DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS
Research and Development

Final Project Report

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CSG 15

4 JUL 2003

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Project title	Digestion and absorption of feed components in broilers
DEFRA project code	**CS0119**
Contractor organisation and location	ADAS Nutritional Sciences Research Unit Alcester Road, Stratford-on-Avon Warwickshire, CV37-9RQ
Total DEFRA project costs	£ 223,576
Project start date	01/05/01
Project end date	31/03/03

Executive summary (maximum 2 sides A4)

- Genetically modified (GM) crops are of global importance. The area grown increased from 4 million ha in 1996 to 59 million ha in 2002. Although studies have confirmed both compositional and nutritional equivalence of GM and conventional crop varieties, concern has been expressed about the use of GM crops in animal feeds. One of the issues raised was whether transgenic DNA (tDNA) and/or transgenic protein could be transferred to and accumulate in milk, meat or eggs derived from animals fed GM crops?

- Only a limited number of studies have been conducted to determine the presence or absence of tDNA in animal-based products from animals' consuming GM diets. To date tDNA has not been detected in these products. Also, there is limited information available on the degradation of DNA in the GI tract of animals.

- The objectives of the current study were (1), to develop and validate techniques for the detection of transgenes in broiler tissues and digesta. (2), to determine the presence or absence of specific transgenes in broiler tissues and digesta. (3), to determine if any detected transgenes are present transiently in broiler tissues or integrated into the host genome.

- The study involved the use of GM soyabean meal (containing the *cp4epsps* gene for Monsanto Roundup Ready® Soybean event GTS-40-3-2) and GM maize grain (containing the *cry1a(b)* gene for Monsanto YieldGard® Bt Maize event MON 810. In addition the near isogenic (non-GM) counterparts, Bronson soybeans and Dekalb maize hybrid DK626 were also used.

- The GM and non-GM soyabean meal and maize grain, together with a range of miscellaneous feed ingredients, were used to prepare four experimental treatment diets (T1-T4). T1, contained non-GM maize

and soyabean meal; T2, contained non-GM maize and GM soyabean meal; T3, contained GM maize and non-GM soyabean meal and T4 contained GM maize and soyabean meal. Each treatment diet was re-formulated during the course of the study to meet the nutritional requirements' of the broilers from 0-2, 2-4 and 4-6 weeks of age (starter, grower and finisher).

- The study comprised of two experiments carried out in succession. In Experiment 1 only T1 was fed to 12 broiler birds while in Experiment 2, each treatment diet was fed to 24 broiler birds. Also in Experiment 2, the source(s) of the GM ingredients was removed 96 h prior to slaughter for 0.5 of the broilers receiving treatment diets T2-T4.

- The broilers were slaughtered after 43 days and samples of blood, tissue (heart, liver, kidney, bursa, spleen, breast and gizzard) and digesta (gizzard, duodenal, small intestine and large intestine digestas) collected for DNA extraction, polymerase chain reaction (PCR) methodologies and DNA separation and visualisation, following staining with ethidium bromide.

- PCR was used to determine the presence or absence of target DNA sequences. Primers were selected to amplify small (~200 bp) fragments from single-copy genes coding for soya lectin, maize high-mobility protein, and the *cp4epsps* and *cry1a(b)* genes from the GM crops, and the multi-copy genes coding for the poultry (*Gallus gallus*) mitochondrial cytochrome b and rubisco.

- Separation, by agarose gel electrophoresis, of the genomic DNA extracted from the maize grain showed that the DNA was present in fragments greater than 23 kb. In contrast the isolated DNA from the soyabean meal samples was highly degraded with DNA fragments from 2 kb to 500 bp.

- PCR analysis confirmed the presence of the constructs *cp4epsps* and *cry1a(b)* in the GM soyabean meal and maize grain respectively and therefore, confirmed their correct provenance. PCR analysis showed that there was a low level of GM maize DNA in four samples of non-GM maize tested for the MON 810 amplicon. Semi-quantitative analysis showed that one of the samples contained between 0.1 and 1% GM maize. A low level of GM soya bean DNA was confirmed in the non-GM soyabean meal at a level between 0.1 and 1%.

- No true positive detections of any of the single-copy genes including transgenes was made in the WBC and serum blood fractions.

- Except with a single exception, no true positive detections of any of the single-copy genes including transgenes was made in the tissue samples analysed. The single positive detection (one positive PCR result in a total of 3780 tissue PCRs) was recorded for lectin in bursa. The multi-copy rubisco gene was detected in a proportion of the samples examined from each tissue type. The mean proportion of positive rubisco detections was 0.23 across all tissue samples and treatment diets.

- With the exception of the duodenum, DNA fragments from the specific single-copy genes were detected up to the large intestine. Poor amplification of DNA fragments in duodenal digesta was observed and concluded to be due to PCR inhibition by the presence of bile salts in the isolated DNA. Transgenic DNA was detectable in gizzard digesta 96 hours after the last feeding of diets containing GM feed ingredients. Rubisco was detected in each type of digesta studied and the proportion of positive detections in the small and large intestine digesta was high (0.98 and 0.89 of total PCRs respectively).

- The current study has shown that within current levels of detection, tDNA could not be detected in blood or tissue samples. However, DNA fragments from the single- and multi-copy genes could be detected in digesta up to the large intestine. The multi-copy rubisco gene was detected in a proportion of all sample types studied. It is concluded that the detection of rubsico is a function of the abundance of chloroplast DNA in each cell but also that it is not fully degraded during digestion and that it is absorbed.

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Scientific report (maximum 20 sides A4)

The full Scientific Report is given in the CS0119 Project Report attached to this document (total numbered pages is 77).