The Food Safety of Transgenic Animals: Implications from Traditional Breeding

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ABSTRACT: The genetic events associated with traditional selection have implications for the food safety of transgenic animals. Selection has been empirical, relying on the use of the best animals for breeding. Molecular techniques are now being used to identify the genes selected and to describe the differences between alleles that are important in selection to improve quantitative traits. The results of such analyses provide background details of the genetic and physiological effects of the traditional selection of animal lines. Examples of the kinds of genes that may be subject to selection are those coding for peptide hormones, steroid metabolic enzymes, the calcium-channel gating protein, and genes of the major histocompatibility complex. Unselected genes, sometimes with undesirable alleles, may be carried along as "hitchhikers" if they are closely linked to the selected gene. In spite of this potential for physiologically dangerous genetic changes in selected animals, hereditary food toxicity has never been associated with a selected line of the common food animals. This is probably because the allowable physiological range of results of selection is limited by the requirement for healthy, productive animals. Based on these limitations, foods from healthy transgenic animals produced for the purpose of herd improvement are likely to be as safe as the foods from the untransformed parental line. Animals are important indicators of their own food safety.

Key Words: Transgenics, Food Safety, Animal Breeding, Quantitative Traits, Restriction Fragment Length Polymorphisms

Introduction

Transgenesis provides a shortcut to the development of lines of animals with many desired traits, including, but not limited to, increased disease resistance, more efficient production, leaner meat, and even expanded ecological ranges. Since the discovery of restriction enzymes in the early 1970s, recombinant DNA techniques have continued to expand the methods for introducing specific genetic changes into animal lines and for characterizing the genetic variability in the animal genome. Genetic mechanisms that were largely speculative only 20 yr ago can now be tested, and molecular techniques have been incorporated into selection strategies (Dentine, 1992). The impact of molecular techniques on animal breeding is just beginning to be widely appreciated.

Anticipating the marketing of transgenic food animals, in 1990 I described some of the considerations for evaluating the food safety of these animals (Berkowitz, 1990). One of the important points was that transgenic animals are not entirely new, but are part of the continuum of the time-proven breeding of safe food animals. Of particular concern was the notion that the use of molecular techniques represents a "disjunction" of some sort in the development of new food-producing breeds. This view reflects our ignorance of the detailed genetic mechanisms and physiological changes accompanying traditional selections. Whereas transgenesis is described entirely in molecular terms, traditional breeding descriptions have been almost entirely empirical. Since 1990, many descriptions of the molecular events associated with traditional breeding have been published. The "new" and the "old" methods are similar at the molecular level, so the food safety questions should be viewed in the context of the continuum. First I describe some of the genetic and physiological events associated with traditional breeding. Then, the food-safety questions related to transgenesis are reviewed in the historical context.

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Traditional Breeding

The ancients knew that the characteristics of animals are passed on to offspring from parents, and that the choice of parents is very influential in determining the qualities of the offspring. For quantitative traits such as growth rate or quantity of milk production, animals carrying the most desirable traits are artificially selected (as opposed to natural selection) for breeding. The breeding of these animals increases the frequency of the desirable alleles at quantitative trait loci (QTL), the genetic sequences responsible for the desirable traits in the animal population. Detailed molecular descriptions of the actual genetic events and the identities of the QTL are emerging, but many of the physiological systems affected are those expected based on classical genetics and physiology. For example, it is no surprise that many cows traditionally selected for high milk production also have elevated levels of somatotropin (Barnes et al., 1985). Population variations in circulating growth hormone levels have also been described in mice (Sinha et al., 1974).

At the DNA level, restriction fragment length polymorphisms (RFLP) are being used to identify QTL. This has produced a number of interesting results, in spite of the fact that the association of a polymorphism with a QTL is often quite complicated. An interesting example is provided by RFLP associated with the somatotropin gene in a strain of mice selected for rapid growth (Salmon et al., 1988). Using a probe for the growth hormone gene, polymorphisms were found in DNA fragments produced by seven different restriction enzymes. The polymorphisms occur both upstream (5') and downstream (3') from the growth hormone structural gene, as well as in the structural gene itself. Surprisingly, in the F2 generation, the haplotype carrying the polymorphic sites that were fixed in the high-growth line segregated in the low-growth rather than in the high-growth animals, which implies that this haplotype does not carry the QTL (Winkelmann et al., 1990). Nevertheless, the distribution of polymorphic sites is interesting, because the implication from the locations of the polymorphisms is that these genetic changes could alter the levels, the activity, or even the tissue expression pattern of somatotropin. The polymorphisms in the mouse growth hormone gene's upstream (5'-end) flanking region contain DNA elements that mediate the control of growth hormone transcription by thyroxine (3,5,3'-triiodo-L-thyronine) (Casanova et al., 1985). Thus, not only could the level of expression of somatotropin be altered, but the control of the level of expression by other endocrine components could be altered.

The results of a study of polymorphisms in or near the prolactin gene in high-producing dairy cattle is interesting for other reasons (Cowan et al., 1990, 1992). Prolactin stimulates lobulo-alveolar growth and milk secretion in the mammary gland (Cowie, 1972), so it is reasonable to guess that the prolactin locus might be related to polymorphisms associated with high milk production. In fact, the authors found a RFLP using a prolactin DNA probe and demonstrated with crosses that one haplotype is associated with increased milk production, the other with decreased milk production, and the heterozygote with an intermediate level of production. Subsequent analysis showed that the QTL associated with increased production is not in the prolactin gene itself, but is closely linked to it. The identity of the QTL is still not known. However, other interesting genes highly linked to the prolactin gene and to the QTL are carried along when the QTL is selected. These linked genes include the bovine major histocompatibility locus (MHC) and the steroid 21-hydroxylase gene. Polymorphisms in these linked genes could have effects on the immune responsiveness of the animal (MHC) or on progesterone or aldosterone levels (steroid 21-hydroxylase). Although the QTL itself has not yet been identified, this example illustrates the wide range of physiological systems and pathways that can be affected by traditional selections, and it demonstrates that these involve not only the selected locus, but also closely linked genes that come along as "hitchhikers" (Smith and Haigh, 1974).

Undesirable alleles of genes linked to desirable QTL can be and are carried along as hitchhikers. In Holstein cattle selected for high milk production, an allele of a leukocyte adhesion glycoprotein (CD18) gene causes a severe immunodeficiency due to impaired leukocyte function in animals homozygous for the allele (Shuster et al., 1992). A test for this allele has been used to screen bulls so that only noncarriers may be used for breeding. In swine, a QTL associated with increased muscularity and carcass yields carries a malignant hyperthermia gene (MH) as a hitchhiker. In animals expressing the disease, the meat is referred to as pale, soft, exudative pork. The undesirable allele codes for a hypersensitive calcium-channel gating protein (Ryanodine receptor) (Fuji et al., 1991). These are additional examples of the types of genetic and physiological changes produced by classical breeding. Again, I emphasize that all these changes and interactions are induced by traditional selections; recombinant DNA techniques have merely made it possible to examine the molecular changes brought about by these traditional selections.

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3This principle was not uniformly practiced. In 16th- and 17th-century England, the production of desirable food animals was attributed to good feeding. The best animals were marketed, and the least desirable were reserved for breeding. However, to improve horses, Henry VIII ruled in 1535 that only horses meeting a minimum height were to be kept alive. This began improvements in English horse breeding that were not adopted for food animals until the 18th century by Robert Bakewell. Bakewell's ideas later influenced Charles Darwin's work (Hall and Clutton-Brock, 1989).
The effects of the transgene, a single foreign gene, may have been exaggerated by the enthusiasm for molecular methods. In some cases, not enough emphasis has been placed on the interaction of the transgene with the rest of the genome. Sewall Wright wrote, "I recognize that an organism must never be looked upon as a mere mosaic of unit characters, each determined by an individual gene, but rather as a vast network of interaction systems" (Wright, 1978). Thus, even with transgenesis, a quantitative genetic analysis will usually be essential to obtain information on the interactive components of the genetic variance. The phenotypic expression of a transgene is determined, as for any other gene, not only by the introduced gene but also by the interactions of the transgene with the rest of the genome. The example of the thyroxine regulatory interaction with the 5'-flanking region of the somatotropin gene altering somatotropin levels was discussed above.

**Transgenic Animals**

When the food safety of transgenic animals is viewed in the context of the above discussion, it appears unlikely that the addition of a transgene that alters the physiological balance of an animal would raise concerns. Enormous numbers of the allowable mutations and variations have been represented during the long history of animal agriculture. Physiological changes not compatible with a productive animal would have resulted in abortion or in an unhealthy, productively disadvantaged food animal. I am not aware of any records of a hereditary food safety hazard derived from a safe parental animal. The addition to the genome of a single gene that affects the physiology of the animal without impairing the health of the animal is unlikely to affect the food safety of the flesh. The only exception may be transgenes coding for allergens. However, because the transgene product is known, testing can be performed when it is available and useful.

The food safety assessment of transgenic animals benefits from the knowledge and high degree of characterization of the introduced gene. In traditionally bred animals, identifying the genetic loci responsible for quantitative traits is complicated, and more than one locus is usually involved. Ascertaining the cause of a physiological effect or identifying the primary physiological system mediating the effect can be quite difficult. In transgenic animals, knowledge of the function of the transgene focuses the safety investigation on the gene product, which can usually be measured, and on the correct physiological system, making it easier to discover the effects and consequences of the gene product.

Genetic effects resulting from the location and direction of insertion of a transgene must also be considered in a discussion of food safety. The model for this comes from plants, many of which produce toxins as defenses against predation. The levels of the toxins probably respond genetically as quantitative traits, as shown for solanine in potatoes (Sanford and Siden, 1972). The widespread presence of insertion elements in plants suggests that the insertional process could activate latent toxin pathways, if such pathways exist. Genetic insertions also occur naturally in animals, prompting questions about the production of high levels of orally active hormones or peptides that might compromise food safety. However, many naturally occurring genetic events, including all the interactive effects, and deletions, inversions, and translocations as well as insertions, can alter the levels of expression of genes in animals. Gene insertions in animals have been shown to cause deleterious developmental (Schnieke et al., 1983) and fertility and neurological effects (Gordon et al., 1990). Nevertheless, the range of genetic changes selected in food animals is probably limited by the requirement for healthy, productive animals.

Given the kinds of genetic events and genes affected by traditional selections, the absence of toxic animals may be somewhat surprising. The safe food history of animals has probably resulted because the animals are, themselves, important indicators of toxicity. If toxic levels of hormones or other substances were produced, this would override the animal's own homeostatic equilibrium, which would be reflected in the health of the animal. In genetic selection, the health of the animal is an important indicator of its own food safety. (Obviously, if an animal carries a toxin to which it is resistant, such as tetrodotoxin in pufferfish, this would not be reflected in the health of the animal. But, in this case, the toxin level would be a normal consideration in establishing the safety of food from the traditional animal line). For transgenic animals, knowing the transgene product is an added safety factor because it focuses the safety inquiry on the correct physiological system.

**Literature Cited**


