Carry-Over of DNA-Fragments from Maize to Laying Hens and Broilers

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Background

With the introduction of genetically modified plants (GMP) onto the European market a delicate public discussion has arisen concerning safety and advantages of therefrom produced food. The novel food regulation of the EU (EG 258/97) arranges the special requirements for GMP-derived food. Within the last years several approaches were published detecting recombinant plant DNA in food for man e.g. produced from Bt-maize [1, 2]. Simultaneously, such genetically modified plants like maize and soybeans will be used for feeding of farm animals. A possible nucleic acid transfer from these plants into the human organism can be assumed, but the significance of this process is still unknown. Farm animals ingest up to grams of nucleic acids via feed day per day. From recent reports it seems possible that such foreign DNA-fragments could be integrated into cells of the immunesystem of rodents [3, 4] and were detected for several hours within different other organs. This makes it necessary to think about the fate of plant DNA in farm animals especially concerning recombinant feed plants.

Objectives

The aim of this study was to evaluate the possible transfer of plant DNA into laying hens and broilers with second special emphasis to detect recombinant Bt-maize material in secondary animal products like meat or eggs. Therefore a quick and sensible PCR assay was introduced to test for the presence of residual universal plant and Bt-maize genes in animal samples.

Methods

Animals and diets: Twelve male broilers (Lohmann Meat) and twelve female laying hens (LSL, Lohmann White) were caged und standard conditions and fed during the experimental period as indicated [5, 6] (broiler: 50 % maize conventional Cesar hybrid or B Cesar (Novartis), 5 % wheat, 39 % soybean-extract, 2.8 % soybean oil, 3.2 % premix; hens: 50 % maize conventional Cesar hybrid Bt-Cesar, 18.7 % wheat, 18.7 % soybean-extract, 12.6 % mineral-vitamin-aminoacid-premix). The daily feed uptake was 115 g. the end of the period all chicken were slaughtered and several tissues (liver, spleen, muscle) were carefully prepared for DNA analysis and stored at –20 °C.

DNA extraction and gene-specific PCR: Tissue samples (100-300 mg wet weight) from chicken were extracted for total DNA usi commercial kits (Macherey & Nagel, Düren, Germany; Qiagen, Hilden Germany) and quantitated by UV-spectroscopy. Differe PCR amplifications for a ubiquitous plant chloroplast gene (plant1 and 2) and the specific gene sequence to detect Bt-maize (CryI were performed:

Chloroplast-specific primers: plant 1 fd: 5'-CGA AAT CGG TAG ACG CTA CG-3'; plant 1 rev: 5'-GGG GAT AGA GGG AC TGA AC-3'; product: 532 bp for maize; plant 2 fd: 5'-GGA AGC TGT TCT AAC GAA TCG-3'; plant 2 rev: 5'-CTC GAA AA AAT GAA TTG AAG G-3'; product: 199 bp for maize and other plants. *Bt-maize-specific primer pair, according to* [1]: CryIA3 5'-CCG CAC CCT GAG CAG CAC-3'; CryIA4 rev: 5'-GGT GGC ACG TTG TTG TTC TGA-3'; product: 189 bp. All result PCR products were separated on 10 % PAGE containing ethidium-bromide documented under UV-illumination or isolated a sequenced commercially (MWG-Biotech, Ebersberg, Germany or Qiagen, Hilden, Germany).

Results and Discussion

In the following investigations only the small plant chloroplast primer-pair (plant 199 bp) was used to screen for plant DNA chicken tissues. Additionally, a Bt-maize-specific primer pair (CryIA) was selected that amplifies a comparable small PCR produ (189 bp) under similar cycle conditions.

Searching for plant- and Bt-maize-specific gene fragments in chicken samples: In all investigated broiler tissues plant DNA fragments were successful amplified: leg and breast muscles, liver, spleen and kidney were remarkable highly positive for the plant amplicon. In contrast, no Bt-maize-specific gene fragment was ever detected in these tissues. Observing the hen samples similar pictures appeared: all organs (muscle, liver, spleen, kidney) proofed positive for plant DNA fragments. Similar as in the previous experiment, the Bt-maize-specific gene construct (CryIA) was never found in any of the hen samples.

Two main reasons for the high incidence of detecting plant DNA fragments in all chicken organs may be 1.) the application of unprocessed whole corn. 2.) the speciality of the gastrointestinal tract of chicken with a very short digestion path.

In summary this study leads to the assumption that forage-plant DNA fragments are detectable in animals if the amount of starting material is high enough (e.g. chloroplast-DNA) and the degradation stage of the DNA at the place of uptake (intestine) is not too high (DNA fragmentation by silage and increased gastrointestinal storage time).

In chicken organs the detection of chloroplast plant DNA was always successfully performed, but not for eggs. Relatively low levels of starting DNA material, as it will be the case for the single Bt-maize-gene construct within the huge maize genome (CryIA; approx. < 50 000x lower than the corresponding plant chloroplast gene fragment), make it not possible to detect specific CryIA fragments in the animal samples investigated in this study.

Conclusions

The evolution has confronted animals since the beginning with huge amounts of foreign DNA e.g. uptake through the gastrointestinal tract (mg-g/per day) and such an uptake of high amounts of foreign gene material must be accepted as a normal event as well during development of man [7]. Depending on the animal species and kind of food a more or less significant transfer of foreign food DNA can be presumed. Different hypotheses have been raised how foreign DNA can reach the mammalian organism through gastrointestinal or placental portals and which consequences can be considered [7]. It is of major interest that the fate of foreign DNA is not only degradation, but a chromosomal integration and placental transfer would be as well possible as published recently [8, 9]. To our knowledge only rodents with a monogastric digestion system have been investigated. In our study not a single pulse of foreign DNA was applied, but a continuous administration of Bt-maize material over a longer period was realised.

The chance of the DNA detection is limited by the mechanisms within the gastrointestinal tract and the turnover rate of DNA within the organisms. Therefore we decided to choose very short PCR-amplicons to avoid poor sensitivity (both plant and Bt-maize primer pairs amplified a 199 bp and 189 bp fragment, respectively). Only such short amplicons increased the chance of detecting highly degraded and diluted DNA in complex samples.

Possible problems of contamination during sample collection could be excluded during our sample collection due to separated areas. Additionally to minimise false positive signals we did not increase the PCR cycle number nor used recycling. We would like to emphasize that our results are the very first attempt to diagnose DNA-uptake in farm animals. Quantitative results cannot be expected from this approach and will be very laborious to realize.

References

[1] Studer E, Dahinden I, Lüthy J, Hübner P (1997) Mitt Gebiete Lebensm Hyg 88:515-524

[2] Hupfer C, Hotzel H, Sachse K, Engel KH (1998) Z Lebensm Unters Forsch A 206:203-207

[3] Schubbert R, Lettmann C, Doerfler W (1994) Mol Gen Genet 242:495-504

[4] Schubbert R, Renz D, Schmitz B, Doerfler W (1997 Proc Natl Acad Sci USA 94:961-966

[5] Aulrich K, Halle I, Flachowsky G (1998) VDLUFA Kongreßband 1998, VDLUFA-Verlag, Darmstadt 465-468

[6] Halle L, Aulrich K, Flachowsky G (1999) Proc. 5. Tagung Schweine- und Geflügelernährung, Fachverlag Dr. Fleck, Niederkleen 265-267

[7] Doerffler W (2000) Foreign DNA in Mammalian Systems. Wiley-VCH.

[8] Tsujamoto M, Ochiya T, Yoshida S, Sugimura T, Terada M (1995) Nature Genetics 9:243-248

[9] Schubbert R, Hohlweg U, Renz D, Doerfler W (1998) Mol Gen Genet 259:569-576