

Institution: University of Huddersfield		
Unit of Assessment: UoA8		
Title of case study: Arabinoxylans, oligosaccharides and xylanases: enhancing prebiotic benefits in animal feed		
Period when the underpinning research was undertaken: September 2014 – present		
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 Grant Campbell	Professor of Chemical Engineering	September 2014 – present
Dr Mohammad Alyassin	Knowledge Transfer Associate	April 2019 – present
Dr Nikolina Cukelj	Post-doctoral researcher	Sept 2014 – July 2015
Period when the claimed impact occurred: September 2014 – present		
Is this case study continued from a case study submitted in 2014? No		
1. Summary of the impact <p>Work undertaken in Huddersfield in collaboration with AB Vista has established protocols for extracting arabinoxylans from a range of feedstocks and for analysing the degradation products produced by AB Vista's xylanase enzymes, in support of AB Vista's marketing, customer support and new product development based on detailed scientific understanding of the efficacy and modes of action of their xylanases in animal nutrition. The research has helped identify a mechanism of cell signalling of gut microbiota via oligosaccharides released by the xylanases. This new paradigm of enzyme efficacy has informed AB Vista's commercial partnerships and redirected their product development, supported by the new analytical techniques developed in Huddersfield for quantification of prebiotic molecules in animal feed.</p>		
2. Underpinning research <p>AB Agri is the agricultural division of ABF, a diversified, international food, ingredients and retail group with sales of £15.4bn, 133,000 employees and operations in 50 countries across Europe, southern Europe, southern Africa, the Americas, Asia and Australia. AB Vista is a division of AB Agri that markets enzymes into the animal feed industry, and commissions research to enhance their understanding of their enzymes, to support market and product developments, and to give scientific credibility as a distinctive element of the business strategy.</p> <p>Since 2004, AB Vista has grown to be a top-three player in feed enzymes and invests heavily in research and development in order to expand its portfolio of new products. A major product family is based on xylanases, which hydrolyse Arabinoxylans (AX), fibre components of cereals that contribute to animal feed (either directly as whole grains or as by-products of cereal processes).</p> <p>Earlier work by Campbell and colleagues had shown that AX-based products could be commercially viable co-products of integrated cereal biorefineries, offering a new class of food ingredients and non-food materials. Nutritionally, fibre is good for humans but less desirable in animal feed; the emergence of biorefineries offers the opportunity to transfer fibre from the animal food supply into the human food supply, while enhancing biorefinery economics – a win-win-win situation.</p> <p>Following this previous work, AB Vista commissioned work in Huddersfield, undertaken by Campbell and Cukelj from 2014-15, to produce a significant quantity (1 kg) of AX-rich extract</p>		

from maize, with which to test the mode of action and efficacy in animal feed of its commercial xylanase (Econase). To their surprise, their Econase appeared to show no hydrolysing activity on the maize AX, in contradiction to the accepted understanding that xylanase enhances maize as an animal feed by reducing the size of AX polysaccharides, hence reducing feed viscosity and enhancing nutrient uptake. Further BBSRC-funded work by Campbell, Cukelj and collaborators also investigated effects of xylanase on AX extracted from wheat bran and sugarcane bagasse, and introduced design methodologies for integrated co-production with bioethanol [1, 2].

Investment awarded to Campbell to construct a pilot-scale bioethanol plant allowed upscaling of the work and prompted AB Vista to support Campbell's work including the award of a PhD studentship investigating the production of xylo-oligosaccharides (XOS) in wheat-based Distillers Dried Grains with Solubles. This work led to the development of a new chromatographic method for quantifying XOS molecules, and quantified for the first time the action of Econase in producing these prebiotically functional oligosaccharides, co-published with scientists from AB Vista [3] and in an AB Vista-commissioned book [4]. This work also showed that the Econase was contaminated with an exo-acting enzyme that caused production of monomeric xylose, which is not functional in animal feed formulations. A simple process to remove the contaminating enzyme was proposed and tested in the lab, and is now being scaled up and tested in chicken feeding trials.

The collaboration has continued via a Knowledge Transfer Partnership with AB Vista since April 2019. It has become clear that the level of production of oligosaccharides at commercial levels of Econase usage is insufficient to account for the observed prebiotic benefits in animals. This has led to a new proposed mechanism in which microbial cells in the gut are signalled through the Econase-derived oligosaccharides that there is a suitable feedstock around, thus stimulating them to produce their own enzymes. This has led AB Vista to propose a new word and concept, "stimbiotic", and to pursue commercial ventures to add oligosaccharides, in combination with enzymes, in new product formulations. This new paradigm of enzyme efficacy is influencing the commercial and research directions being pursued by AB Vista, supported by the KTP.

3. References to the research

Huddersfield authors are shown in **bold**. Employment dates: Campbell 2014-present; Cukelj Sept 2014-July 2015; Alyassin April 2019-present.

The three papers are published in international peer reviewed journals, the book chapter in a peer-reviewed conference proceedings.

[1] Martinez-Hernandez E, Tibessart A and **Campbell GM** (2018) "Conceptual design of integrated production of arabinoxylan products using bioethanol pinch analysis." *Food and Bioproducts Processing* 112:1-8, <https://doi.org/10.1016/j.fbp.2018.08.005>

[2] **Campbell GM**, Čukelj Mustač N, **Alyassin, M**, Gomez LD, Simister R, Flint J, Philips DJ, Gronnow MJ and Westwood NJ (2019) "Integrated processing of sugarcane bagasse: Arabinoxylan extraction integrated with ethanol production." *Biochemical Engineering Journal* 146:31-40, <https://doi.org/10.1016/j.bej.2019.03.001>

[3] **Alyassin M**, **Campbell GM**, Masey O'Neill H and Bedford M (2019) "Simultaneous determination of cereal monosaccharides, xylo- and arabinoxyloligosaccharides and uronic acids using HPAEC-PAD." *Food Chemistry* 315, 126221, <https://doi.org/10.1016/j.foodchem.2020.126221>

[4] **Alyassin M** and **Campbell GM** (2019) "Challenges and constraints in analysis of oligosaccharides and other fibre components." Chapter 15, Pages 257-277 in *The Value of Fibre: Engaging the Second Brain for Animal Nutrition* (Gonzales-Ortiz G, Bedford MR, Bach

Knudsen KE, Courtin CM and Classen HL, Eds.), Wageningen Academic Publishers, The Netherlands, https://doi.org/10.3920/978-90-8686-893-3_15 [can be supplied on request]

4. Details of the impact

The collaboration between AB Vista and the University of Huddersfield has had three overlapping elements, entailing significant scientific, engineering and business challenges and close collaboration: (1) production of AX at scale for animal feeding trials; (2) analysis of xylo-oligosaccharides; and (3) support of new product and market development.

1. AB Vista's original need was to obtain sufficient AX, of sufficient purity, to which to apply Econase to produce XOS, which could then be fed to chickens, for comparison with feed containing Econase, to establish whether the effect of Econase lay in its production of XOS. References [1-3] in Section 5 report on the production and specification of this maize AX, and its response to xylanase treatment (measured by AB Enzymes). The maize AX was unexpectedly unresponsive to Econase, prompting further SEM studies performed by the Biorenewables Development Centre along with scale-up of the processing to produce material for feeding trials [4]. The inability to measure XOS directly prompted the next element of the collaboration. Meanwhile, AX was also extracted from sugarcane bagasse and fed to chickens in a trial at Nottingham Trent University [5]. The purchase (for £117K) of a GUNT pilot-scale bioethanol plant at UoH facilitated this and all subsequent scale-up work. The impact of this work, in conjunction with related work, was new understanding of the mode of action of Econase, and hence new directions for enzyme development across the range of work commissioned and supported by AB Vista worldwide.

2. Measuring xylo-oligosaccharides is challenging. [3] illustrates how the capability within the AB group was inadequate in 2015, reflecting a wider limitation within the scientific literature. The research turned to Distillers Grains as a feedstock of commercial interest, with a focus on developing analytical methods to measure XOS. A 2017 LBNet-funded Business Interaction Voucher project with Innovative Physical Organic Solutions (IPOS) within UoH applied a gel permeation method [6]; however, the research changed instead to HPAEC, developing a method with superior resolution and scope to previously published work, and applying this to generate new knowledge about Econase action on XOS standards and on AX derived from Distillers Grains. The impact of this work, and that in the previous section, was twofold: (i) to guide AB Vista's business activities as it moved in the direction of XOS-based products; and (ii) to contribute to the scientific credibility that underpins AB Vista's strategy for distinguishing itself in the marketplace [10].

3. Throughout these studies, AB Vista and others in the AB group would send samples of commercial interest to UoH to analyse with our increasing capabilities. [7] and [8] are examples of ad hoc analyses that supported AB Vista's commercial developments in 2018, while [9] confirms that the UoH analytical method has been introduced in AB Enzymes and is supporting their customer service and new product development; they write:

"the long-standing collaboration...has helped to take our work on enzymes into new directions and has given us new approaches for understanding the behaviour of our enzymes and of the XOS products that they produce, and hence the opportunities for new enzyme development and for commercial products that combine enzymes and XOS. This is an area of active development, and the method developed and new insights they are generating, are key to the progress we are making in scientific understanding and new products when working with AB Vista".

In 2017 AB Vista entered into a joint venture to produce XOS product and combine it with Econase, to give a new product, Signis, that is now the fastest growing product in the company's portfolio [10]. The KTP project was initiated in 2019 to continue to support this new product development. The Research Director at AB Vista, writes in his letter of support [10]:

“The collaboration with Huddersfield has contributed to transforming our understanding of the action of xylanase enzymes and oligosaccharides in animal nutrition. The initial work with maize supported a growing realisation that the conventional understanding, that xylanases enhance feed conversion by breaking down AX and thereby reducing viscosity, was insufficient, and that XOS was more important than previously recognised. The PhD work then developed for the first time analytical methods for measuring the small xylo-oligosaccharides; this has transformed our ability to quantify the hydrolysis kinetics of our enzymes and to measure their breakdown products in feed formulations. This has given scientific credibility to our marketing literature and sales teams, as being able to identify the breakdown products that our enzymes produce supports the story that sells the products. More fundamentally, however, the collaboration has driven us in the direction of realising that viscosity- and prebiotic-based mechanisms of activity in animal nutrition were inadequate to explain the observed benefits of XOS at below nutritive levels, and thus proposing this new mechanism and research direction... the unique Huddersfield contribution has been the combination of analytical chemistry and process engineering – the development for the first time of analytical methods for quantifying XOS, the ability to produce materials in quantities sufficient for feeding trials, and the application of both extraction and analytical capabilities to move the company’s science, enzyme development and sales agendas forward”.

In the latest Management meeting for the KTP project, The Research Director at AB Vista reiterates:

“As a consequence [of market reactions to the COVID-19 pandemic], AB Vista need to become ever more competitive in this market, by developing new and unique NSPase and XOS based products, and so this KTP project is as important, if not more important, than ever before. AB Vista therefore will be maintaining and supporting this work actively” [11].

Most recently (December 2020), AXOS fractions separated in Huddersfield have been analysed *ex vivo* in Finland for their stimbiotic activity, as the basis for selecting fractions for *in ovo* studies to be undertaken this year in Hawaii, i.e. to see the efficacy of these fractions for establishing healthy microbial populations in chickens by inserting these stimbiotics within the egg itself, taking the company’s product development in significant new directions.

5. Sources to corroborate the impact

1. 2015 UoH report on maize production and characterisation.
2. 2015 Maize AX specification sheet.
3. 2015 AB Enzymes report on activity of two xylanases on UoH maize AX and commercial wheat AX.
4. 2016 BDC report on scaled-up Econase treatment of maize AX.
5. 2017 AB Agri report on chicken feeding trials investigating effects of AX, AXOS and Econase.
6. 2017 BIV report on IPOS analyses of XOS.
7. 2018 Fibre analysis report
8. 2018 XOS syrup analysis report
9. 2020 Letter of support from AB Enzymes
10. 2020 Letter of support from AB Vista
11. Minutes of KTP management meeting, October 2020