EFSA statement on the fate of recombinant DNA or proteins in meat, milk and eggs from animals fed with GM feed

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1. Background

Regulation (EC) No 1829/2003 on GM food and feed foresees labelling requirements. Decisions on labelling are taken by risk managers with the aim of offering the consumer an informed choice and are outside the scope of EFSA’s work. With the letter of 15 March 2007 (ref. SANCO/E1/SG/cc(2007)D/510144), the European Commission informed EFSA about a petition to label food products (such as meat, milk and eggs) from animals that have been fed with genetically modified feed. There were no references to a potential safety concern or to a risk assessment of an existing GMO product. In this context, however, the Commission was interested in the potential for transgenes or their products to be incorporated into animal tissues or products such as eggs and milk, in view of the fate of recombinant protein and DNA within the gastrointestinal tract of livestock. EFSA was asked to provide a reply and has therefore prepared the following literature survey.

2. Literature survey on the fate of recombinant DNA of GM feed

To date, no recombinant DNA sequences have been found in any organ or tissue sample from animals fed GM plants (Flachowsky et al., 2007). In considering whether recombinant DNA from GM plants, or the derived proteins, can end up in animal tissues, milk or eggs, several aspects have been investigated. These include (1) the fate of the recombinant DNA and protein during the feed processing and ensilaging; (2) the fate of the recombinant DNA and protein in the gastrointestinal tract of animals fed with the GM feed; (3) the potential absorption of the digested pieces of DNA or protein into animal tissues/products and (4) the potential of biological functionality of absorbed DNA and protein fragments.

2.1 The fate of the recombinant DNA and protein during the feed processing and ensilaging

Before consumption by the animal, the GM plant is processed into feed via different treatments. Studies have shown that mechanical treatments had no influence on the stability of DNA, while the process of extraction and toasting (desolventizing) resulted in highly fragmented DNA (Flachowsky et al., 2007). Forage conservation by ensilaging causes a degradation of DNA to small fragments of about 200 bp (Wiedemann et al., 2006, Lutz et al., 2006, Flachowsky et al., 2007, CAST, 2006).
2.2. The fate of recombinant DNA and protein in the gastrointestinal tract of livestock

In considering the fate of recombinant protein and DNA within the gastrointestinal tract of livestock, several aspects need to be taken into account:

2.2.1. Recombinant DNA and proteins in GM feed are not different from other DNA and proteins in the diet

In principle, all feed (and food) contains considerable amounts of DNA and proteins, being essential nutrient sources for animals (and humans) after digestion. Thus, the gastrointestinal tract of animals (and humans) has always been exposed to foreign DNA, proteins and protein fragments from the diet. The DNA introduced into crops through recombinant DNA technology is not different from other sources of DNA in the diet and is considered equivalent to DNA from existing food organisms that have always been consumed (Jonas et al., 2001, CAST, 2006).

2.2.2. Digestion of DNA and proteins into fragments

DNA and proteins are released from plant material by normal digestion processes that take place in the gastrointestinal tract. Ingested DNA and proteins are rapidly cleaved into small fragments by the mechanical processes of mastication along with buccal and gastrointestinal enzymatic digestions and acid hydrolysis. DNA is digested into fragments and nucleotides; proteins into polypeptides, oligopeptides, and amino acids. A number of recent reviews discuss this process (Beever and Kemp, 2000, Jonas et al., 2001, Lutz et al., 2005, CAST, 2006).

2.3. Survival of recombinant plant DNA in the human gastrointestinal tract

Although this statement is focussed on the fate of recombinant DNA and protein in the gastrointestinal tract of livestock, data are also available on the fate of recombinant plant DNA in the gastrointestinal tract of humans (Netherwood et al., 2004). In this study, human volunteers, of whom twelve were healthy and seven had undergone ileostomies (a resection of the terminal ileum and diversion of digesta via a stoma to a colostomy bag), were given meals containing GM soya containing the epsps recombinant gene. For the seven ileostomists, the amount of recombinant DNA that survived passage through the small bowel varied between individuals, with a maximum of 3.7% recovered at the stoma of one individual. The recombinant DNA did not survive passage through the intact gastrointestinal tract of healthy human subjects fed GM soya. Three out of seven ileostomists showed evidence of low-frequency gene transfer from GM soya to the microflora of the small bowel before
their involvement in these experiments. The authors concluded that gene transfer to
the microflora did not occur during this feeding experiment.

2.4. The potential absorption of the digested fragments of DNA or protein into
animal tissues

Rapid breakdown of DNA and proteins during normal digestion as described in
section 2.2 is expected to minimize the opportunity for absorption of intact DNA or
protein. In 2006, the Council for Agricultural Science and Technology (CAST)
published a paper entitled “Safety of Meat, Milk, and Eggs from Animals Fed Crops
Derived from Modern Biotechnology” (CAST, 2006). This report indicates that no
intact or immunologically reactive fragments of recombinant plant proteins or DNA
have been detected in samples of meat, milk, eggs, lymphocytes, blood, and organ
tissue from production animals fed crops modified for agronomic traits using
recombinant DNA technology. In the following paragraphs an overview is given of
research in the area of absorption of DNA and protein via the gastrointestinal tract:

In rodents:

For proteins, there was an initial case report, where purified and orally administered
ovalbumin (non-recombinant) was detected in plasma and lymph fluid of rats in
minute amounts of 0.007-0.008% of the dose (Tsume et al., 1996). Similarly, for
DNA, it was reported that purified M13 phage DNA (non-recombinant) that was
dosed in pharmacologically high concentrations, was detected in white blood cells of
mice (Schubert et al., 1997).

In farm animals:

Under normal conditions in both ruminants and monogastric farm animals, digested
proteins are mostly absorbed as free amino acids and also as di- and tripeptides. The
results of studies with dairy cattle, growing calves, broiler chickens, and swine have
not detected the presence of recombinant protein in products and tissues from farm
animals fed currently available genetically modified crops (CAST, 2006). Ash et al.
studied the fate of genetically modified protein from Roundup Ready soybeans in
laying hens (Ash et al., 2003), Chowdhury et al. studied the fate of genetically
modified protein from Bt11 in calves (Chowdhury et al., 2003); Jennings et al.
analysed the fate of recombinant DNA and protein from YieldGard corn in broilers
(Jennings et al., 2003) and from Roundup Ready soybean meal in swine (Jennings et
al., 2003); and Yonemochi et al. studied the fate of recombinant DNA and protein
from Starlink corn in diary cows and in broilers (Yonemochi et al., 2002). All of
these authors failed to detect recombinant DNA or proteins that were encoded by
recombinant DNA in the tissues of animals fed on transgenic plant material.

For recombinant DNA further studies have been undertaken to determine whether or
not fragments of recombinant DNA could be detected in animal tissues and food
products such as meat, milk and eggs and these have been reviewed by the CAST
A list of studies is given in the review by Flachowsky et al. (Flachowsky et al., 2007), including details on the source of the DNA, the animal species tested, the results of the detection of recombinant DNA and the results of the detection of non-recombinant DNA. It was concluded that even when highly sensitive PCR and Southern blot methodologies were used, no fragments of recombinant DNA from single-copy transgenes were detected in samples of meat, milk, eggs, skin, duodenal tissue, leukocytes, lymphocytes, blood and organs tissue obtained from animals fed with currently available genetically modified crops. As an exception, very small fragments (of 106 and 146 bases) of the recombinant cry1a(b) and cp4epsps genes (3500 and 1800 bases pairs respectively) were found in conventional milk samples, but these sequences were also found in organic milk samples. The source of the detected DNA is suggested to be a faecal or airborne contamination or directly from the natural environment via the soil bacteria B. thuringiensis and Agrobacterium sp., which are the source organisms of the cry1a(b) and epsps sequences (Agodi et al., 2006).

The CAST review notes that there are reports of fragments from natural multicopy plant genes (e.g. chloroplast genes) found in certain animal tissues and fluids (CAST, 2006).

In view of the data described above it is clear that the uptake of DNA fragments or proteins fragments from the intestinal tract into the body is a normal physiological process for animals.

The fact that no intact recombinant DNA has been detected in meat, milk and eggs may be explained because (1) the recombinant sequence is unlikely to stay intact during digestion and (2) the recombinant sequence is present in the GM plants only as a single or low level copy, which makes potential absorption a rare event and therefore difficult to detect. It is noted that this may change if, for example, plants in which the recombinant DNA is incorporated into chloroplast or mitochondrial DNA are released in the future since organelle DNA is present in multiple copies per cell. However, as stated below, this would not imply a safety concern. Emphasising the critical influence of gene copy number and sensitivity of detection (Alexander et al., 2007), it is also noted that no technique is currently available to enable a valid and reliable tracing of animals products (meat, milk, eggs) when the producer animals have been fed a diet incorporating GM plants.

Flachowsky et al. (2007) also concluded that the results of recent publications agree that most of the DNA in a diet is degraded in the gastrointestinal tract, but that some DNA fragments have been found in animal tissues. These fragments came from “natural” plant DNA fragments and were found in some animal species but not in others. No residues of recombinant DNA or protein were detected in any organ or tissue sample including eggs and milk obtained from animals fed with GM feed.
2.5. The potential of biological functionality of absorbed DNA and protein fragments

After digestion into fragments and potential absorption into animal tissues, the hypothesis that absorbed DNA or protein from animal feed would be functional in animal tissue is highly questionable in view of the small size of the fragments. In addition, regarding their potential functionality after absorption, the following aspects need to be taken into account:

- Foreign DNA or proteins are degraded by endogenous restriction nucleases or proteolytic enzymes respectively, these enzymes being part of natural defence systems that have evolved to destroy foreign DNA and proteins (Jonas et al., 2001, Alexander et al., 2007).
- For recombinant DNA to become functional, a genomic integration would be the pre-requisite for expression. The probability of such a horizontal gene transfer from a plant or bacterial gene into the animal genome can be considered very low because the principal mechanisms for DNA incorporation into a genome is via homologous recombination.
- Furthermore, for incorporated DNA to be functional, the absorbed DNA must be inserted in a transcriptionally active region. Unless it carries its own expression system, incorporated DNA will also need a location in juxtaposition with an appropriate promoter, transcriptional site and ribosomal binding site (Beever and Kemp, 2000).
- Currently, there is no evidence that any plant proteins are expressed in tissues of animals that have consumed plant material. Indeed, no plant gene (or fragment thereof) has ever been detected in the human genome or that of any other animal species (Beever and Kemp, 2000).
- Also in the context of the studies mentioned above, where multicopy plant DNA was found in certain animal tissues, neither chloroplast DNA nor maize genes are present endogenously within the wild-type poultry genome (Klotz et al., 2002).
- It has also been reported that when mice were fed for eight generations with large amounts of a unique recombinant DNA construct, no functional expression was observed nor germline transfer of this orally-administered DNA (Hohlweg and Doerfler, 2001).

Conclusions

(1) Biologically active genes and proteins are common constituents of foods and feed in varying amounts. After ingestion, a rapid degradation into short DNA or peptide fragments is observed in the gastrointestinal tract of animals and humans.

(2) To date, a large number of experimental studies with livestock have shown that recombinant DNA fragments or proteins derived from GM plants have not been
detected in tissues, fluids or edible products of farm animals like broilers, cattle, pigs or quails.

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References


