**The Enviropig: An Environmentally Friendly Pig That Utilizes Plant Phosphorus More Efficiently**

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[This document has been modified from its original form to include information from the

article “Guelph Transgenic Pig Research Program.”]

**A biotech breakthrough at the University of Guelph in reducing the environmental impact**

**of manure produced by pigs**: Researchers at the University of Guelph have developed

transgenic lines of Yorkshire pigs trademarked EnviropigTM that use plant phosphorus more

efficiently (Golovan et al., 2001a; Golovan et al., 2001b). Non-transgenic pigs are unable to use

an indigestible form of phosphorus called phytate present in the cereal grain diet. Therefore

producers add supplemental phosphate to meet the dietary phosphorus requirement for optimal

growth and development. The novel trait of the EnviropigTM enables it to degrade the indigestible

phytate and absorb the phosphate eliminating the need to supplement the diet with readily

available phosphate, and as a consequence the phosphorus content of the manure is reduced by

as much as 75%. Digestion of the phytate also leads to improvements in digestion of minerals,

proteins and starch in the diet.

**The Environmental Problem**: Manure from farm animals is an important natural fertilizer for the

growth of crops. The manure from monogastric animals such as pigs and chickens, contains a

higher concentration of phosphorus than is suitable for repetitive field application because

indigestible (phytate) phosphorus passes through the digestive tract of the animal while other

nutrients are absorbed. Therefore, the phytate phosphorus is concentrated in the manure.

Consequently, at high application rates of manure to land in areas of intensive pork production,

the potential for pollution of local surface water and ground water with phosphorus becomes a

serious problem (Sims et al., 1998). When runoff and leachate from drainage tiles of fields that

have a high phosphorus content drain into ponds and streams extensive plant and algal growth

occurs, tainting the water and robbing it of oxygen leading to death of fish and other beneficial

aquatic organisms (Jongbloed and Lenis, 1998; Kornegay, 2001). Although rare, if there is

flooding and rupturing of manure storage reservoirs more serious situations can arise (Mallin,

2000).

A low phosphorus concentration in fresh water systems is key to clean water because its absence limits algal growth (Hudson et al., 2000). If phosphorus is not present at a growth-limiting higher concentration extensive eutrophication can occur, leading to the production of methane and

nitrous oxide potent greenhouse gases (Huttunen et al. 2001; Steenbergen, et al. 1993).

Eutrophication arising from agricultural sources also occurs in estuaries and near shore marine

environments with production of nitrous oxide (Naqvi et al., 2000). The projected growth of the

livestock industry (Delgado et al., 1999; Tilman et al., 2001) is expected to accelerate

environmental problems on a global scale. It therefore is critical that agricultural practices be

modified to reduce such environmental impacts.

**The Current Strategy to Reduce the Phosphorus Content of Pig Manure**: The current

practice to reduce excretion of fecal phosphorus by pigs is to decrease the supplemental

phosphorus and to simultaneously include in the feed the fungal enzyme called phytase, which is

available commercially. This enzyme acts to digest dietary phytate releasing phosphorus in the

stomach of the pig. The net effect is improved phosphorus absorption in the small intestine by

approximately 20 to 40% at phytase concentrations of 500 to 1000 Units per kilogram of feed

(Ketaren et al., 1993; Simons et al., 1990). The reduced content of phytate in the small intestine

decreases complexes formed between phytate and trace minerals, proteins and starch, thereby

improving their absorption as well. Phytase is currently added to the swine diet in many countries.

Currently crops are being developed that contain phytase in the seeds, however, there is a

problem with stability of the enzyme during pelleting and storage. Research is also in progress on

the development of phytate-reduced cereal grains, for example, corn that contains 65% less

phytate (Raboy et al., 2000) which reduced the need for added phytase, however,

supplementation was still beneficial for pigs (Sands et al., 2001) and poultry (Huff et al., 1998) .

The potential of low phytate cereals is not fully resolved, since at least low phytate corn exhibits a

lower germination and reduced yield as compared to unmodified lines of the corn.

**What is novel about the Enviropig?:** The Enviropig produces the enzyme phytase in the

salivary glands that is secreted in the saliva. The enzyme acts in the stomach in the same way as

fungal phytase added to the feed, except it is synthesized in larger quantities in the salivary

glands (perhaps as much as 100,000 Units per kg of feed consumed) than the amount commonly

added to the diet. The Enviropig was produced in the following way: A transgene constructed by

linking a small portion of a mouse gene responsible for production of a salivary protein in the

parotid, sublingual and submaxillary salivary glands to a phytase gene from a non-pathogenic

strain of the common intestinal bacterium Escherichia coli (strain K12). This transgene was

introduced into fertilized pig embryos, which were subsequently implanted into pseudopregnant

surrogate sows. The offspring were tested for the presence of the gene by analysis of DNA from

the piglets, and by testing saliva for phytase. Initially thirty-three different Enviropigs were

produced with the same transgene. The transgene probably was introduced into a different

location of the chromosome of each of these pigs, therefore, each pig is considered to be a

different line. Several of these lines have been studied in more detail. They produce sufficient

phytase to digest practically all of the phytate in a cereal grain diet. Phosphorus in feces from

young grower pigs not supplemented with phosphate was reduced by 75% while that in finisher

pigs was reduced by 56 to 67% when fed diets not supplemented with phosphate. The enzyme is

reasonably stable and fully active in the stomach, but is degraded in the small intestine by

pancreatic proteases, preventing excretion from the pig. Furthermore, because of the high

specificity of the transgene promoter, the phytase is produced primarily in the salivary glands with

only trace concentrations (less than 0.1%) in the major tissues such as muscle, liver, heart, skin,

etc..

Image of how Enviropig Works

**Are the Enviropigs Healthy?:** All indications are that the pigs have a similar health status to that

of non-transgenic pigs. They grow at rates similar to non-transgenic pigs and they appear to have

similar reproductive characteristics.

**Benefits of the Enviropig TM**:

1. They excrete as much as 75% less phosphorus in the manure as compared to nontransgenic pigs when fed a diet not containing supplemental phosphorus, producing a fertilizer with a higher ratio of nitrogen to phosphorus, which is better suited for long-term repetitive application to agricultural land. Pigs receiving a typical industry standard diet without supplemental phosphorus excreted fecal material with 64 to 67% less phosphorus.
2. They utilize practically all of the phosphorus present in soybean meal and do not require supplemental phosphate for growth on a standard diet consisting of corn, barley, wheat and soybean meal, with a saving of $1.14 per pig (CDN) for supplemental phosphorus, or an equal or greater saving in the cost of phytase. Furthermore, added phytase to the diet at the concentrations normally used does not release phosphorus from dietary phytate as effectively as the salivary phytase
3. We expect the pigs will utilize dietary trace minerals, proteins and starch more efficiently.
4. Because the Enviropig can utilize plant phosphorus efficiently it may be of great benefit in countries with low phosphorus resources, and which often lack currency for the purchase of phytase, and furthermore, which often lack the infrastructure for precise mixing and distribution of feed containing phytase.

**When will the Enviropig be available to Pork Producers?:** We predict it will be three to five

years before this line of pigs will be available to swine breeders. The research is at an early

stage, but some questions have been answered:

1. The phytase gene is stably transmitted.
2. The phytase functions effectively in the stomach of the pig.
3. The phytase protein is largely limited to the salivary glands and to the digestive system as far as the small intestine. It is destroyed before it reaches the large intestine.
4. All indications are that the Enviropig exhibits similar growth and carcass characteristics to non-transgenic market pigs.

The Enviropigs are subject to the Canadian Environmental Protection Act (CEPA) under the

auspice of Environment Canada. When these animals or samples of tissue, blood or even fecal

samples are moved from one University of Guelph facility to another, under the present

arrangement, tracking documents must be maintained for each animal and each sample collected

from them. The animals are subject to the Health of Animals Act under the auspice of the

Canadian Food Inspection Agency. When the phytase pigs reach the stage of testing to

determine suitability as a food for humans, they will be subject to the Novel Food Regulations

(http://www.hc-sc.gc.ca/food-aliment/) of the Federal Food and Drug Act under the auspice of

Health Canada. These stringent requirements will assure that pork from these animals, will be

safe when it is eventually approved for the consumer.

Publications forthcoming from Health Canada:

1. Guidelines for the safety assessment of novel foods. Volume III. Genetically modified livestock animals and fish.
2. Guidelines for the slaughter and disposal of livestock animals and fish derived from modern biotechnology

**Animal Welfare Issues**: All animal experiments are conducted following the strict guidelines of

the Canadian Council on Animal Care (http://www.ccac.ca/. Guidelines on the production of

transgenic animals may be downloaded from the site. Pigs are raised in accordance with the

Canadian code of practice for environmentally sound hog production

(http://www.canpork.ca/codes.html).

**Who is supporting the research?:** The research was supported by Ontario Pork, Ontario

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Natural Sciences and Engineering Research Council of Canada, Agriculture and Agri-Food

Canada, and the Food Systems Biotechnology Center at the University of Guelph. The University

of Guelph provides the expertise and facilities.

**Some Regulatory Issues**: I will make some personal comments regarding issues relating to the

development of a protocol for assessing food safety of transgenic animals.

The Key Aspects of Novel Food Assessments:

1. The host organism.
2. The donor organism.
3. The modification process.
4. DNA analysis of the transgene.
5. The genetic stability of the modified organism.
6. Expressed material/effect.

I believe that the protocol for assessing transgenic plants can be applied to transgenic animal,

except for the method of sampling tissues, pretreatment prior to sample analysis, and the extent

of testing.

1. Will the assessment be performed on whole animals or tissues? With plants I understand that the whole raw seed is analyzed if the whole seed is to be eaten. In the case of fish, I would assume that the whole animal might be ground and assessed because large pieces of the fish are eaten. However, in the case of animals, such as the Enviropig where organs, such as liver, kidney, or muscle are eaten separately there may be a desire to carry out assessment of separate organs rather than grinding up the whole carcass and sampling that. In the case of the Enviropig TM the salivary glands would be candidate organs to assess. In preparation for developing guidelines I recommend a broad survey of the published literature to assess the variation in composition between organs.
2. Pork and chicken meats are always cooked before consumption. Therefore, I think it is reasonable that the samples should be cooked before testing. In contrast, plant samples are edible in the raw form and therefore sampling of the raw material is reasonable.
3. Some scientists have stated that transgenic animals will be more difficult to assess than transgenic plants because of their greater complexity. I contend that because the animal is more complex it will be easier to assess than plants. Arguments:
   1. Plants can produce toxic compounds without affecting their growth and appearance.
   2. Transgenic plants are normally tested for safety by feeding to animals. Therefore, it may be argued that transgenic animals serve as their own internal control of food safety. Thus a healthy animal with a normal growth rate likely is safe to eat.
   3. I expect that after the first transgenic animal has been approved by the regulatory system, you will no longer need such a stringent analysis for vitamin content, amino acids composition etc. I suspect that a vitamin deficiency would affect the growth and would to symptoms before the content of a vitamin would have decreased dramatically. This comment leads to suggestion that it would be very useful to do an extensive literature survey of the nutritional literature to determine the relation between minimal requirements for essential nutrients and tissue composition of that nutrient. The survey will help to set a baseline for whether it is necessary to analyze the chemical composition of all tissues.