SOAEFD flexible fund project RO 818

Audit of data produced at the Rowett Research Institute

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Audit committee:

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1. The project

1.1 In 1995 SOAEFD commissioned a three-year multicentre project "Genetic engineering of crop plants for resistance to insect and nematode pests: effects of transgene expression on animal nutrition and the environment". The collaborating institutions were the University of Durham, the Rowett Research Institute (RRI) and the Scottish Crop Research Institute (SCRI).

1.2 Provision of genetically-modified crops and measuring the environmental impact of the transgenes was the responsibility of the University of Durham and SCRI. The role of RRI was to examine the substantial equivalence of the transgene products and to determine any antinutritional effects on rats in a series of short-term (10 day) and long-term (3 month) feeding trials.

2. The genetically modified plant material

2.1 Candidate genes known to encode for three lectins, selected on the basis of their known effects on insect pests and differences in the severity of their effects on the mammalian gastrointestinal tract were proposed for study. These were the snowdrop lectin (GNA), the jackbean lectin (concanavalin A [ConA], a known mitogen) and the *Phaseolus vulgaris* lectin (PHA, known to be toxic to higher animals). Target crops were potato, oilseed rape and strawberry. Only GM potato and oilseed rape were supplied to RRI and only potato expressing GNA and ConA in amounts sufficient for feeding studies.

2.2 The SCRI, using a binary vector (pBIN19) gene construct supplied by the University of Durham, prepared transgenic potato plants expressing the ConA gene under the constitutive CaMV35s promoter. The construct was introduced into potato cells by a standard *Agrobacterium*-mediated transformation. Transgenics were selected by resistance to kanamycin (pBIN19 contains the *nptII gene* encoding neomycin phosphotransferase under the *nos* promoter). Transgenic plants containing the GNA lectin gene also expressed under the CaMV35s promoter, were provided by the University of Durham and propagated by the SCRI.

2.3 The concentration of foreign lectin (GNA) in transformed potato was measured at RRI by ELISA. GNA was expressed at 25μg/g dry matter in the first generation tubers of line 71 falling to 16-17.5 μg/g dry matter in the second generation and at 12.7-17.2 μg/g dry matter in the second generation of line 74. The concentration of GNA in the leaf and stem was at least 10-fold higher than in tubers. Cooking tubers of line 71 at 100^0C for one hour reduced the GNA concentration detectable by ELISA to 4.9 μg/g dry matter while cooking line 74 at 110^0C for 20 minutes essentially destroyed the lectin (0.08 μg/g dry matter). Denaturation of the lectin by heat sufficient to prevent recognition by the anti-lectin antibody was assumed to indicate the total loss of biological activity. While this may be a reasonable assumption it was not confirmed in any other system.

2.4 The single potato line examined expressing Con A (Con A4) had a ConA tuber concentration of 0.15 μg/g dry matter.

2.5 Parent and GM potato lines 71 and 74 were shown to be substantially equivalent with respect to native potato lectin content, protease inhibitor concentration, gross composition and amino acid content. One other possible confounding factor in feeding experiments, the glycoalkaloid content, was not measured. The extent of expression of *nptII* also was not measured in any of the potato samples used in the feeding experiments and potato transfected with the empty vector was not used as a control although this had been considered and the construct prepared. However, in all material prepared for feeding experiments, the parent potato was grown alongside the transformed line which would be expected to minimise any differences in composition caused by growth conditions.

3. Feeding experiments: effects on growth and body composition

3.1 A total of 12 feeding experiments were completed, of which ten were short-term (10 day) and two long-term (110 days). The first seven experiments used control tubers not containing the foreign lectin genes and were concerned with the design of potato-based diets which both supported growth of the rats and met the animal welfare requirements of the Home Office.

3.2 No feeding studies were made with transgenic potato expressing ConA.

3.3 Addition of Con A at 800 μg/g diet to a cooked potato diet or to lactalbumin-starch diet resulted in a small but significant depression in growth of 5.4% ($P<0.05$) and 6.1% ($P<0.001$) respectively over a 110 day period (D214). A raw potato diet also included in this experiment failed to adequately sustain the growth of the rats and was abandoned after 63 days.. The absolute weight of organs, adipose lipid and skeletal muscle was reduced in rats given ConA but, when expressed as a proportion of total body weight , there were no significant differences. In a short-term experiment (D232), Con A was added to a lactalbumin-starch diet at 400 μg/g diet with no effect on weight gain after 10 days.

3.4 The presence of GNA, whether added to potato-based diets or occurring as a component of transgenic tuber line 71 (D227) or line 74 (D237, D242), had no significant effect on weight gain, weight change or organ weight compared to parental controls. Addition of GNA at 1200 μg/g diet to lactalbumin-starch diet also had no significant effect on rat growth after 110 days (D237) or at 400 μg/g diet after ten days (D232). The one apparently anomalous result, in which rats fed an uncooked transgenic potato (D242) performed significantly better than animals fed the uncooked parent potato with or without GNA addition was explained by examination of the *empty* body weights after 10 days. These showed no significant differences between the groups. Rats fed the uncooked transgenic potato apparently accumulated the diet in the digestive tract during the last four days for

reasons which are unknown.

4. Feeding experiments: lymphocyte proliferation assay

4.1 The major test of immune function of rats fed different diets, including transgenic potato, was the well-established lymphocyte proliferation assay adapted for use with rat blood and using ConA and PHA as the stimulating mitogens. Some information on circulating cytokines (IL-1β) was obtained from a single experiments (D214), but was preliminary in nature and involved only rats fed diets with added Con A.

4.2 Data from the lymphocyte proliferation assay was available for three experiments (but see 4.5). However, in one experiment (D232), too few cells were obtained to allow complete replication and, as a result, none of the results reached statistical significance.

4.3 Addition of ConA (800 μg/g diet) to a cooked potato diet apparently reduced significantly the capacity of the lymphocytes to respond to stimulation by Con A compared to the lectin-free control after 110 days of feeding (D214). However, this effect was not reproduced in lymphocytes from rats fed a lactalbumin-starch diet. PHA used as a mitogen in lymphocyte proliferation assays gave more consistent results and lymphocytes from rats fed both lactalbumin and cooked potato diets with added Con A showed a much reduced response to stimulation with this mitogen compared to the corresponding controls ($P<0.01$).

4.4 The short-term feeding experiment involving the transgenic line 74/2T expressing GNA (D242) provided inconsistent data. *This was the only experiment involving the feeding of transgenic material for which lymphocyte proliferation data were available (but see 4.5).* With Con A as the mitogen, lymphocytes from rats fed the uncooked parent potato with and without added GNA were equally stimulated, whilst those from the transgenic potato showed a much reduced stimulation. This pattern was not duplicated in lymphocytes from animals fed the equivalent cooked-potato diets. As previously (4.3), results with PHA as the mitogen appeared more consistent than with ConA with the apparent extent of lymphocyte stimulation, in decreasing order, being the uncooked parent alone followed by uncooked parent + GNA and finally the uncooked transgenic line. However, the inherent variability of the results meant that many of the differences between mean values which appeared to show the greatest effect did not reach statistical significance.

4.5 The lymphocyte proliferation assay for the long-term feeding study with GNA transgenic potato (D237) was completed on 20th August 1998 immediately before this audit and these results were not previously available. These results clearly demonstrate that although lymphocytes from the lactalbumin-fed animals were stimulated by ConA and PHA as mitogens there was no statistically significant response from lymphocytes obtained from any of the cooked potato groups regardless of the presence or

absence of GNA.

4.6 The considerable variability associated with the lymphocyte proliferation assay in these experiments was partly the result of inter-animal variation but also reflects some extreme variability between the responses of replicated cell populations derived from the same animal.

5. Conclusions

5.1 Addition of concanavalin A to diets of rats at 800 μg/g diet does cause a small but significant reduction in growth over 110 days. However, the concentration used was some 5000-times greater than was measured in tubers of Con A4, the experimental potato line expressing this lectin.

5.2 GNA is a more likely candidate for use in commercial crops. This lectin did not have any deleterious effects on the growth of rats in three short and one long-term feeding experiments even when added at 100-times the concentration expressed in the tubers of the transgenic products.

5.3 The significant effect on digesta retention of feeding raw transgenic GNA line 74/T2 to rats in one short term experiment remains unexplained, but was not observed in a second experiment involving line 71.

5.4 Given the known mitogenic effect of some lectins, the intention to examine whether consumption of transgenic constructs expressing lectins has any effect on the immune response of higher animals was entirely valid. However the results obtained were, in most cases, far too variable to reach statistical significance and too inconsistent to draw *any* meaningful conclusions.

Therefore, the audit committee is of the opinion that the existing data do not support any suggestion that the consumption by rats of transgenic potatoes expressing GNA has an effect on growth, organ development or immune function