

The Enviropig physiology, performance, and contribution to nutrient management advances in a regulated environment: The leading edge of change in the pork industry^{1,2}

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ABSTRACT: The Enviropig is a transgenic pig that synthesizes phytase in the salivary glands and secretes active enzyme in the saliva. This capability enables pigs to utilize practically all the P in cereal grains and soybean meal and to excrete fecal material usually containing 60% less P than nontransgenic pigs fed the same conventional diet lacking supplemental phosphate. By computer simulation, it was determined that 33% less land would be required to spread manure from transgenic phytase pigs, and if the diet was modified to decrease crude protein, even less land would be required. Introduction of Enviropig genetics may be perceived as leading to an expansion of the pork industry, but perhaps a more realistic view is that introduction of the transgenic phytase pig would enhance sus-

tainability of the industry in a world with increasingly stringent soil nutrient management legislation. The transgenic phytase pig is probably on the leading edge of the production of various types of genetically modified animals that will reduce the environmental footprint of animal agriculture through enhanced metabolic capabilities. These pigs, and other transgenic animals under development elsewhere, will require safety and quality testing in the country of origin and in countries to which the product is exported to ensure that they do not have a deleterious effect on human health and the environment. Consumer surveys suggest that transgenic technology directed to issues involving environmental sustainability and food safety will receive the greatest support.

Key Words: Environment, Phosphorus, Phytase, Pigs, Poultry

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Developments in Monogastric Nutrient Management Strategies

As knowledge of the precise nutritional requirements of food animals increases and the nutritional status of feedstuffs improves, there is the continuing objective to refine the dietary needs of animals in order to increase productivity and simultaneously reduce any environmental impact by decreasing the output of nutrients in fecal and urinary excretions. Various approaches may be taken to accomplish this, including 1) formulation of rations to more precisely meet the

dietary requirements of the animal (e.g., reduction of the concentration of supplemental phosphate in the ration [Shen et al., 2002] or replacement of a portion of the crude protein by essential amino acids [Lenis and Jongbloed, 1999]); 2) improvement in feed digestibility by the addition of supplemental enzymes, including phytase (Simons et al., 1990) or β -glucanase and xylanase (Bedford, 2000); 3) feeding more digestible cereal grains (e.g., low-phytate corn) developed by genetic mutations [Sands et al., 2001]; and 4) expressing genes coding for enzymes that enhance metabolic potential of food animals (Ward, 2000). The expression of genes coding for novel enzymes in food animals is a rational, albeit controversial, strategy to enhance digestive capabilities. Research on the Enviropig represents the leading edge of a revolution that will ultimately change the feed industry; such research directly tackles the elusive goal of producing animals with reduced environmental impact. The development of animals with novel characteristics unveils new opportunities, but harbors uncharted challenges.

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Initial Research on the Genetic Modification of Monogastric Animals to Enhance Digestion

Hall et al. (1993) explored the expression in mice of an endoglucanase gene, endoglucanase E, from *Clostridium thermocellum* to improve the capacity of monogastric animals to digest β -glucans present in cereal grains and supplements. This transgene, which was composed of the exocrine pancreas-specific promoter of the elastase I gene linked to the endoglucanase gene, gave rise to expression of glucanase in the pancreas of the mouse with secretion into the duodenum (Hall et al., 1993). The glucanase secreted into the small intestine was stable in the presence of intestinal proteases, including elastase, trypsin, and chymotrypsin. This research was extended to expression of the xylanase gene XYLY from *C. thermocellum* in transgenic mice, and localized expression in the pancreas was again achieved (Fontes et al., 1999).

With a similar objective, but with the intent to achieve regulation of synthesis linked to energy consumption, Zhang et al. (1999) constructed an endoglucanase transgene by fusing the 2.5-kb amylase Amy 2.2 promoter and signal peptide from the mouse amylase gene to the *Bacillus subtilis* endo-(1-4)- β -glucanase gene C6.5 (Zhang et al., 1997). The transgenic mice generated with this construct expressed glucanase activity with a high degree of specificity for the pancreas. The activity was consistent with dietary starch inclusion, although expression was at a very low level. These studies on glucanase expression in the mouse model demonstrated that organ-specific expression of microbial hydrolase genes in animals is feasible.

Glycanase and xylanase expression are appropriate target genes for poultry since these enzymes reduce the high viscosity of glycan polymers in the gastrointestinal tract that interfere with digestion. An indirect benefit is reduced soiling of eggs because the excreta are less watery and sticky. These genes, however, provide limited nutritional benefit for growing and finishing pigs and variable results for weanlings since pigs have less viscous intestinal contents (Bedford, 2000). Likewise, β -glucanases contribute little to direct energy production because animals lack the β -glucosidases necessary for cleavage of low-molecular-weight $\beta(1,3)$ - and $\beta(1,4)$ -linked oligosaccharides products into readily digestible glucose.

Phytase is an Important Target Gene for Expression in Pigs and Poultry

Cereal grains, such as corn and barley, and plant-based protein supplements fed to pigs and poultry contain between 33 and 80% of their P in the form of myo-inositol hexakis dihydrogen phosphate complexed with minerals (phytate) (Jongbloed and Kemme, 1990). Pigs do not digest phosphorus in this form; instead, it is concentrated in the feces by a factor of

three- to fourfold (unpublished data). As a consequence of the poor digestibility, supplemental phosphate has been included in diets to meet the dietary requirements for optimal growth (NRC, 1998). The resulting high-P manure makes an excellent fertilizer when spread on land. When the P concentration exceeds the anion-binding capacity of the soil, however, the P can leach into normally phosphate-limited freshwater and marine systems, causing eutrophication with the death of fish and aquatic animals and impacting water quality (Jongbloed and Lenis, 1998; Diaz, 2001; Sundareshwar et al., 2003).

The amount of supplemental phosphate needed in the diet can be reduced by inclusion of the enzyme phytase, usually at 250 to 1,000 U/kg of feed, which hydrolyzes a portion of the phytate, thereby releasing readily digestible phosphate. This will lead to a 25 to 50% reduction in fecal P for growing and finishing pigs and poultry (Simons et al., 1990; Ketaren et al., 1993). With higher concentrations of phytase added, the extent of hydrolysis can be improved, albeit with diminishing returns (Kornegay, 2001). These studies demonstrate that a single discrete gene coding for a phytase enzyme is able to provide the full benefit attainable, a feature of prime importance to simplify the generation of an efficient transgenic animal.

Site for Action of Phytase

Results of postslaughter and cannulation experiments with pigs have shown that dietary phytase is predominantly active in the stomach (Kornegay, 2001). The pH of the pig stomach varies from a low of 2.3 just prior to eating to as high as 4.6 after eating (Clemens et al., 1975), whereas in the chicken, the gizzard has a similar pH of 2.5 to 2.8 (Farner, 1942). The low pH is essential for phytase action since at pH values above 4.0, phytic acid begins to precipitate with multivalent cations, and at pH 6.0, it is mainly present as inaccessible precipitates (Siener et al., 2001). Maenz et al. (1999) demonstrated that multivalent cations at neutral pH are potent inhibitors of microbial phytase due to the formation of phytate-mineral complexes.

An ideal phytase enzyme for expression in animals, therefore, needs to be expressed in a gastric or pregastric location, be active and stable at pH values between 2 to 5, and resistant to pepsin. From a survey of microbial phytases reported by Wodzinski and Ullah (1996), and more recently by Lei and Stahl (2001), the *Escherichia coli* appA-encoded phytase has desirable characteristics for expression in animals not shared by other phytases, which include a high activity (Golovan et al., 2000) exceeding that of recently cloned fungal enzymes (Lassen et al., 2001) and resistance to pepsin at low pH (Rodriguez et al., 1999; Golovan et al., 2000) (Table 1). These in vitro characteristics are borne out by animal studies showing that the AppA phytase is as effective as the commercially available *Aspergillus*

Table 1. Important characteristics of an enzyme product and a transgene for expression in animals

| | |
|------------------------|---|
| Protein product/enzyme | |
| 1. | Protease resistance. |
| 2. | Suitable pH optimal depending upon site of action. |
| 3. | Low K_m (Michaelis constant). |
| 4. | High V_{max} . |
| 5. | Low molecular weight. |
| 6. | Monomeric. |
| 7. | Temperature optimal for high activity at 37°C. |
| 8. | Signal sequence for export if extracellular activity is a prerequisite. |
| 9. | Post-translational sites should not affect secretion to desired location, and activity and stability. |
| Transgene | |
| 1. | Promoter with appropriate recognition and regulation. |
| 2. | Enhancer with desired strength and tissue specificity. |
| 3. | Strong Kozak consensus sequence. |
| 4. | Locus control region present. |
| 5. | Sequences necessary for correct post-translational modification of mRNA. |
| 6. | Genomic copy of structural gene if of eukaryotic origin. |
| 7. | Codon usage and distribution suitable for expression in a monogastric animal. |
| 8. | Sequences affecting expression removed. |

phytase (on a per unit basis) in improving the P bio-availability in diets of chicks (Leeson et al., 2000), broiler and layer poultry (Igbasan et al., 2001), and in diets of young pigs (Igbasan et al., 2001).

Promoter Selection, Secretion Signals, and Construction of the Transgene

Promoter selection and specifications for construction of a transgene are critical (Table 1) to obtain organ-specific excretion of a target protein at a regulated and effective level. In the case of phytase expressed in the salivary glands, efficient unidirectional export from acinar cells into a pregastric site of the gastrointestinal tract was essential because phytate and a number of lower inositol phosphates have important roles in intracellular metabolism including 1) signaling mobilization of Ca^{2+} ; 2) regulation of membrane trafficking and cytoskeleton organization; 3) gene expression; and 4) export of messenger RNA from the nucleus (York et al., 2001). Therefore, any intracellular expression of the phytase could be lethal to the embryo.

No porcine salivary promoters were available for pregastric expression of phytase; however, two murine promoters with appropriate characteristics for salivary expression were available: the rat proline-rich-protein promoter (Tu et al., 1993) and the mouse parotid secretory protein (**PSP**) promoter (Laursen and Hjorth, 1997). The *E. coli appA* structural gene, including the signal peptide sequence, which has a codon usage profile close to that of eukaryotic genes (S. P. Golovan, unpublished data), was inserted downstream of each of these promoters (Golovan et al., 2001a). The inducible proline-rich-protein promoter-appA construct was initially introduced into mice by pronuclear microinjection (Hogan et al., 1986) to test whether induction of phytase synthesis would be deleterious.

Healthy transgenic offspring were obtained using this promoter, and phytase was secreted in the saliva after induction by injection of isoproterenol, documenting that phytase expression in animals was not lethal. The constitutive parotid secretory protein promoter-phytase transgene (**PSP-APPA** transgene, Laursen and Hjorth, 1997) was then successfully introduced into mice. Two healthy transgenic offspring were produced; one of these synthesized phytase and secreted the enzyme in the saliva, which demonstrated that constitutive synthesis of salivary phytase was not deleterious and at the same time resulted in reduced fecal P.

Generation of Pigs Producing Salivary Phytase

The PSP-APPA transgene (Golovan et al., 2001a) was used for the generation of transgenic pigs by pronuclear microinjection following the procedure of Wall et al. (1985). Thirty-three independent founder (G_0) transgenic piglets were obtained. Many of the individual G_0 animals were tested periodically for salivary phytase throughout the growth phase. Figure 1 illustrates the erratic results obtained by collecting saliva from the mouth of a pig with a cotton swab. Despite the variation in sampling, the ability to conduct a convenient, rapid, and noninvasive test for salivary phytase continues to be particularly useful. The figure also shows the percentage reduction of P in fecal samples from the same transgenic pig during the weaning, growing, and finishing phases compared with non-transgenic littermates when fed a conventional diet containing supplemental P. This data demonstrated the potential of founder (G_0) pigs to digest phytate P. When weanling and growing-finishing pigs from the G_1 generation of one line (WA line) of transgenic pigs were tested for true digestibility of dietary P in soybean meal as the sole source of P using an ileal cannu-

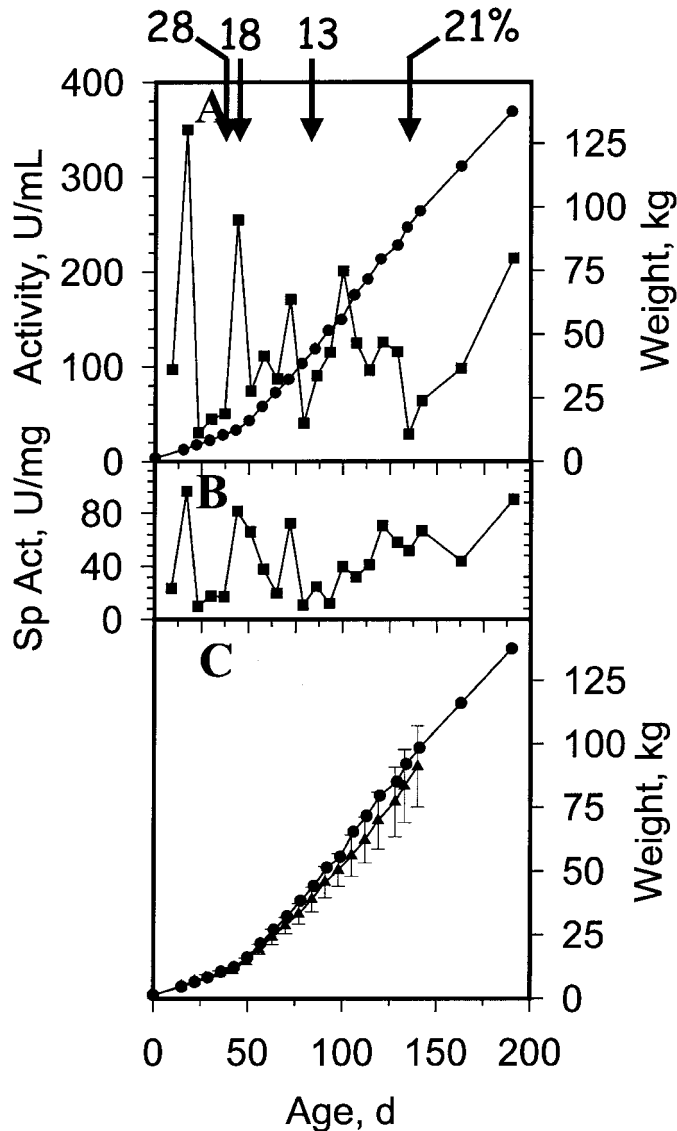


Figure 1. Salivary phytase production, growth, and fecal P reduction by the transgenic G_0 pig 421-06 compared with nontransgenic littermates receiving conventional diets with supplemental phosphate. A) Phytase activity (■) and weight gain (●); B) phytase specific activity; C) weight gain of transgenic pig (●) and nontransgenic littermates (▲, $n = 4$). The percentage values at the top of the figure indicate the reduction in fecal P compared with nontransgenic littermates.

lation methodology (Fan et al., 2001), they were found to digest 88 and 99% of the dietary P, respectively, compared with nontransgenic pigs that digested 49 and 52%, respectively (Golovan et al., 2001b). Fecal material from the weanling and growing-finishing phytase pigs contained a maximum of 75 and 56% less P, respectively, than that of nontransgenic pigs fed the same diet. Since the transgenic phytase pigs digested practically all of the dietary P, the P entering the terminal ileum of these pigs presumably consisted primarily of differentiated enterocytes released from the

mucosa during the process of continual epithelial regeneration (Ramachandran et al., 2000).

Boars and gilts transgenic for the phytase gene that were fed a conventional cereal grain diet lacking supplemental P during the finishing phase had fecal P concentrations that were 67 and 64%, respectively, less than the corresponding nontransgenic pig in the same trial (Golovan et al., 2001b). It is worth noting that the preliminary observations on the G_0 pigs were borne out by the more comprehensive data obtained from feeding trials with G_1 and G_2 weanling and growing-finishing transgenic pigs. The amount of P excreted in the urine was not determined in these studies, but more recent research on weanling, growing, and finishing pigs has shown that they excrete slightly more phosphorus in the urine than nontransgenic pigs (A. Ayodele, unpublished data). It has been reported that urinary P accounts for 6, 9, and 27% of P excreted by weanling pigs, growing pigs, and sows, respectively (Poulsen et al., 1999). The data clearly document the innate capability of the phytase pigs to extensively digest dietary phytate P.

Reduction of the Environmental Impact of Pork Production

Although P is the third most expensive nutrient fed to pigs, the cost of phosphate is not a major constraint, and overfeeding of this compound has been a common practice. In all jurisdictions, the land base for spreading of manure is a serious limitation. To assess the benefit of Enviropig genetics in terms of land area for spreading manure, we have used the NMAN 2001 manure management computer simulation program developed by the Ontario Ministry of Agriculture and Food. The simulation was for a 350-sow, farrowing-to-finishing pig operation with given defaults (Table 2). As shown in Table 3, the spreading of manure from nontransgenic pigs on low erosion soil theoretically requires 151 ha and this minimum area is necessary to avoid application of excess P. If transgenic phytase pigs were raised in place of conventional pigs, the land area required for spreading could be reduced by 33% before manure N would be applied in excess. It is generally recognized that for each 1% decrease in CP in the diet, there is an 8 to 10% reduction in manure N (Lenis and Jongbloed, 1999; Le Bellego et al., 2001). Using the NMAN program to simulate the relationship between decreasing manure N and the reduction in land required to spread manure, it can be seen that if the N content of the manure was reduced by up to 40%, the area of low erosion soil for spreading could be reduced by 60% (i.e., to 100 ha) before P would be applied in excess (Figure 2).

The benefit of competing technologies, in terms of reduced land base for spreading of manure, is not as great. For example, corn that contains 50 to 75% less phytate than unmodified strains of corn fed to monogastric food animals provide for fecal P reductions up

Table 2. Parameters for the NMAN nutrient management program for assessing the effect of the phytase pig characteristics on the land base required for the spreading of manure^a

| | |
|----|---|
| 1. | The simulation was for a 350-sow, farrowing-to-finishing farm producing 8,570,913 L of 4.2% DM manure/yr (MSTOR calculation). |
| 2. | Transgenic (Tg) phytase pigs produce manure with 60% less P. Program default values were used for the concentrations of N, P, potassium, and ammonium N (NH ₄ -N) in swine manure. |
| 3. | Continuous corn with an average yield of 8.13 metric tons per hectare and manure applied once a year in spring before seeding. |
| 4. | Liquid loading was not considered because it can be split into two or more applications. |
| 5. | Nitrogen from previous manure applications was taken into account. When lowering N both total N and NH ₄ -N were lowered by the same percentage. |

^aNMAN2001 (Feb 22, 2002 version) is a manure nutrient management computer simulation program developed by the Ontario Ministry of Agriculture and Food <http://www.omafra.gov.on.ca/scripts/english/engineering/nman/default.asp>.

to 50%, depending on the diet formulation (Raboy et al., 2001). Supplementation of low-phytate corn with phytase enhances the digestibility further (Sands et al., 2001). These data show that low-phytate cereals have great potential. However, to achieve a reduction in fecal P comparable to the phytase pigs, supplemental phytase is necessary. The current problems with some low-phytate cereals, as exemplified by low-phytate corn, are a lower germination rate (Lott et al., 2000) and a 4 to 23% reduction in seed weight (Raboy et al., 2000). A separate problem with low-phytate cereals may be the added cost incurred in handling them as separate commodities from conventional cereals. The reductions in fecal P as a result of feeding supplemental phytase (see above) are usually no greater than that observed for low-phytate barley. These data demonstrate the superior performance of Enviropig genetics, but this benefit is counterbalanced by the substantial investment for introduction of the new biotechnology.

Quite separate from short-term considerations, an impending problem facing the industry is the dwindling source of economically recoverable mineral P that Smil (2000) predicted would last only 80 yr at the current rate of extraction. A related problem with lower quality mineral deposits is the increasing con-

tamination of the P by trace metals, particularly cadmium (Smil, 2000). More efficient recycling of P through a combination of phosphorus technologies will forestall this impending shortage and at the same time reduce environmental eutrophication.

Enhancing the Metabolic Capability of Food Animals

Recent advances have been made in the methodology for the generation of transgenic food animals (Wheeler and Walters, 2001; Houdebine, 2002), including poultry (Harvey et al., 2002). Even the classical pronuclear microinjection may have been improved, as was demonstrated by Chang et al. (2002). They reported that spermatozoa incubated with monoclonal antibodies recognizing a specific surface antigen will bind DNA. By surgical oviduct insemination of gilts and by artificial insemination of chickens, the gene of interest was efficiently transferred to oocytes. In all cases, up to 25% of born animals or birds were transgenic and expressed the transgene. Furthermore, problems including ectopic expression and silencing of transgenes, which are often caused by position effect (e.g., integration close to an endogenous transcriptional control element), are being solved with

Table 3. Land base necessary for spreading of manure from transgenic (Tg) and non-Tg phytase pigs given the same diet, except that supplemental phosphate was omitted from the diets of the phytase pigs

| Pigs | Starting soil test | Manure applied, L/ha | Hectares for spreading manure | Reduction in land for spreading, % |
|--|---------------------------|------------------------|-------------------------------|------------------------------------|
| Low erosion potential soil | | | | |
| Non-Tg | 101 ppm of P ^a | 56,827.5 ^b | 150.8 | — |
| Tg | 101 ppm of P | 85,240 ^c | 100.4 | 33 |
| Non-Tg | 10 ppm of P | No change ^e | No change | — |
| Tg | 10 ppm of P | No change | No change | 33 |
| High erosion potential soil ^f | | | | |
| Non-Tg | 101 ppm of P | 25,003 | 343 | — |
| Tg | 101 ppm of P | 65,918 | 130 | 62 |
| Non-Tg | 10 ppm of P | 56,828 | 151 | — |
| Tg | 10 ppm of P | 85,238 | 101 | 33 |

^aThis is regarded as an excessive level of P.

^bApplication above this amount exceeds the P limit.

^cApplication above this amount exceeds the N limit.

^dThis is a medium level of P.

^eSame as above because 154 kg over crop removal is the limiting factor if the P limit is low.

^fSoil erodes at the rate of 36 metric tons/ha annually.

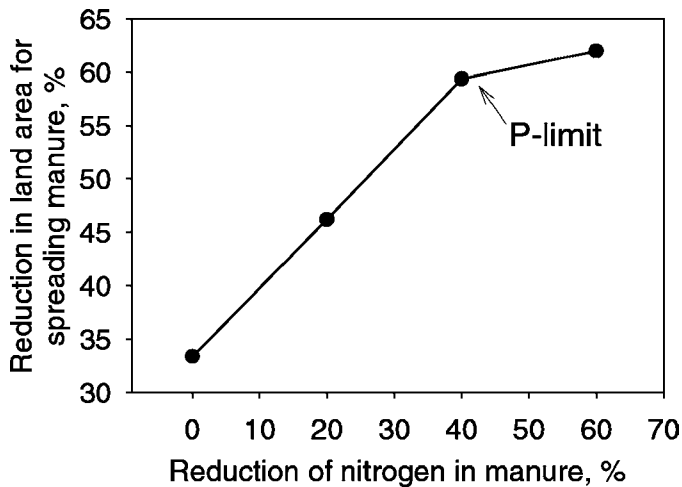


Figure 2. Effect of reducing the N in manure of transgenic phytase pigs on the land area for spreading manure. Assumes that phytase pigs excrete 60% less P and spreading is on a low erosion soil.

insulator genes (Matske et al., 2000; Bell et al., 2001) and artificial chromosomes (Huxley, 1997).

These methodologies, in addition to cloning techniques, provide the opportunity to enhance the digestive physiology, growth, fertility, and disease resistance characteristics, and milk and meat composition of domestic food animals (Wheeler and Walters, 2001; Houdebine, 2002).

Hurdles from Concept to the Meat Counter

Genetically modified food animals are subject to animal health, environmental, and food safety legislation. The criteria for a valuable transgenic food animal are good health, disease resistance, reduced impact on the environment, desired physiological characteristics, and safe consumption.

Health of the transgenic food animal is central to production because an unhealthy animal would usually be less productive. For example, transgenic pigs harboring a transgene encoding bovine GH, although exhibiting a 10 to 15% increase in daily weight gain and a 16 to 18% increase in feed efficiency, suffered serious physiological defects and were unsuitable for commercial food production (Pursel et al., 1989; Pinkert et al., 1994). To circumvent this type of problem, which in some cases may be due to recessive insertional mutations, strategies of selecting, breeding, and dissemination of transgenic livestock have been explored in detail (Van Reenen et al., 2002).

To meet environmental regulatory requirements, transgenic animals must be documented as having no deleterious effects on the environment and human health, either directly or indirectly. In Canada, they must satisfy the requirements of the Canadian Environmental Protection Act (**CEPA**), which is the joint

responsibility of Environment Canada and Health Canada (www.ec.gc.ca/substances/nsb/eng/reg_e.htm) to determine that the animal is not a CEPA toxin (CEPA 99, Section 64). In the United States, the FDA administers similar requirements (www.fda.gov/cvm/biotechnology/bio_drugs.html), the environmental requirements being under 21 CFR, part 25 (www.access.gpo.gov/nara/cfr/waisidx_01/21cfr25_01.html). Transboundary movement of living genetically modified (**GM**) animals, although presently administered by individual countries, may in the future be administered through the Cartagena Protocol on Biosafety to the Convention on Biodiversity, which is a treaty under the United Nations Convention on Biological Diversity (www.biodiv.org/biosafety/protocol.asp and www.biodiv.org/convention/articles.asp), if the convention and protocol are ratified by major trading countries. The convention and protocol are, in part, designed to provide a framework within which living modified organisms (**LMO**) can be traded in a safe and responsible manner with due regard for protection of environmental biodiversity.

Recently, the NAS (2002) reported on risk issues surrounding transgenic and cloned animals and fish, including food safety, environmental safety, and animal welfare. The major food safety issue noted was the potential for allergenicity or hypersensitivity responses in some consumers. Products from somatic cell cloned cattle were not considered to be a food safety concern. Environmental concerns revolved around fitness traits, such as increased growth rate, in highly mobile species (i.e., fish carrying an up-regulated growth hormone transgene). Lesser environmental concern was expressed regarding fitness traits, such as phytase expression in less mobile animals such as the pig, although it was noted that feral pig populations do exist (Brisbin and Mayer, 2001) that in the past have caused environmental damage. Animal welfare issues were also discussed. An issue underlying scientific considerations was the need for an ethical framework since resolving and implementing science-based decisions ultimately requires public involvement.

The strategy for the safety assessment of foods derived from GM plants is well established under the Codex Alimentarius commission of the Food and Agriculture Organization (www.codexalimentarius.net/reports.asp, document ALINORM 03/34). These guidelines are very similar to the legislated assessments used in Canada (www.hc-sc.gc.ca/food-aliment/mhdm/ofb-bba/nfi-ani/e_novel_foods_and_ingredient.html) and in the United States (Chassy, 2002). Pre-market food safety evaluations consider the issues listed in Table 4. Extensive studies have now been conducted to assess the safety of GM DNA for human consumption and its persistence in the environment. Studies with human volunteers show that no GM DNA survived the passage through the entire human digestive tract. Although some DNA survived

Table 4. Characteristics considered in a premarket safety assessment of a food derived from a genetically modified organism

-
-
1. Safety of the source organism and gene(s)
 - (a) Safety of the inserted DNA
 - (b) Safety of DNA ingestion
 - (c) Safety of the antibiotic resistance marker (if used)
 2. The food safety issues of the newly introduced product(s)
 - (a) Potential for toxicity (protein product)
 - (b) Potential for allergenicity (protein product)
 - (c) Safety of any unintended effects
 3. Equivalence of composition
 4. Retention of nutritional value
 5. The human dietary exposure
-

in laboratory created environments that simulated human or animal gastrointestinal tracts, the research concluded that the likelihood of functioning DNA being taken up by bacteria in the human or animal gut is extremely low (Food Standards Agency, 2002). With a reduced focus on the safety of DNA, the assessment process probably will focus strongly on the protein(s) expressed from the introduced novel genes. As GM animals are relatively new compared to GM plants or microorganisms, the various governmental agencies are still in the process of setting policies and of drafting guidance documents for the assessment of these animals. In the interim, there have been assessments on a case-by-case basis; for instance, the GM pig developed by Bresatec in Australia and the AquAdvantage salmon developed by Aqua Bounty (www.aquabounty.com/abbounty.htm) in the United States.

The GM pig developed by Bresatec (now BresaGen) is transgenic for a GH that can be switched on by addition of zinc to the diet, which allows for increased production of meat and enhanced feed efficiency (Nottle et al., 1999). Bresatec provided data to the Australian authorities to document that the GM pork was substantially equivalent to pork from nontransgenic pigs, but the Australia and New Zealand Food Authority (ANZFA) did not approve the Bresatec pork because approval was outside the Authority's charter (www.affa.gov.au/docs/operating_environment/armcanz/gene/appendix2.html).

The AquAdvantage salmon is transgenic for a salmon GH gene under control of a promoter from the ocean pout's antifreeze protein gene. The transgene is expressed in the liver and provides for year-round secretion of GH. The transgenic salmon grow at four to six times the rate of nontransgenic salmon to reach the same weight at maturity (Fletcher et al., 1999). Aqua Bounty has submitted an application to the Center for Veterinary Medicine of the FDA for the market approval of the AquAdvantage salmon, where it is under assessment as a "new animal drug" (Kleter and Kuiper, 2002).

A recent interim report by Health Canada on the preparation of guidelines for food safety assessment of transgenic livestock and fish (Health Canada, 2001)

confirms the similarity of approaches for GM plants and animals; however, there will be differences to account for the different physiological characteristics of plants and animals. A key ingredient, particularly with the consideration of pigs, is the similarity to humans in terms of their nutritional (Miller and Ullrey, 1987), physiological (Higgins and Cordell, 1995; Tumbleson, 1986), and immunological (Helm et al., 2002) characteristics. Therefore, the well being of transgenic pigs would seem to be a persuasive indicator of food safety for many aspects, except perhaps for the toxicity/allergenicity of the newly expressed protein.

Allergenicity

A novel protein, such as the *E. coli* phytase present in the Enviropig, will be considered as self in the pig (Goldsby et al., 2000) and will not present an allergenic challenge to the pig. However, the phytase expressed in the pig may act as an allergen when humans consume the pork, since it has not previously been consumed as a food constituent, except through the accidental presence of *E. coli* in food consumed or because of its presence as part of the normal flora in the lower gastrointestinal tract (Tannock, 2001). A weight of evidence approach is taken to assess allergenicity of a protein, which focuses on the source of the gene, sequence homology with known allergens, physicochemical properties of the protein (such as heat stability), and digestive stability. Additional criteria include immunoreactivity of the novel protein with serum IgE from individuals with known allergies to species that are broadly related to the source of the transferred DNA and the immunogenicity of the novel protein in appropriate animal models (Taylor, 2002; www.fao.org/es/ESN/food/risk_biotech_papers_en.stm). This decision process, although well established, is likely to change as new information appears, such as the recent finding of the lack of a stringent relationship between the stability of proteins in simulated gastric fluid and allergenic potential (Dearman et al., 2002).

Salivary phytase presents an interesting case for allergenicity testing because in its native state, it is highly resistant to stomach proteases, retaining greater than 90% of its activity after exposure to a 1,000-fold excess of pepsin at pH 2.5 (Golovan et al., 2001b). However, salivary phytase is denatured by heating to 100°C, and after this treatment, is sensitive to proteases (J. P. Phillips, R. G. Meidinger, and C. W. Forsberg, unpublished data), a characteristic shared by native nonallergenic proteins (Astwood et al., 1996). It is probable that a native intact protein will have more allergenic potential than a denatured protein after digestion by pepsin. However, pork is always cooked prior to consumption because of a range of infectious microorganisms potentially present, including viruses, bacteria, fungi, and parasites (Kumar et al., 2002). Thus, rather than assessing the allergenicity of novel proteins in uncooked pork, as is done

with transgenic plant products, the cooked pork may be a more realistic product for testing.

Consumer Acceptance of Genetically Modified Foods

The ongoing debate over the human and environmental safety of GM foods is complex and multifaceted, but several recent reports show a positive view. Santerre and Machtmes (2002) surveyed 576 people, including members of community organizations, undergraduate students, graduate students, and extension educators, to see how their knowledge and attitudes toward genetically enhanced foods changed after receiving an hour of scientific instruction on food biotechnology. After this introduction, respondents were more accepting of the regulatory process; furthermore, 90% stated they would eat and serve GM foods to their families and 90% believed that their families would benefit from GM foods within the next 5 yr. The importance of providing consumers with scientific information on GM foods is borne out by a recent Ipsos-Reid study on Canadian public attitudes toward pork production. Respondents were more receptive to the future consumption of GM pork once they were aware that it would come from pigs that produced less polluting manure. Environmentally friendly applications of biotechnology were also viewed positively by U.S. consumers (Bruhn, 2002).

Implications

Transgenic technology has the potential to enhance the role that the animal industries have in world food production system. This paper describes the science, thought process, reasoning, and outcomes that evolved during the development of the Enviropig. The importance society places on solving the environmental, food safety, and food quality challenges supports the contention that this particular transgenic technology should be directed first and foremost to these issues rather than the customary economically based production area. This paper emphasizes the general understanding and value of transgenic animal technology in helping to ameliorate the environmental impact of animal production.

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