the UK"*. Separately, **Brearley** has provided training in analytical methods to AB Enzymes (a market leader as reflected in their turnover exceeding USD112,000,000 (08/2019)), partner on Grants A-C. This allowed for new methods to be adopted by the R&D department of AB Enzymes and in turn led to AB Enzymes testing novel applications of Quantum Blue [S4]. In addition, **Brearley** has transferred data on novel phytases for further investigation to Roal Oy, partner via AB Vista on Grant C.

Company success: The changes in R&D, know-how and marketing, all arising from the new understanding of AB Vista's Quantum Blue phytase provided by **Brearley's** research, have enabled AB Vista to sell more of its product. This has been achieved by advertisement of the efficiency of Quantum Blue in degrading phytate and releasing inositol [S3]: *"The credibility that UEA research brings to our products means that customers who are at the forefront of technology are far more likely to buy from us, ...Charles' analysis of inositol phosphates and inositol, his further development of methods suitable for tissues and organs and his interaction with our other academic partners contributes to our literature, presentations and conferences, informing existing customers regarding their use of our products, and keeping them abreast of technological developments"* (AB Vista's Research Director [S1]). **Brearley's** research has therefore been central to AB Vista's growth to become a top three supplier of phytase [S1, S5], in a market projected to exceed USD2,000,000,000 (11-2019) by 2024. *"I can confirm that the research undertaken at UEA has enabled AB Vista to move from being the 6-7th largest enzyme supplier in the world to number 2-3 in the last seven years"* (AB Vista's Research Director [S1]).

Benefiting the global poultry and pig industry: The impact of **Brearley's** work extends beyond AB Vista to its customers and competitors in the poultry and pig sectors. For example, industry-wide adoption of Superdosing and the contribution of inositol to this technique is evidenced in a competitor's 2017 marketing presentation, which stated, *'Use of Ronozyme® HiPhos increases blood levels of myo-inositol by a quick break-down of phytate in the gut'* [S6]. Moreover, phytase sales benefit customers as well as producers. AB Vista's Global Sales Director has quantified the value of phytate degradation to customers, *"Estimates suggest the anti-nutritional effects of phytate could be costing the industry as much as €2 billion [EUR2,000,000,000 (05/2014)] per year in lost performance, and that the potential still to be captured from near-complete phytate*

*destruction is as high as €5 [EUR5.00 (05-2014)] per tonne of feed manufactured" [S7]. Lastly, **Breareley**'s research on the role of inositol in the aetiology of two muscle diseases in chicken, "Woody Breast" and "White Striping" [R5], hold potential for substantial future benefits to the industry. As AB Vista's Research Director has stated of **Breareley**'s presentation of this work to an industry conference in Taiwan in 2019, *"The event in Taiwan helped more than one hundred participants... The sales increment could be substantial if we find that dosing two to three times higher than current 'superdosing' norms prove beneficial"* [S1].*

5. Sources to corroborate the impact

S1 Letter from the Research Director, AB Vista (30.12.20).

S2 Walk CL, Santos TT, Bedford MR (2014) Influence of superdoses of a novel microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in young broilers. *Poultry Science* 93: 1172-1177. DOI: 10.3382/ps.2013-03571 [This article is AB Vista's first report of the contribution of inositol release to the efficacy of Superdosing, as referred to in S1's paragraph 3, line 7, sentence beginning, *"By providing us..."*.]

S3 AB Vista website and marketing materials: A Superdosing infographic from AB Vista from abvista.com (accessed 21.11.20). [The use of Breareley's data in AB Vista's marketing materials is referred to in S1's paragraph 4, line 5, sentence beginning, *"Charles' analysis of ..."*.]

S4 Research Scientist, AB Vista, partner company AB Enzymes.

S5 Feed industry assessment of AB Vista market position; article from globenewswire.com, 2019 (accessed 21.11.20). [Paragraph 6: Quantum Blue has helped AB Vista become second in world.]

S6 Marketing presentation by the enzyme company DSM; DSM/Novozymes Enzyme School from dsm-feedback.dk, 1-2.3.17 (accessed 21.11.20). [Quote is on slide 7.]

S7 AB Vista press release; AB Vista's Quantum Blue: Revolutionary New Phytase from thepoultrysite.com, 16.5.14 (accessed 21.11.20). [Quote is from paragraph 3.]