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Safety Considerations Regarding Use of *Bt*176 Maize as Broiler Feed

K. Aeschbacher, R. Messikommer, L. Meile[1] and C. Wenk

Institute of Animal Sciences
Nutrition Biology
ETH Zurich, Switzerland

ABSTRACT. *The use of genetically modified (GM) feed for animal production is expected to increase in the future. But acceptance of such feeds is being debated in the society. The question whether the process of genetic modification could have unintended effects on feed quality or nutrient composition of the grain is of great practical relevance. In addition, recent studies showed that native plant-DNA-fragments of different size could be associated with animal tissues. It was therefore of interest to study the way of inactivation of nucleic acids from feedstuffs in the digestive tract of chicken. For this purpose, feeding experiments with Bt176 maize and its isogenic control were performed. Bt176 maize hybrids express a gene that enables the plants to produce an insecticidal protein, which is similar to that produced in nature by Bacillus thuringiensis. Two kinds of feed with a portion of either 60% conventional or 60% Bt176 maize were fed to broilers. Neither the nutrient composition of the maize grain and feed, nor feed intake, bodyweight or feed conversion ratio showed significant differences between the two treatments. The performance did not differ between the two groups of broilers. The maize-specific gene ivr (226 bp) could be detected in maize, feed and in digesta samples as far as the small intestine. The Bt-specific gene bla (479 bp) could only be detected as far as the crop. From this study it can be concluded that the two maize types can be considered as "substantially" equivalent as originally defined. The transgenic maize and its isogenic control maize had no detectable differences in effect on the performance and the metabolism of the chicken in this study.*

INTRODUCTION

In recent years, agricultural biotechnology has produced several new varieties of crop plants with enhanced features such as protection against pests, diseases and herbicides or improved quality. Therefore, the use of genetically modified (GM) feed for animal production is expected to increase in the future. To date, 46% of the soya-products on the world-market, and 7% of the maize-products are transgenic (www.transgen.ch). In the year 2001 the area under crops for genetically modified plants increased from at least 19% to 52.6 million ha (www.transgen.ch). Therefore, more and more such products are becoming available for livestock feeding. However, genetically modified plants in the society is a controversy. The concerns regard the substantial equality of the plants, the risk of allergens or of antibiotic resistance transfers, the threat for the biodiversity and for non target organisms.

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[1] Institute of Food Science and Nutrition, Laboratory of Food Microbiology, ETH Zurich, Switzerland.

DNA of all living organisms, including that from GMO's, is composed of the same four nucleotides. Genetic modification results in the re-assortment of sequences of nucleotides, but the chemical structures stay unchanged. Therefore, DNA from GMO's is chemically equivalent to any other DNA. The only difference concerns the DNA sequence. The human being consumes daily between 0.1 and 1 g of DNA from GMO's (Doerfler, 2000). This includes more or less degraded fragments of genes of plants, animals and microorganisms. Humans and animals have been confronted with foreign DNA for millions of years. There is no specific risk of genetic engineering as genetic modification will not increase the dietary intake of DNA.

So far no relevant differences in performance or nutrient utilization of farm animals have been observed when genetically modified feedstuffs (plants with input traits: 1st generation) were compared with the isogenic original crops (OECD, 1993). However, when subject to strict scientific-analytical criteria minor differences can be detected, if foreign genes are integrated (Clark and Ipharraguerre, 2001; Flachowsky and Aulrich, 2001). Recent studies have shown that native plant-DNA-fragments of different length could be associated with animal organs and tissues like spleen and muscles in cattle, pigs and poultry (Einspanier *et al.*, 2001; Reuter *et al.*, 2002). It has been therefore of interest, to study the way of inactivation of nucleic acids in the digestive tract of chicken.

A new type of maize was developed for the control of the corn borer; in particular the European corn borer. This is a major lepidopteran pest in the USA, Canada and Europe. The annual cost of European corn borer damage is very high (Mason *et al.*, 1996). Maize hybrids derived from Event 176 express a gene that enables the plants to produce an insecticidal protein, the *Cry1Ab* protein. It is similar to that produced in nature by certain subspecies of the common soil bacterium *Bacillus thuringiensis* (*Bt*). Upon ingestion, the *Cry1Ab* protein is selectively toxic to the larvae of the European corn borer and certain other lepidopteran pests, whereas other species are unaffected (Brake and Vlachos, 1998). This selectivity is due to the presence of receptor-like sites, with affinity for the *Cry1Ab* protein, in midgut membranes of the larvae. After ingestion of the protein, the midgut membrane is disrupted and the larvae desist from feeding and die. The commercial insecticides (*Bt*-microbial preparations) are safe and lack toxicity to nontarget organisms like mammals, birds or beneficial insects (U.S. Environmental Protection Agency, 1986). *Bt*176 maize produces the protein mainly in green plant tissues and pollen. Other plant tissues produce only trace quantities of the insecticidal protein. The *Bt*176 maize was genetically modified by the introduction of three genes. Firstly, the *Bt* toxin gene *Cry1A(b)* (from *Bacillus thuringiensis*), secondly the ampicillin resistance gene *bla* (β-Lactamase) and thirdly the herbicide resistance gene *bar* (from *Streptomyces hygroscopicus*). The latter two genes serve as markers during the development of the genetically modified plant. The *bla* gene is necessary for selection in bacterial transformations and the *bar* gene allows to distinguish between the transformed and the non-transformed plants.

That the process of genetic modification introduces no detectable, unintended effects on food or feed quality or nutrient composition of the grain had to be demonstrated prior to commercialization. Hybrids were shown to be equivalent to their conventional counterparts in total fat, protein, fibre, carbohydrate, amino acid composition, fatty acid composition, carotenoid content, selected minerals and vitamins. Feed trials conducted with various animal species confirm the equivalence of current commercially available

GM-feedstuffs, including *Bt176* maize, to their conventional counterparts (for review, Clark and Ipharraguerre, 2001; Flachowsky and Aulrich, 2001; Reuter *et al.*, 2002).

Therefore the objectives of this study were to identify the nutrient composition of the two maize varieties and of the feedstuff; potential effects of ingestion of *Bt176* maize on broiler performance and meat composition; and the degradation of the nucleic acids in the digestive tract.

METHODS

Diets and broiler feeding experiments

Two isogenic varieties of maize (conventional and transgenic *Bt176*) were grown, harvested and stored under the same environmental conditions (at Syngenta facilities, Germany). Precautions were taken to ensure that no mixing of the two grains occured. Two kinds of broiler feed including 60% of either conventional maize or transgenic Event 176 - derived "*Bt*" - maize were prepared. The rest of the diet was the same for both treatments and contained 30% soya groats, 3.5% soya oil, 2.2% potato protein, minerals, vitamins and trace elements. The nutrient composition conformed to the standard requirement. The temperature was kept below 60°C during pelleting of the feeds.

Ninety four male broiler chicken (ROSS 208) were fed for 39 days with the experimental diet prepared either with transgenic maize (treatment) or with nontransgenic isogenic maize (control). The birds were provided continuous access to feed and water and received the experimental diet from the first day onwards. Access to feed ceased approximately 18 h before slaughter. The animals were kept in 12 cages, 7–8 animals per cage (0.92×0.54×0.83 m). They were randomly distributed into the cages at one day of age. Average stable temperature was 22°C and relative humidity was about 42%.

Nutrient analysis and performance recording

Samples from the two maize varieties and the two experimental feeds were gathered for nutrient analysis. Contents of dry matter, total ash, neutral detergent fibre (NDF) and acid detergent fiber (ADF) in maize and feedstuffs were determined according to standard methods (Naumann and Bassler, 1997). Nitrogen content was determined with a C/N analyser by the Dumas method. Crude protein was calculated as 6.25 × N. The gross energy content was assessed through an anisothermic calorimeter. Fat content in muscle samples was determined gravimetrically. The performance of the broilers was measured weekly. In order to compare the performance of the birds bodyweight, daily weight gain, feed conversion ratio (at 14, 28 and 39 days), feed intake, slaughter weight and water consumption were measured. Total cage weight data as well as feed intake data were collected weekly. Water consumption data were recorded daily. The right breast muscle of 82 birds was desected, freeze dried and ground for nutrient composition analysis. The results of the feeding studies were evaluated by t-test analysis using the statistical program Statgraphics plus for Windows.

Detection of maize DNA in the digestive trat of broilers by PCR

To follow up the degradation of the nucleic acids in the digestive tract, two animals of each treatment were slaughtered after 14, 28 and 38 days. Samples from different parts of the digestive tract (crop, gizzard, small and large intestine and excreta) were collected and stored at –20°C. DNA analysis was then carried out by polymerase chain reaction (PCR), according to the method described by Ehlers *et al.* (1997) to search for the maize-specific *ivr* gene (226 bp) (Ehlers *et al.*, 1997), a poultry-specific fragment (227 bp) (Matsunaga *et al.*, 1999), as well as for the *Bt176*-specific foreign gene *bla* (479 bp). With the inclusion of control amplifications, the purity of the DNA extraction could be verified and the existence of inhibitors in the PCR solution could be excluded. For the detection of the transgenic *bla* gene, two primers were constructed: one binding within the *bla* gene and (CGCCCTTTGACGTTGGAGTCCAC), the other with the vector-specific sequence in front of the *bla* gene (CTGTTGAGATCCAGTTCGATGTA). These two primers were designed after sequencing the corresponding *Bt176* maize DNA in the Food Microbiology Laboratory of ETHZ, Switzerland.

RESULTS AND DISCUSSION

Nutrient content of feed stuffs

The nutrient content of the maize varieties is shown in Table 1. The statistical analysis of the nutrient composition showed that the transgenic maize kernels did not significantly differ ($P>0.05$) from the ones of the conventional maize kernels in any of the nutrients analysed. The observed minor differences may have been related to differences in grain density or moisture content, which are known to occur across hybrids.

Table 1. Nutrient content of *Bt176* maize kernels and non-modified isogenic maize (control) kernels.

		Control maize	*Bt176* maize
DM	[g kg^{-1}]	875	876
in the DM:			
OM	[g kg^{-1}]	979	985
CP	[g kg^{-1}]	114	115
NDF	[g kg^{-1}]	163	164
ADF	[g kg^{-1}]	34	36
GE	[MJ kg^{-1}]	18.7	18.9

DM - dry matter; OM - organic matter; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; GE - gross energy

Performance of broilers

There were no statistically significant differences at any time during the experiment between the broilers that received the transgenic maize diet and those that received the nontransgenic maize diet with respect to any of the growth parameters measured (Table 2). Thus no deleterious effect on performance of broilers could be detected with the feed containing transgenic maize.

Table 2. Effect of maize diet on performance parameters of broilers.

		Control maize		*Bt176* maize		Total number of broilers
		$\bar{x}$	sd	$\bar{x}$	sd	
BW d14	[g]	425.7	14.0	425.3	15.5	94
BW d28	[g]	1368.8	76.0	1407.6	55.9	90
BW d39	[g]	2196.3	124.8	2202.9	134.2	86
Weight gain d 1-39	[g animal^{-1} day^{-1}]	55.2	2.5	55.3	4.2	-
FCR d14	[g/g]	1.329	0.06	1.341	0.05	94
FCR d28	[g/g]	1.608	0.11	1.599	0.066	90
FCR d39	[g/g]	3.254	0.872	3.226	0.823	86
Feed intake d 1-39	[g animal^{-1} day^{-1}]	90.4	4.1	91.1	4.9	-
Slaughter weight	[g animal^{-1}]	1581	136.5	1571	142.5	82
Water consumption	[ml g^{-1} feed]	1.8	0.13	1.83	0.14	-

$\bar{x}$ - mean; sd - standard deviation; BW - body weight; FCR - feed conversion ratio; d - day

Table 3 shows the nutrient composition of the broiler meat under the two diets. There was no significant difference between the two diets with respect to average percentage dry matter, crude protein or fat of broiler meat ($P>0.05$).

Table 3. Nutrient analysis of the muscle samples of broilers fed *Bt*176- and isogenic maize.

		Control group	*Bt*176 group
DM	[g/100 g]	90.7	91.2
CP	[g/100 g]	23.5	23.5
Fat	[g/100 g]	2.91	2.46

DM - dry matter; CP - crude protein

PCR analysis of maize and poultry specific DNA in feed and broilers

Table 4 shows the results of the PCR analysis of maize- *Bt176* - and poultry - specific DNA fragments in the experimental diets and digests samples of broilers.

Table 4. DNA-fragments in the feed, digesta and excreta samples (Control/*Bt*176).

Primer	Maize-specific (226 bp)	*Bt*176-specific (479 bp)	Poultry-specific (227 bp)
Maize	++/++	--/++	--/--
Feed	++/++	--/++	--/--
Crop	++/++	--/(+)	++/++
Gizzard	++/++	--/--	++/++
Small intestine	(+)/--	--/--	++/++
Large intestine	--/--	--/--	++/++
Excreta	--/--	--/--	++/++

-- : no signal; (+) : slight positive signal; ++ : positive signal

A maize-specific, a poultry-specific and a *Bt176* maize-specific primer-pair were used in the PCR analysis. Initial tests with both the conventional and the transgenic maize samples served as control for detecting *Bt* maize or maize in general by the invertase gene *ivr*. All maize and feed DNA extracts resulted in a strong positive signal for the maize-specific fragment. In addition, PCR reactions of those maize and feed extract samples with poultry-specific primers never generated any product. The PCR for the *Bt176* maize-specific fragment showed only strong positive signals for the genetically modified maize and feed samples. Thus a cross contamination of the conventional maize could be excluded. With all DNA extracts from the broiler samples poultry-specific fragments were detected. This indicates that there were no inhibitors and PCR could be performed properly. Additional negative and positive controls verified that each PCR reaction was functioning properly.

The maize-specific gene *ivr* (226 bp) could be detected in maize, feed and in digesta samples only as far as the small intestine (only weak signals). The *Bt176* maize-specific gene *bla* (479 bp) could only be detected as far as the crop. The poultry-specific fragment with 227 bp was detected in all digesta samples. It is known that ingested nucleic acids are normally contained within cells and become available after cell lysis. They are extensively broken down in the gastrointestinal tract and the nucleotides are absorbed. Some DNA fragments, though very unlikely to be complete genes, escape breakdown. DNA fragments may be taken up by cells of the intestinal wall, including cells of the immune system. DNA fragments, after passing through the intestinal wall, may be actively removed by cells of the gut immune system. DNA from GMO's is equivalent and chemically identical to DNA from existing food organisms that has always been consumed

with diets. The body handles all DNA the same way, and the breakdown of DNA during food or feed processing (*e.g.*, high temperature or pressure) and passage through the gastrointestinal tract reduces the likelihood that intact genes will survive. There are no scientific indications that genetic modification *per se* has an impact on the digestibility or stability of the nucleic acids. Furthermore, there is no indication that DNA from *Bt176* has allergenic or other immunological properties that would be of relevance for consumption of foods or feeds derived from GMO's.

A few copies of the *bla* gene are present in the genome of *Bt176* maize, whereas the *ivr* chloroplast gene is present at a much higher frequency. This might be an explanation for the positive detection of the *ivr* gene in contrast to the detection of the *bla* gene. Another reason is the sensitivity of the PCR method. It is clear, that as the sensitivity of the detection methods improves, the PCR products from recombinant DNA will be more likely to be detected in meat and other animal products.

Besides the benefits to the farmers as increased yield, lower insecticides usage and better plant health, the transgenic maize may have a benefit to the animal's health, because mycotoxin contaminations are reduced. There is some evidence that the content of some mycotoxins can be significantly reduced in kernels of *Bt* maize compared to kernels from conventional maize if they are grown under unfavourable climatic conditions (Brake and Vlachos, 1998; Munkwold *et al.*, 1999; Valenta *et al.*, 2001; Maag *et al.*, 2002). This could be an important argument for the use of GMO. The results of this investigations correspond well to those described by other authors who carried out poultry feeding studies using *Bt176* maize (Brake and Vlachos, 1998; Aulrich *et al.*, 1999; Halle *et al.*, 1999; Aulrich *et al.*, 2001; Klotz *et al.*, 2002) or other *Bt* maize (Mireles *et al.*, 2000).

CONCLUSIONS

The study shows that the two different types of maize in the feed (*Bt*176 and conventional maize) leads to similar performance (bodyweight, weight gain, feed intake, feed conversion ratio) of the broilers. It had been demonstrated that broilers receiving diets prepared with transgenic maize *Bt176* performed as well as those feeding diets prepared with the isogenic conventional maize. Therefore, the two maize types can be considered as physiologically and substantially equivalent. The digestion process of nucleic acids is efficient in chicken. Feed DNA, be it recDNA or other DNA, is fragmented by nucleases in the gastrointestinal tract. The fragments of the *ivr* gene and the *Bt176*-specific gene could only be detected as far as the crop (*Bt176*) or the small intestines. Afterwards the signal disappeared.

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