POTENTIAL OF NUTRITION TO ALTER GENE EXPRESSION IN POULTRY

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Introduction

Nutrition research in the future will increasingly focus on the ways in which our genes are affected by what we eat or how our animal's genes are affected by what we feed them. Foods and feeds that are safer and more nutritious, new medical treatments, and novel ways to help save the environment are potential benefits expected to result from such research.

Genetic research can be applied to the field of nutrition in many ways, whether it is to increase the nutritional value of a food, to identify ways to reduce an individual's risk of certain diseases, or to identify optimal health or growth-promoting diets. Since the arrival of the first draft of the human genome, a new chapter in understanding health and nutrition has opened. In February 2000 two independent teams of researchers simultaneously published their first "rough drafts" of the human genome. This is an astonishing achievement when one considers that the complete sequence of the human genome consists of 3.2 billion letters and is so enormous that it can only be published on the internet. It has been estimated that if printed in a format equivalent to telephone books that one copy would stack higher than the Washington Monument. Research on the human genome has required a thorough understanding of cell function and reproduction.

What is the Genome?

To aid those not familiar with the field of genetics and to get a basic understanding of the science involved, the following paragraphs very briefly review the structure and function of various aspects of the human genome. There are four possible bases and each one is matched to a specific partner on the opposite chain. Adenine (A) is paired with thymine (T), and guanine (G) is paired with cytosine (C). All of the information needed for a cell to function or reproduce is locked in the sequence made up of these four bases. This sequence is repeated millions and even billions of times throughout the genome. Every life form on the planet uses this same language and hence, the particular order of the bases is important because this is what makes a human a human, rather than a chicken, cow, or pig. In other words, it is the sequence of bases that underlies the diversity of organisms. The DNA sequences hold the secret of every life form from bacteria to humans, and science now has the power to decode these books of life called genomes.

A genome consists of all of the DNA molecules in an organism, including those in genes. A gene is a sub-unit of DNA that determines an individual's inherited characteristics such as eye or hair color. Genomes vary in size depending on their source. For example, the smallest known genome is from a bacterium and has 600,000 DNA base pairs. The human genome has about 3 billion base pairs while the chicken has only 1 billion. Even as genes get a lot of attention, it's the proteins in the cell that actually do all the work. Genes carry information to enable the cell to make proteins. The proteins in turn determine a whole host of features such as what the organism will look like, how well it functions and perhaps even how it behaves.

How to harness genome information

Research using the knowledge from the Human Genome Project will ultimately enable scientists to understand the functions of human genes and how they are switched on and off. This knowledge will provide them with information on how genes and nutrients interact and the effect of individual genetic differences on diet and nutrition. This research will be directly applicable to other species whose genome sequencing projects are underway. Research can help to identify these effects and help to understand why certain nutrients and foods are of benefit to health. Similarly, the genetic basis behind the differences in how some individuals or livestock strains respond to particular foods and nutrients can be identified and used to recommend foods and diets that are most beneficial for each. Studies into gene and nutrient interactions will also help to provide new information for developing more accurate **biomarkers** (indicators) to detect various diseases or growth suppressants much earlier and identify the genes that can be targeted by nutritional intervention to prevent them. These **biomarkers** are of particular importance to animal agriculture since breeding strategies can be tailored to take advantage of superior genetics.

Nutrient-gene interactions

It has been known for some time that diet and specific nutrients can affect the functioning of our genes. We all know that even when people are eating the same diets, some will become overweight, some develop heart disease and some develop allergies, while others will not. Wouldn’t it be wonderful to know why? The anticipated benefits from research into the function of genes include advances in the development of nutritious foods and special functional ingredients, optimal diets for individuals and improved methods for preventing many lifestyle-related diseases.

The mechanism by which nutrients specifically regulate the expression of genes in vertebrates in general is poorly understood. This is a puzzle; due to the basis of our fundamental understanding of how gene expression is regulated resulted from studying how bacteria respond to nutritional changes. The induction of proteins that transport and hydrolyze lactose in response to adding lactose to the growth media was the first examination of gene regulation of any kind (Jacob and Monod, 1961). Research targeting the nutritional regulation of gene expression in eukaryotic cells has progressed considerably slower due to the complexity of mechanisms controlling gene expression and the difficulty in identifying specific metabolites to which the intestinal epithelium (IE) cells respond (Traber, 1997; Sanderson, 1998). The IE cell must respond to the contents of the digesta through specific nutrient interactions and react to this "sensing" mechanism by "signal transduction" pathways to alter gene expression (Yeh and Holt, 1986).

Several genes have been identified as being regulated by nutrient levels in the diet (reviewed by Kelley and Sanderson, 1999). One of the most studied nutrient regulators of gene expression has been the micronutrient zinc (Blanchard and Cousins, 1996; Wu *et al.*, 1998; Reaves *et al.*, 2000). Zinc deficiency has been well characterized clinically and is associated with changes in the expression of many genes including cholecystokinin, uroguanylin, and ubiquinone oxidoreductase (Blanchard and Cousins, 2000).

Nutrient gene interactions-a practical example-Phosphorus

In many biomedical studies diet has been shown to be a significant factor contributing to debilitating diseases such as diabetes and arteriosclerosis but not for all individuals (Roberts *et al.*, 2001). It is generally accepted that these differences in dietary effects within the population are a function of genetics. The future of nutrition is tied to a clearer understanding of the interactions of specific nutrients within specific genetic backgrounds and to harness the power of emerging genomic technologies (Guengerich, 2001). A functional genomic approach will allow the nutritional potential of feeds to be better realized and utilized for selectively manipulating health or body composition of livestock. A driving force for understanding the regulation and mechanisms of nutrient absorption in animal agriculture is for the minimization of key nutrients (particularly phosphorus) in the excreta. This problem could be reduced by manipulation of genes involved in its absorption from the diet. The broiler chicken will serve as the focus of functional genomic analyses of nutrient absorption described in these studies. If these regulatory mechanisms can be determined and exploited, overfeeding of phosphorus (P) to broilers could be eliminated and excreta nutrients minimized.

The primary function of the gastrointestinal tract is the absorption of nutrients, a task that is the responsibility of the cells of the IE. The IE cells are the first to directly encounter nutrients present in the digesta. They must absorb and transfer nutrients via circulation to the entire organism therefore the intestine is the logical place to look for nutritional effects on gene expression. Many nutritional studies have focused on genes involved in the absorption of calcium (Ca) and P and the relationship between these micronutrients. Ca absorption is limited by transport across the membrane of the IE cell where the Ca-ATPase transporter is up regulated when an animal is fed a Ca restricted diet (Matkovits and Christakos, 1995). Similarly, the Na^+/P co-transporter, found in the IE cell, is up regulated in response to low dietary phosphate (Katai *et al.*, 1999). In addition to those genes responsible for the mechanical transport of nutrients by the IE, many novel genes, likely regulatory in nature, have been discovered by other approaches as responding to dietary nutrients (James *et al.*, 1986). A gene called PiUS which specifically up regulates the absorption of P (Norbis *et al.*, 1997) and several additional novel genes like diphor-1 that are regulated by dietary P have been described (Custer *et al.*, 1997).

Phosphorus (P) is an essential nutrient and possesses numerous essential functions, both structural and metabolic, in all living creatures. In broilers fed diets containing normal levels of P and calcium, apparent ileal absorption of P is only about 40-50%. The mechanisms of P absorption in the small intestine are not well defined. We have cloned and sequenced a full-length gene involved in phosphorus absorption designated GeneX. After GeneX was cloned, it was compared to the Genebank database and is homologous to a family of genes previously identified in other species at both the nucleotide sequence and amino acid level. To determine if GeneX was specifically expressed in intestinal tissues a multiple tissue Northern Blot was prepared using RNA samples taken from duodenum, jejunum, ileum, kidney, lung, liver, brain, heart, pancreas, and skeletal muscle (Figure 1). This analysis indicated that GeneX is expressed at the highest levels in the duodenum, followed by the jejunum and ileum and not in the other tissues tested. This trend in expression pattern is common with what is known regarding the localization of similar genes identified in the gut of other species. This observation was verified by quantitating the gene expression level by real-time polymerase chain reaction (PCR) and primers designed to amplify a unique region of GeneX.

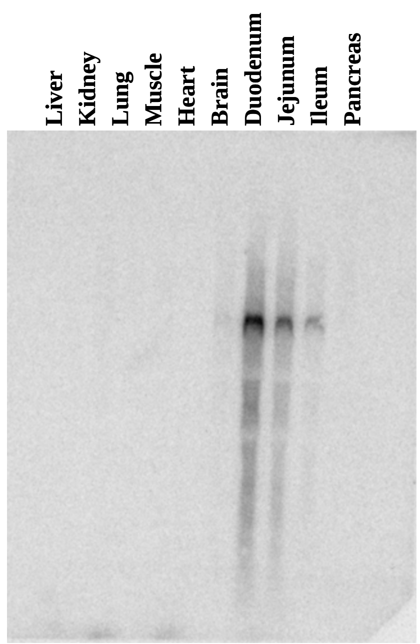

Figure 1. Tissue distribution of GeneX.

Northern analysis was conducted by isolating total RNA from multiple chicken tissues, separating the 10ug of the RNA by electrophoresis, and hybridizing a radiolabeled probe for GeneX.

To identify the specific cellular location of GeneX mRNA in the small intestine, in situ hybridization was performed with tissue samples taken from duodenum, jejunum, and ileum (Figure 2). The mRNA for GeneX is expressed at levels high enough to be detected with in situ hybridization and was found to be expressed throughout the intestinal epithelium cell layer.

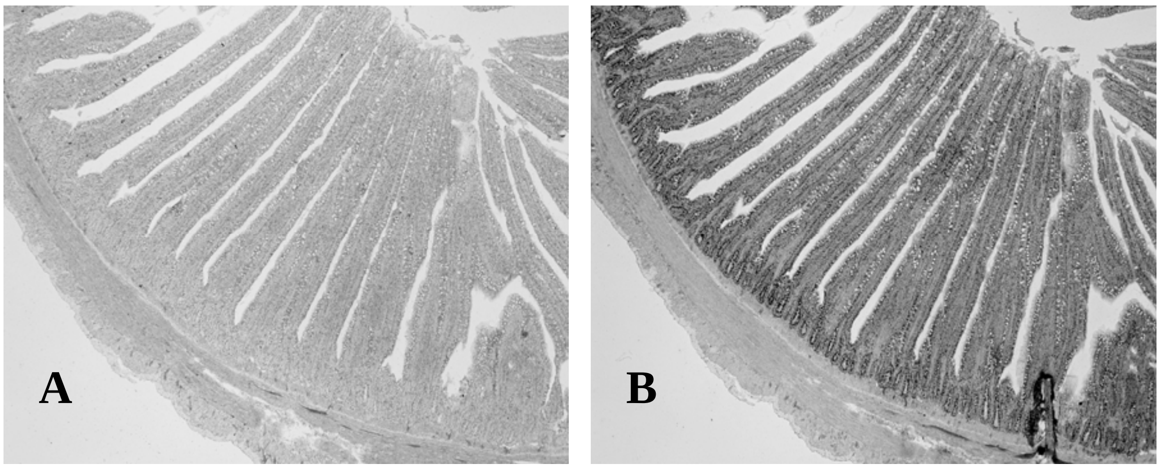

Figure 2. In situ hybridization of GeneX involved in phosphorus absorption. Duodenum was collected from a 2 week old broiler chicken and preserved in paraformaldehyde (4%). Tissue segments were embedded in paraffin, sectioned and mounted to glass slides. RNAs corresponding to the sense (coding) sequence for GeneX and the anti-sense (complimentary) sequence were labeled and hybridized to the tissue sections. Adjacent sections are shown above with the location of GeneX expression in Panel B (anti-sense) in the intestinal epithelium and not in surrounding smooth muscle or in the negative control (sense) shown in Panel A

Utilizing GeneX as a **biomarker**, either through real-time PCR, Northern, or in situ hybridization measures, for predicting phosphorus absorption potential will be of significant economic impact. First, many chicken lines will be screened for superior dietary P utilization. Superior animals will be crossbred and the progeny tested for P utilization to confirm the association with GeneX. Implementation of this **biomarker** in a commercial setting will then be sought through an industry partnership.

Summary and implications for animal agriculture

Animal meat is the primary source of protein in the U.S. diet. Animal agriculture is the largest consumer of feedstuffs world wide, comprising the major cost of animal production. Any increase in the efficiency of nutrient utilization and thus reduction in feed costs is of significant global economic importance. Additionally, rising environmental concerns have focused attention on animal waste and its high nutrient content, specifically nitrogen and phosphorus. Many states now have legislation limiting the amount of manure based on nitrogen and phosphorus content, that can be applied to crops reducing the amount that can be applied by as much as 70% in areas of intensive agriculture (Delmarva Peninsula, North Carolina, etc.). The limitation of available croplands may cause the added expense of manure transport out of these regions. Maximizing nutrient utilization from the diet is key to both decreasing feed costs as well as decreasing nutrient content of manure.

The ability to select (using **biomarkers**) and breed superior animals that are able to better utilize dietary phosphorus would be of significant impact to animal agriculture worldwide. The estimates for savings to the U.S. broiler industry alone are on the order of 400 million dollars annually. Similar biomarkers for litter size in swine and marbling in beef cattle have proved lucrative for those individuals that discovered them as well as their corporate partners. These continuing studies will identify genes expressed in the small intestine, identify the etiology of cell types expressing these genes, and characterize the influence of these genes over production traits. Once **biomarkers** are identified that are involved in P absorption in the chicken novel approaches will have to be taken to manipulate them. This manipulation may include the generation of transgenic animals or gene silencing through RNA interference, a technique that essentially shuts down specific gene expression. Regardless of the method, significant economic and environmental benefits would result from modulating specific nutrient absorption and retention.

While genomic research can appear daunting, and even frightening, to some people, improved understanding of the techniques involved and the potential benefits to be derived from this research will hopefully show that it is an area that will advance rapidly and lead to significant breakthroughs in nutrition and improved efficiencies in animal agriculture.

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