Institution:
UNIVERSITY OF LIVERPOOL

Unit of Assessment:
UOA6 - Agriculture, Veterinary and Food Science

Title of case study:
Prevention of weaning related disorders and maintenance of gut health in domestic animals: a role for artificial sweeteners

1. Summary of the impact

Research undertaken at the University of Liverpool (UoL) has identified a new class of intestinal nutrient sensors that influence nutrient absorption properties of agricultural species. Specific sensor systems were stimulated by artificial sweeteners with positive effects on intestinal function and whole animal wellbeing. This work has provided the rational basis for the supplementation of animal feed with artificial sweeteners, which has led to their worldwide deployment in the diet of early-weaned piglets. This innovation has (i) improved health and survival rate of piglets by preventing post-weaning intestinal disorders, and (ii) created significant economic benefits for the animal production industry. A patent has been granted, there has been extensive collaboration with the animal feed company Pancosma, and industry is also actively investigating inclusion of sweeteners in feed for ruminants.

2. Underpinning research

In intensive pig production, piglets are weaned at an early age (2-4 weeks), when the gut structure and function have not fully developed. Whilst this practice allows more piglets to be born, it leads to a number of disorders including nutrient malabsorption, resulting in diarrhoea, malnutrition and dehydration, accounting for 10% mortality. The UoL research, all undertaken under the leadership of Professor Soraya Shirazi-Beechey in the period 1998-2012, has investigated molecular pathways controlling intestinal nutrient (glucose) absorption and gut development.

Central to the research has been the intestinal glucose transporter protein, **S**odium/**GL**ucose **T**ransporter **1**, SGLT1, which is the major route for absorption of glucose (and galactose) from the lumen of the intestine into absorptive epithelial cells. Further, glucose absorption by SGLT1 activates sodium chloride and water absorption, restoring electrolyte balance and hydration.

The research showed that enhancement in expression of SGLT1 and the capacity of the gut to absorb dietary monosaccharides is initiated by the activation of a glucose sensor protein [1]. The identity of the sensor molecule was discovered and showed to consist of two subunits, **T**aste **1** family **R**eceptor **2** (T1R2) and **3** (T1R3), expressed on the luminal membrane of enteroendocrine (sensor) cells [2] leading to Shirazi-Beechey obtaining a patent (PCT/EP2005/054760).

The next phase of the UoL research investigated the potential role of the T1R3 sub-unit and gustducin as components of the glucose sensor protein using knockout mice (provided by RF Margolskee at Mt. Sinai School of Medicine) in which the genes for T1R3 and gustducin (the G-protein associated with T1R2-T1R3) were deleted. Work carried out at UoL showed that wild-type mice fed a high carbohydrate diet had a several fold increase in expression of SGLT1 mRNA, protein and function compared to mice kept on a low carbohydrate diet. It was also found that artificial sweeteners, when included in the diet, also increased SGLT1 expression. However, in mice lacking either T1R3 or gustducin, neither the high carbohydrate diet nor addition of artificial sweeteners had such effect [3]. Further research demonstrated that activation of T1R2-T1R3 by artificial sweeteners releases a gut hormone, glucagon-like peptide-2 (GLP-2), from the enteroendocrine cells, that stimulates intestinal growth and gut maturity and enhances SGLT1 expression [3,4]. Hence, mechanisms were identified that show how the addition of dietary artificial sweeteners can enhance the absorption of glucose, salt, water and promote intestinal cell growth.

These studies provided the basis for further research (2008-2012) demonstrating that pig intestine, as well as the intestine of other mammalian species [4,5,6] (except feline) [6], expresses the sweet receptor, signalling elements and GLP-2, required for upregulation of SGLT1 expression. Specifically, it has been shown that the artificial sweetener SUCRAM, which consists of saccharin and neohesperidin dihydrochalcone (NHDC), when included in the feed or the drinking water of

weaning piglets, enhances the expression of SGLT1, gut structural maturity and the capacity to absorb glucose [5] (Pancosma commissioned research, 2008-2010). Furthermore, current research (2010-) in the laboratory is demonstrating that maintaining piglets on the diet containing SUCRAM can positively influence piglet's innate immunity due to enhancement in gut population abundance of lactobacilli.

Postdoctoral fellows Dyer, Vayro, Daly, Moran and PhD students Salmon, Arora, Batchelor, Zibrik and Al-Rammahi and the technician Coulter were involved in the studies.

3. References to the research

1. **Dyer J**, **Vayro S** (joint first), King TP and **Shirazi-Beechey SP**. Glucose sensing in the intestinal epithelium. Eur. J. Biochem: 270, 3377-3388, (2003). http://www.liv.ac.uk/media/livacuk/iib/efdg/docs/Eur_J_Biochem_(2003)_270.3377-3388.pdf Citations: 60 Impact Factor: 3.58

2. **Dyer J**, **Salmon KSH**, **Zibrik L** and **Shirazi-Beechey SP**. Expression of sweet taste receptors of the T1R family in the intestinal tract and enteroendocrine cells. Biochem. Soc. Trans. 33: 302-5, (2005). http://www.liv.ac.uk/media/livacuk/iib/efdg/docs/biochem_soc_trans_(2005)_33.1.302-306.pdf Citations: 161 Impact Factor: 2.587

3. Margolskee RF, **Dyer J**, Kokrashvili Z, **Salmon KS**, Ilegems E, **Daly K**, Maillet EL, Ninomiya Y, Mosinger B & **Shirazi-Beechey SP**. T1R3 and gustducin in gut sense sugars to regulate expression of Na+/glucose cotransporter 1. Proc Natl Acad Sci U S A. 104:15075-80, (2007). http://www.liv.ac.uk/media/livacuk/iib/efdg/docs/pnas_104.38.15075-80_(2007).pdf
This paper was highlighted as the leading edge select paper in Cell (2007, 130:965-966) with a commentary entitled "Gut reaction to a sweet sensation"
Citations: 270 Impact Factor: 9.737

4. **Daly K**, **Al-Rammahi M**, **Arora DK**, **Moran AW**, **Proudman CJ**, Ninomiya Y & **Shirazi-Beechey SP**. Expression of sweet receptor components in equine small intestine: relevance to intestinal glucose transport. Am J Physiol Regul Integr Comp Physiol. 303: R199-208 (2012). Citations: 7 Impact Factor: 3.284

5. **Moran AW**, **Al-Rammahi MA**, **Arora DK**, **Batchelor DJ**, **Coulter EA**, **Daly K**, Ionescu C, Bravo D & **Shirazi-Beechey SP**. Expression of sodium/glucose co-transporter 1 (SGLT1) is enhanced by supplementation of the diet of weaning piglets with artificial sweeteners. Br J Nutr. 104: 637-46 (2010). http://www.liv.ac.uk/media/livacuk/iib/efdg/docs/BJN.104.637-646(2010).pdf Citations: 20 Impact Factor: 3.302

6. **Batchelor DJ**, **Al-Rammahi MA**, **Moran AW**, Brand JG, Li X, Haskins M, **German AJ** & **Shirazi-Beechey SP**. Sodium/glucose cotransporter-1 (SGLT1), sweet receptor (T1Rs) and disaccharidase expression in the intestine of the domestic dog and cat: two species of different dietary habit. Am J Physiol Regul Integr Comp Physiol. 300: R67-75 (2011). http://www.liv.ac.uk/media/livacuk/iib/efdg/docs/Am.J.Phys.Reg.Int.Comp.Physiol.300.R67-75.pdf Citations: 13 Impact Factors: 3.284

Key Research Grants: Shirazi-Beechey (PI) for all the grants below:

1997 – 1999. **Wellcome Trust**. Dietary regulation of intestinal glucose transport, £65,826 (part of a larger grant, transferred with Shirazi-Beechey's move to Liverpool on 01.09.97)

1997 – 1999. **Biogenesis UK Ltd**. Nutrient and gene interaction in the intestinal epithelium, £32,602.

2001 – 2003. **Wellcome Trust**. Transcriptional regulation of intestinal Na^+/glucose

cotransporter (SGLT1) gene expression by dietary sugars, £195,111

2003 – 2005. **Wellcome Trust**. Nutrient regulation of gene expression in the intestinal epithelium, £18,000

2007 – 2010. **Horserace Betting Levy Board**, PhD studentship, and **Oversees Research Scholarship**. Molecular characterisation of the equine intestinal sweet taste receptor, £122,460

2008 – 2011. **Republic of Iraq Ministry of Education**, PhD studentship. Structural and functional changes in the small intestine of animals of different dietary habits during development and with weaning, £51,900

2008 – 2014. **Panscoma SA**. Intestinal glucose (sweetener) sensing and signalling and regulation of swine intestinal glucose transport, £572,151

2010 – 2014. **Panscoma SA**. Effect of dietary change on swine large intestinal microbiota and gene expression during development, with weaning and in response to sweetener supplementation, £402,183

Patents

PCT/EP2005/054760, Identification of intestinal sweet sensor.

USA #61/695; France #1350349 Use of an additive, sweetener, in the feed and the drink of ruminant animals.

4. Details of the impact

The research described here has changed substantially the fundamental understanding of the mode of action of artificial sweeteners used in animal nutrition. It is now understood that, rather than being a non-specific 'palatant', sweeteners such as SUCRAM at a small concentration (0.015% w/w) are potent activators (600-fold>glucose) of the intestinal glucose (sweet) sensor. This research, since 2008, has had major impacts on the health and wellbeing of the animals concerned, as well as having significant economic impacts on the pig production industry and the animal feed company, Pancosma, with whom Prof Shirazi-Beechey collaborates.

Economic Impacts

Post weaning mortality constitutes an on-going substantive financial loss to the pig industry. Shirazi-Beechey's research has demonstrated that sweeteners contribute to health maintenance and increased growth mainly due to optimization of feed utilization. This has major economic benefits for the animal industry as their return on investment is significantly increased by the use of SUCRAM [9].

The novel mechanisms discovered have also enhanced customers' understanding of the mode of action and benefits of artificial sweeteners in piglet feed, thereby increasing the sale of SUCRAM. Since the implementation of Shirazi-Beechey's research in 2008, annual sales have more than doubled from €5.9m in 2008 to €12m in 2012; the largest demand is in China, Brazil, South Korea, Thailand, Philippines, Spain, Russia, Netherlands, Mexico, Italy, Japan and Indonesia. Shirazi-Beechey, in collaboration with Pancosma, has been pivotal in promoting the benefits of SUCRAM to potential international customers in the animal feed industry, creating a demand responsible for the increased sales figures [11]. The benefits to animal breeders will be many times this, as the net return on investment has been shown to be 400% of expenditure on SUCRAM [9]. The importance of the role of sweeteners on pig gut development has received wide industry recognition with high profile articles in pig industry journals [10]. Global animal feed companies such as Cargill, Purina and CP Group are incorporating SUCRAM into their products [7,8,12,13].

The partnership between Shirazi-Beechey and Pancosma has had additional economic benefits to

the Company that go beyond sales figures. Her research has scientifically validated the Company's promotion of the product; giving customer's the assurance that the decision to include SUCRAM in their animal feed will have a quantifiable impact on their pig production. Her research has also allowed the Company to remain competitive in the animal feed industry, by helping to realign the Company's values to focus more on science and research, rather than just on production and sales. This is demonstrated in their updated motto, "Customer driven, science minded", with a new Company focus on "gut health" [11]. Their reputation as a world-leading, scientifically forward Company has been significantly aided by Shirazi-Beechey's initiation and organisation of annual meetings (Paris 2010; Paris 2011, Madrid 2012) where topics relating to important research underpinning animal gut health are presented and discussed. Audience members include scientists, individuals from animal feed industries, EU regulators, policy makers, administrators and valued customers [11].

These benefits would not have been achieved without the fundamental insights into the underpinning molecular mechanisms. The global animal feed company, Cargill, states that "this is an excellent example of how relatively 'deep' science can have a very close connection with, and impact on, practical animal agriculture." [7]. The knowledge that artificial sweeteners trigger molecular pathways is leading to industrial realisation that the research outputs are relevant to other major aspects of agriculture, including the dairy industry [7,8].

Animal Welfare Benefits

Subsequent to research, a large number of field trials (involving more than 4500 piglets) have indicated that the artificial sweetener, SUCRAM, is effective, by over 90%, in reducing post weaning intestinal disorders, leading to enhancement of growth and wellbeing of early weaned piglets.

5. Sources to corroborate the impact

Each source listed below provides evidence for the corresponding numbered claim made in section 4 (details of the impact).

7. Letter: Cargill Innovation Center. Confirms the connection between the science and practical animal agriculture.

8. Letter: Purina. Confirms the importance of the research, and interest in other applications of artificial sweeteners in animal diets.

9. Pancosma report on economic value of SUCRAM: https://www.dropbox.com/s/ze1dqewakhrogxq/World%20feed%20production%20in%202012-final.pdf

10. Report on the benefits of SUCRAM in animal feed: Pig Progress, 28(6): 2012: http://www.pigprogress.net/Breeding/Piglet-Feeding/2012/8/Gut-development-is-essential-for-weaner-pigs-PP009215W/

11. Letter: outlining the benefit of research to Pancosma

In addition to Cargill and Purina, other major customers of Pancosma using SUCRAM in animal feed are willing to confirm the importance and impact of research. For example:

12. Contact: CP Group, Thailand.

13. Contact: Euronutec, Mexico.