THE MANIPULATION OF INTERMEDIARY METABOLISM BY TRANSGENIC TECHNOLOGY TO ALTER NUTRIENT REQUIREMENTS IN ANIMALS

K.A. WARD

Summary

This paper describes the potential for the manipulation of the intermediary metabolism of animals by the use of transgenic technology. The aim is to introduce into animals the genetic information required for the enzymes involved in the biosynthesis of organic nutrients that must at present be supplied by the diet. The general concept requires the isolation of functional genes from bacteria, modification for expression in animals and transfer to embryos to create transgenic animals. Examples given are the introduction of a cysteine biosynthesis pathway and a glyoxylate cycle to mammals. It is envisaged that the general principles may also be applicable to poultry.

I. INTRODUCTION

For intensive animal industries such as the poultry, pig, feedlot cattle and aquaculture industries, the supply of inexpensive feedstuffs is a major factor in overall production costs and thus becomes a critical factor in any producer's economic survival. Much of the expense of these feedstuffs derives from the need for supplementation with various organic nutrients that cannot be synthesised by the animal and are either completely absent or not present in sufficient quantity in normal inexpensive feed sources. Such nutrients must be supplied by the diet because animals have during evolution lost the genetic information necessary for the enzymes involved in the relevant biochemical pathways. There are two obvious ways to improve this situation for the producer. The first approach is to alter the genetic properties of the primary feedstuff source (predominantly plants) so that they contain appropriate levels of the missing nutrients. The other approach is to modify the genetic properties of the animal itself so that it becomes capable of synthesising the missing nutrients de novo, thus removing the dietary requirement. The latter approach requires modification of the intermediary metabolism of the animal.

The modification of intermediary metabolism by the introduction of a new biochemical pathway is a significant challenge because the physiological homeostasis of an animal is maintained by the complex interaction of many different biochemical and hormonal regulatory systems. It is reasonable to expect that the introduction of a new pathway to the existing biochemical repertoire could upset this delicate balance. Nevertheless, the potential improvement in productivity and reduction in feedstuff costs makes such an objective worthy of the attempt, provided that the techniques for such manipulations are available. Normal evolutionary processes are very unlikely to restore any of the lost genetic information to full functionality, so conventional selection approaches would be unlikely to succeed. However, the development of transgenic technology (Palmiter and Brinster, 1986) now makes this possible because it provides a mechanism for the transfer of genes between organisms that cannot breed by conventional methods. Utilising these new techniques it is now possible to introduce a missing biochemical pathway into an animal by identifying and isolating a functional equivalent of the missing genetic information from any organism in nature and then transferring it to the target organism. Two examples of such manipulations that have achieved some measure of success are summarised in the following text.

CSIRO Livestock Industries, Locked Bag 1, Delivery Centre, Blacktown, NSW 2148.
II. INTRODUCTION OF A CYSTEINE BIOSYNTHETIC PATHWAY TO ANIMALS

Sheep require a large supply of the amino acid cysteine to maintain optimal wool growth because of the high content of this amino acid in the keratin proteins that contribute more than 90% of the mass of the wool fibres. The amino acid can only be synthesised in an animal from the sulphur amino acid methionine and the amount of methionine in most feeds is insufficient to provide an adequate cysteine supply. Cysteine cannot be synthesised from other substrates because animals lack two crucial enzymes, serine transacetylase and O-acetylserine sulphydrylase. However, the genes for these enzymes are still present and fully functional in bacteria. In *Escherichia coli*, the *cysE* gene encodes the enzyme serine transacetylase and the *cysK* gene encodes O-acetylserine sulphydrylase. The carbon pathway for cysteine biosynthesis is shown in Figure 1.

![Figure 1](image1.png)

**Figure 1.** The carbon pathway for cysteine biosynthesis in *E. coli*.

The *cysE* and *cysK* genes have been isolated and modified for expression in mammals (for review see Ward, 1999). The DNA construct used is shown in Figure 2.

![Figure 2](image2.png)

**Figure 2.** The plasmid MTCEK1 containing the genes from *E. coli* that encode serine transacetylase and O-acetylserine sulphydrylase.
When inserted into transgenic mice the recombinant DNA provides the genetic information necessary for the production of the two enzymes required for cysteine biosynthesis. That the new biochemical pathway was fully functional was tested experimentally by placing transgenic and non-transgenic mice on a synthetic diet which was supplemented with sodium sulphide as a sulphur source but which contained no cysteine and only trace amounts of methionine. As expected, non-transgenic mice were unable to survive on such a diet for any extended period of time. On the other hand, the transgenic mice containing the introduced cysteine biosynthetic pathway were capable of synthesising their entire cysteine requirement for the full duration of the experiment (Ward et al., 1994). This research demonstrates that, at least in some instances, it is possible to introduce new biochemical pathways into animals to remove a dietary requirement.

III. THE INTRODUCTION OF A GLYOXYLATE CYCLE TO ANIMALS

The second example of a modification to mammalian biochemistry is summarised to indicate the extent to which new pathways can be designed to interface with existing intermediary metabolism. This research aims to introduce to mammals a functional glyoxylate cycle which allows the biosynthesis of glucose from the volatile fatty acid acetate. While this is mainly relevant to ruminant animals that have very large concentrations of acetate circulating freely in their blood stream, it may have some application in non-ruminants in tissues that are highly dependent on glucose as an energy source and have access to reasonable quantities of acetate. The overall concept underlying this project is similar to that described above for the biosynthesis of cysteine in that the glyoxylate cycle is non-functional in animals because the genes encoding two critical enzymes, isocitrate lyase and malate synthase, are both missing. These genes are fully functional in E. coli, the aceA gene encoding isocitrate lyase and the aceB gene encoding malate synthase. These genes have been isolated and modified for expression in animals, after which they have been transferred to mice and expression analysed in a variety of tissues from the transgenic animals. Expression of both genes at the mRNA and protein level (Saini et al., 1996) has been found in the intestinal epithelium of these animals, this being the most likely site for expression of the transgene because of the metallothionein gene promoter that was used to regulate its expression. While no dietary experiments have yet been carried out on these animals, the results to date indicate that it is possible to introduce genes that encode enzymes that interact directly with fundamental components of the animal's biochemistry which, in the case of the glyoxylate cycle, is the tricarboxylic acid cycle.

IV. THE APPLICATION TO POULTRY FEEDSTUFFS

Transgenic techniques are more difficult to carry out in poultry than in mammals but considerable progress has been made in the last 5 years or so in the development of suitable methods. Therefore, it is feasible to consider the application of the principles outlined above to poultry if problems relevant to the industry can be identified. It is clear that the biochemical pathways for the synthesis of various amino acids, such as lysine, could be introduced to poultry, thus removing the need for dietary supplementation of these components. While the pathway for lysine biosynthesis presented a significant challenge several years ago because of the number of enzymes required (at least 7), new developments in the past couple of years have made it possible to create fusion proteins that fold to contain several different enzymic activities in the one protein molecule. The use of fusion proteins can greatly reduce the size and complexity of the recombinant DNA needed for metabolic pathway construction. This concept has been under active research within the author's group,
for example, to create a single fusion protein containing both the serine transacylase and O-acetylserine sulphydrylase activities needed for cysteine biosynthesis and progress in this work has been most encouraging. Therefore, it is not beyond realistic expectation that the biochemical pathways for some of the more complex molecules needed by poultry for adequate growth might be introduced by this transgenic approach.

A further possibility for this approach is to introduce a gene combination which allows the biochemical processing of a compound in feedstuffs that cannot at present be utilised by poultry. The introduction of cellulases to degrade the cell walls of plant material is a simple example of such an approach and attempts to achieve this in monogastric animals have already made interesting progress (Hall et al., 1993). More complex enzyme combinations can be envisaged that would allow the processing of much of the plant material that at present is not usable as a nutrient source for poultry.

V. SOCIAL IMPLICATIONS OF TRANSGENESIS

At present society is skeptical of the value of transgenic organisms and remains reluctant to consume plants or animals that contain recombinant DNA. This situation will slowly alter as evidence accumulates that there are no inherent dangers in this sort of genetic manipulation. However, it is unlikely that animals with modified biochemical pathways will be readily accepted for some time yet. When introducing new enzymic pathways to animals it is clearly important to ensure that the new pathway is compatible with the existing biochemistry of the target organism, that no novel and potentially hazardous biochemical intermediates are likely to be produced and that the new enzymes that are synthesised in the animal’s tissues present no dietary problems to consumers if eaten. It is critical for public acceptance of this technology that all experimental results be readily available for full scrutiny and that public opinion be sought at all stages of the work. Under such circumstances the benefits of this approach may be more fully appreciated and eventually accepted by most consumers.

REFERENCES