Effect of Bt Corn on Broiler Growth Performance and Fate of Feed-Derived DNA in the Digestive Tract

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ABSTRACT The aim of the study was to evaluate the effect on broiler performance of transgenic *Bacillus thuringiensis* (Bt) corn containing the Cry1A(b) protein compared with the corresponding near isogenic corn and to analyze the degradation of the *Cry*1A(b) gene in the digestive tract. Ross male broilers (432) were fed for 42 consecutive days with diets containing Bt or isogenic corn. Diet, Bt corn, and the isogenic form of the Bt corn were analyzed for composition and aflatoxin B_1, fumonisin B_1, and deoxynivalenol contents. Broiler body weight and feed intake were recorded at regular intervals (d 0, 21, and 42). The presence of the *Cry*1A(b) gene and plant-specific genes *Zein* and *Sh*-2 in gut contents of crop, gizzard, jejunum, cecum, and samples of blood was determined in 10 animals per treatment at the end of the trial using a PCR technique. Chemical composition was not different between Bt and its isogenic form, whereas the fumonisin B_1 content for Bt was lower than for isogenic corn (2,039 vs. 1,1034 ppb; $P < 0.05$). The results of the growth study showed no difference for average daily weight gain (129.4 vs. 126.0 g/d), feed intake (63.4 vs. 61.8 g/d), and feed conversion ratio (1.95 vs. 2.02) among the groups. No significant relationship was observed between mycotoxins content and growth performances.

Feed-derived DNA is progressively degraded along the digestive tract. Detection frequency of short fragments of maize-specific high copy number *Zein* gene was high but significantly decreased in distal sectors. An 1,800-bp fragment of the *Cry*1A(b) gene, corresponding to the minimal functional unit, was detected only in crop and gizzard of birds fed Bt corn. *Sh*-2 showed the same detection frequency of *Cry*1A(b) and was also found in birds fed isogenic corn. Blood samples were positive with low frequency only for the *Zein* gene fragment. No significant difference in DNA detection was observed between birds fed Bt and isogenic corn, indicating that DNA derived from transgenic feed undergoes the same fate as isogenic feed.

(*Key words*: broiler performance, Bt corn, DNA traceability, mycotoxin)

INTRODUCTION

Under European environmental conditions, the European corn borer (*Ostrinia nubilalis*) is the primary corn pest (Mason et al., 1996). It is often the cause of major production losses, but its control, through the use of conventional or microbial pesticides, is difficult because treatment should be performed when the young larvae are still present on the plant surface. Indeed, once they penetrate inside the plant, the treatment is no longer effective. Production losses due to this parasite may reach up to 30% (Bohn et al., 1999); furthermore, the affected plants are highly susceptible to secondary infections caused by pathogens, such as the mold *Fusarium spp.* or *Ustilago*

maydis. In addition, growth of mold of the *Fusarium* genus may lead to the production of fumonisin, a mycotoxin linked to lethal leukoencephalopathies in horses, pulmonary edema in pigs, decreased performance in birds, and carcinogenicity in man (Marasas, 1995).

Genetically modified (GM) corn, providing resistance against *Ostrinia nubilalis*, has been introduced on the market (Rice and Plicher, 1998; Graeber et al., 1999; Magg et al., 2001). This corn contains genes derived from the common soil bacterium *Bacillus thuringiensis* coding for a crystalline protein [Cry1A(b)] with specific activity against certain Lepidopteran corn pests, including *Ostrinia nubilalis* and *Sesamia nonagrioides*. The insecticidal action of the Cry1A(b) protein is due to the presence of a special binding site in the epithelial membrane of the digestive tract of susceptible insects. After uptake of

Abbreviation Key: ADG = average daily gain; EE = ether extract; FCR = feed conversion ratio; GM = genetically modified.

Cry1A(b), the gut membrane is damaged with severe physiological alterations, especially in the main transport mechanisms. In other organisms, endotoxins produced by *Bacillus thuringiensis* are normally digested like all other proteins and, therefore, do not induce damage. Furthermore, Fearing et al. (1997) showed that the presence of endotoxins is no longer detectable after 4 mo of ensilaging, most likely because of the degradation processes that are involved in fermentation. In trials on dairy cows that were fed fresh transgenic corn, Faust and Miller (1997) found no Cry1A(b) protein in milk.

Despite the potential benefits offered by biotechnology to improve food quality, public and scientific concerns have been raised about the environmental and food safety of GM crops (Conner et al., 2003). One of the major concerns in the use of GM organisms in human and animal nutrition is the unknown effect that newly introduced sequences may have on the organism (Beever and Kemp, 2000). Dietary exposure to foreign DNA occurs everyday in the organism. The ingested DNA, as part of the digested feed and food components, is broken down in the gut into small fragments by the mechanical processes of mastication, acid hydrolysis, and gastrointestinal enzymatic activity. Schubbert et al. (1994, 1997) showed that, upon oral administration of naked bacteriophage M13 and vector DNA to mice, DNA persists in fragmented form in different sectors of the gastrointestinal tract and may be found in cells of the intestinal wall, peripheral white blood cells, spleen, and liver. Fragments of up to 1,600 bp of the rubisco (ribulose-1,5-biphosphate carboxylase) gene have been detected in the intestinal digesta of mice fed soybean leaves (Hohlweg and Doerfler, 2001). Other studies with farm animals fed conventional and transgenic meals showed that only 200 bp from a chloroplast gene could be detected in blood cells and milk but not in organs of cows.

Similarly, nontransgene plant genomic DNA was found in organs of broilers, whereas sequences of the transgene were not identified (Einspanier et al., 2001). Further experiments showed that recombinant DNA could be detected in gut contents of pigs fed Bt maize (Reuter and Aulrich, 2002). Duggan et al. (2003) reported that a fragment of 1,914 bp from the *Cry*1A(b) gene could be detected in ruminal fluid of sheep fed maize grains. Only a shorter fragment of the transgene (211 bp) could be recovered from the rumen of sheep fed with a silage preparation of the same maize, indicating that long DNA fragments are unlikely to survive in silos.

The aim of our study was to evaluate the effect on broiler performance of transgenic *Bacillus thuringiensis* (Bt) corn (event MON 810) containing the Cry1A(b) protein compared with the corresponding near isogenic corn (isogenic) and to analyze degradation of the *Cry*1A(b) gene in the digestive tract.

MATERIAL AND METHODS

Bt Corn

The Bt corn used in this study was grown from a homogeneous mixture of 3 transgenic Bt corn hybrids produced by Dekalb Genetics, imported to Italy by Dekalb Italy S.p.A. under the Italian Ministry of Agriculture policy authorization (no. 35791 of December 4, 1998). The Dekalb hybrids, identified as T 512 EZ, T 591 EZ, and T 653 EZ, contained the Cry1A(b) protein (event MON 810). The Bt and nontransgenic near isogenic corn (isogenic) were grown by Monsanto Italy (Milan, Italy) in the Po Valley of northern Italy on farms located in the provinces of Lodi (farm A), Cremona (farm B), and Venezia (farm C). The isogenic corn was a conventional hybrid corn derived from the same inbred parents as the transgenic line. The environmental conditions of growth and processing in field were the same for the 2 types of corn. Isolated harvesting and storage were performed using standard procedures and conditions. The analyzed characteristics of Bt and isogenic corn are given in Table 1.

Experimental Design

The study was carried out in the facilities of the CERZOO Research Centre. A total of 432 male, 1-d-old, Ross broiler chicks coming from the same parent stocks, vaccinated at hatch, was randomly distributed into 6 homogeneous groups of 72 birds each. Each group was divided into 4 replicates of 18 birds and kept together in one pen. The pens were assigned in a complete randomized block design using the randomized procedure of SAS Institute (1999). The experimental design consisted of diets made from 6 types of corn (Bt corn produced on farms A, B, and C and its isogenic form produced on the same farms).

Diets

Birds were provided continuous access to feed and water from one feeder and automatic drop drinker for each pen. In the first 10 d of age, a supplemental bell drinker was used in each pen to ensure unlimited access to water. The isonitrogenous and isoenergetic crumbled diets were formulated according Ross Breeder's Netherlands (1989) requirements and produced in the CERZOO facility. Birds were fed starter diets for 21 d, followed by grower-finisher diets for the remaining 21 d. The composition and analyzed characteristics of the diets are shown in Tables 2 and 3.

Samples (500 g) of Bt and isogenic corn grown on the 3 farms and the experimental diets were sampled before the beginning of the trial, during the experimental periods, and at the start of the first (1 to 21 d) and second (22 to 42 d) growing phases. The samples were analyzed for DM, CP, ether extract (EE), crude fiber, ash, sugars, starch, and mycotoxin contents. The analyses were carried out in compliance with the Ministry of Agriculture and Forestry (1994) and AOAC (1993) methods. The mycotoxins were determined according to the Commission of the European Communities (1992) method for aflatoxin B_1, the method of Shephard et al. (1996) for fumonisin B_1, and the method of AOAC (1990, 1993) for deoxynivalenol. Mycotoxins were also evaluated in the refused feed at

TABLE 1. Proximate analysis (on DM) and mycotoxins content of transgenic (Bt) and isogenic (Iso) corn

	Farm A (Lodi)		Farm B (Cremona)		Farm C (Venezia)	
	Iso corn	Bt corn	Iso corn	Bt corn	Iso corn	Bt corn
Chemical composition (% of dry matter)						
DM, %	87.40	86.87	87.87	87.64	89.22	88.03
CP, %	8.65	9.17	9.23	10.52	9.18	8.24
Ether extract, %	4.21	4.75	3.97	5.05	4.52	4.02
Crude fiber, %	3.42	2.47	3.33	3.79	2.52	3.42
Ash, %	1.48	1.50	1.57	1.73	1.65	1.30
Nitrogen-free extract, %	82.24	82.11	81.90	78.91	82.13	83.02
Mycotoxin content						
Deoxynivalenol, ppb	151	195	245	229	196	170
Fumonisin B_1, ppb	7,499	760	16,113	2,463	9,489	4,395
Aflatoxin B_1, ppb	ND[1]	ND	ND	ND	0.54	ND

[1]Not detectable.

the end of the study; samples were taken directly from the feeders.

Housing

Birds were housed on wood shavings in 2 similar rooms of the same facility with controlled climate. Each room was 8×6 m with 12 pens of approximately 2 m^2 each; the broiler density was 0.10 m^2/bird. Environment (temperature, relative humidity, and air exchange) was automatically controlled; the temperature and relative humidity data were recorded every 30 min during the study by

software. The bird received 10 h of light during all of the experimental period; infrared light as heat source was provided during the first 10 d of age. Experiments were conducted in accordance with the principles of the Italian law on the care of laboratory animals (Presidenza della Repubblica, 1992).

Measurements

Performance. Birds were individually weighed in a fed state at 1, 21, and 42 d of age. At the same 3 times, feed intake per pen for calculating the feed conversion

TABLE 2. Composition (%), proximate analysis (as fed) and mycotoxin content (on DM) of the diets employed in the first growing period (from 1 to 21 d)

	Farm A (Lodi)[1]		Farm B (Cremona)		Farm C (Venezia)	
Ingredient	Iso corn	Bt Corn	Iso corn	Bt Corn	Iso corn	Bt Corn
Corn, %	50.20	50.70	50.70	52.20	51.20	48.70
Soybean meal (48%), %	40.00	39.50	39.50	38.00	39.00	40.80
Corn oil, %	5.00	5.00	5.00	5.00	5.00	5.70
DL-Methionine, %	0.30	0.30	0.30	0.30	0.30	0.30
Limestone, %	1.70	1.70	1.70	1.70	1.70	1.70
Monocalcium phosphate, %	1.80	1.80	1.80	1.80	1.80	1.80
Sodium chloride, %	0.20	0.20	0.20	0.20	0.20	0.20
Sodium bicarbonate, %	0.30	0.30	0.30	0.30	0.30	0.30
Premix,[2] %	0.50	0.50	0.50	0.50	0.50	0.50
Dry matter, %	88.22	89.10	89.34	89.11	89.38	88.94
Crude protein, %	24.81	23.72	24.70	25.91	25.48	24.77
Ether extract, %	6.83	7.32	6.88	6.78	6.66	7.05
Crude fiber, %	3.37	3.66	3.34	3.23	3.06	3.37
Ash, %	6.55	6.92	7.12	6.01	6.63	7.32
Nitrogen-free extract	46.66	47.48	47.3	47.18	47.55	46.43
Starch, %	40.9	43.06	41.65	41.67	42.27	41.4
Sugars (as sucrose), %	5.76	4.42	5.65	5.51	5.28	5.03
ME,[3] kcal/kg	3,288	3,332	3,314	3,347	3,338	3,302
Mycotoxin content						
Deoxynivalenol, ppb	50	64	103	163	167	210
Fumonisin B_1, ppb	2,520	464	8,229	906	5,790	1470
Aflatoxin B_1, ppb	ND[4]	0.40	0.53	0.40	ND	0.25

[1]Iso = isogenic; Bt = transgenic.

[2]Content per kilogram of mixed feed: vitamin A, 20,000 IU; vitamin D_3, 4,000 IU; vitamin E, 20 IU; vitamin K_3, 2 mg; vitamin B_1, 0.80 mg; vitamin B_2, 6.40 mg; pantotenic acid, 8 mg; niacin, 40 mg; folic acid, 0.40; vitamin B_{12}, 0.02; Co, 0.60 mg; Fe, 38 mg; I, 1.60 mg; Mn, 80 mg; Cu, 40 mg; Se, 0.60 mg; Zn, 100 mg; butylated hydroxytoluene, 20 mg; ethoxyquin, 13 mg.

[3]Metabolizable energy (ME) was evaluated according to the equation proposed by Italian Ministry of Agriculture and Forestry (1994). ME (kcal/kg as fed) = $37.04 \times$ % CP + $81.93 \times$ % EE + $39.86 \times$ % starch + $31.07 \times$ % sugars (expressed as sucrose).

[4]Not detectable.

TABLE 3. Composition (%), proximate analysis (as fed) and mycotoxin content (on DM) of the diets employed in the second growing/finisher period (from 22 to 42 d)

	Farm A (Lodi)[1]		Farm B (Cremona)		Farm C (Venezia)	
	Iso corn	Bt Corn	Iso corn	Bt Corn	Iso corn	Bt Corn
Corn, %	59.50	60.20	60.70	62.70	60.70	58.70
Soybean meal, 48%	30.90	30.50	30.00	28.00	30.00	31.00
Corn oil, %	5.30	5.00	5.00	5.00	5.00	6.00
DL-Methionine, %	0.30	0.30	0.30	0.30	0.30	0.30
Limestone, %	1.20	1.20	1.20	1.20	1.20	1.20
Monocalcium phosphate, %	1.80	1.80	1.80	1.80	1.80	1.80
Sodium chloride, %	0.20	0.20	0.20	0.20	0.20	0.20
Sodium bicarbonate, %	0.30	0.30	0.30	0.30	0.30	0.30
Premix,[2] %	0.50	0.50	0.50	0.50	0.50	0.50
Dry matter, %	88.60	88.65	89.24	88.33	89.24	88.43
Crude protein, %	19.76	19.89	19.82	18.60	18.90	19.54
Ether extract, %	7.57	7.73	7.27	8.02	7.70	8.45
Crude fiber, %	3.32	3.36	3.08	3.42	3.20	3.25
Ash, %	5.56	5.54	5.5	5.70	5.35	5.81
Nitrogen-free extract	52.39	52.13	53.57	52.59	54.09	51.38
Starch, %	49.12	48.86	50.3	49.52	50.63	47.92
Sugars (as sucrose), %	3.27	3.27	3.27	3.07	3.46	3.46
ME,[3] kcal/kg	3,411.6	3,419.2	3,436.3	3,415.3	3,456.5	3,433.7
Mycotoxin content						
Deoxynivalenol, ppb	23	242	ND[4]	260	391	ND
Fumonisin B$_1$, ppb	2,621	485	10,517	1,582	7,210	2,494
Aflatoxin B$_1$, ppb	0.10	0.10	ND	0.12	0.13	0.35

[1]Iso = isogenic; Bt = transgenic.

[2]Content per kilogram of mixed feed: vitamin A, 20,000 IU; vitamin D$_3$, 4,000 IU; vitamin E, 20 IU; vitamin K$_3$, 2 mg; vitamin B$_1$, 0.80 mg; vitamin B$_2$, 6.40 mg; pantotenic acid, 8 mg; Niacin, 40 mg; folic acid, 0.40; vitamin B$_{12}$, 0.02; Co, 0.60 mg; Fe, 38 mg; I, 1.60 mg; Mn, 80 mg; Cu, 40 mg; Se, 0.60 mg; Zn, 100 mg; BHT, 20 mg; Ethoxyquin, 13 mg.

[3]Metabolizable energy (ME) evaluated according to the equation proposed by Italian Ministry of Agriculture and Forestry (1994). ME (kcal/kg as fed) = 37.04 x % CP + 81.93 × % EE + 39.86 × % starch + 31.07 × sugars (expressed as sucrose).

[4]Not detectable.

ratio (FCR) and the average daily weight gain (ADG) during the 3 experimental periods (1 to 21 d, 22 to 42 d, and 1 to 42 d of age) for each replicate were also recorded. Remaining starter and grower/finisher diets in the feeders were discharged and weighed after 21 and 42 d. The adjusted FCR were calculated and considered the total feed intake per pen divided by the sum of animal live weights and the body weights of animals that died during each period for each replicate. For this evaluation, all birds that died during the study were weighed within 24 h of death. During the experiment 7 birds died, 2 in the Bt group and 5 in the isogenic group.

Blood Sampling. At the end of the growth phase, 10 broilers per treatment with similar live weights were immobilized for blood collection from the cephalic vein of the wing into a Vacutainer.[2] An average of 3 to 5 mL of total blood for each broiler was collected in sterile tubes and immediately stored at −20°C.

Feed Collection in the Digestive Tract. At the end of the study the same birds used for blood collection were slaughtered and immediately hung upside-down to guarantee a complete bleeding. Birds fed isogenic corn were slaughtered first, followed by birds fed Bt corn, to avoid accidental cross-contamination. Between the 2 slaughtering phases, the place and the surgical tools were cleaned. Different sectors of the gut (cecum, jejunum, gizzard, and crop) were identified and then isolated, bound, and cut. Samples of the gut contents were collected in the following order: cecum, jejunum, gizzard, and crop. This order was followed because the target genes to be detected were assumed to be more concentrated toward the oral cavity because of the degradation activity of the digestive process. In this way, accidental contamination among different sectors was avoided. The samples were collected in sterile test tubes and immediately stored at −20°C after removal. All birds at the end of the study were disposed with according to Italian law.

DNA Extraction. Genomic DNA was isolated from intestinal contents of crop and gizzard and blood samples following the Wizard Genomic DNA Purification procedure,[3] except for intestinal contents of jejunum and cecum, which were processed with the QIAamp Stool Mini Kit[4] according to the manufacturer's protocols. DNA isolations were quantified on a 0.8% (wt/vol) agarose gel using known amounts of λ DNA.[5] The samples derived from birds fed with Bt corn and the isogenic corn were processed in 2 different places to avoid accidental cross-contamination. Mortars, pestles, homogenizer, and place of work were carefully cleaned before every new sample. New disposable gloves were used for each sample.

[2]Becton and Dickinson, Franklin Lakes, NJ.
[3]Promega, Madison, WI.
[4]Qiagen GmbH, Hilden, Germany.
[5]Amersham Biosciences, Little Chalfont, UK.

PCR Analysis. To avoid contamination, PCR reactions were assembled in a ultraviolet-sterilized hood. Filter tips against sample aerosol and sterile disposable tubes were used during pipetting. The reaction mixtures contained 50 ng of genomic DNA, 10 pmol of primers, 0.4 mM dNTP, 1.5 mM MgCl$_2$, 1× PCR buffer, 5% (vol/vol) dimethylsulfoxide (DMSO), and 1 unit of Hot Star Taq polymerase[6] in a 30-μL final volume. Primers designed from Genbank (Accession number M23537) were used to amplify the $Zein$ gene fragment of about 439 bp. Because $Zein$ is of a family of high copy number of genes, the same primers, under certain experimental conditions (i.e., PCR on blood), may give a pattern of different fragments ranging from 200 to 600 bp because of their partial homology with the various $Zein$ genes. Detection of the right size fragment was confirmed with Southern blot analysis. Primers (designed from United States Patent Office number 5689052) were used to amplify a Cry1A(b) gene fragment of 1,815 bp. Primers (designed from Genbank Accession number M81603) were used to amplify an adenosine diphosphate glucose pyrophosphorylase (Sh-2) gene fragment of 1,830 bp. Primers (designed on Genbank Accession number J00895) were used to amplify a chicken ovalbumin gene fragment of 482 bp. Reaction conditions for $Zein$ were: 95°C for 15 min, followed by 40 cycles consisting of 1 min at 95°C, 1 min of annealing at 57°C, 1 min of extension at 72°C, and a final step of 10 min at 72°C. Annealing temperature for Cry1A(b) was 63°C and for Sh-2/ovalbumin was 55°C.

All the reactions were performed in an Eppendorf Mastercycler Gradient.[7] PCR products were detected by electrophoresis in 2% (wt/vol) agarose gel after staining with ethidium bromide. Southern blots were performed according to standard procedure (Sambrook et al., 1989). The amplified PCR products were cloned into the pGEM-T Easy Vector System,[3] sequenced, labeled with Ready-To-Go DNA Labeling Beads,[5] and used as probes.

Statistical Analysis

Data for live weight, feed intake, ADG, and FCR were statistically analyzed to determine the differences between Bt and isogenic corn diets from corn in the same provinces of production and among the 3 provinces; interactive effects were also considered. Birds were individually weighed, but pen was the experimental unit.

Statistical analysis was performed according to the GLM procedure of SAS Institute software package (1999) with corn sources as independent variables in a 2-way analysis of variance within a randomized complete block design, using between-room variation as the error term. The treatment means were compared using Student's t-test. Bird live weight at the start of the study was used as covariant because inside each dietary group (isogenic or Bt) bird weight was homogeneous (45 g for isogenic

and 44.3 g for Bt), but among different provincial groups (province A, B, or C) there were some differences. Statements of statistical significance were based upon $P < 0.05$. The correlation between mycotoxin contamination and growth performances was carried out using PROC CORR of the SAS statistical package (1999). Statistical analysis of the traceability of Cry1A(b), $Zein$, and Sh-2 genes in the gastrointestinal tracts was performed using the chi-squared test: the number of PCR positives over 3 repetitions for each target gene constituted the frequency sample for 10 birds per diet (isogenic or Bt). The number of positives for each target gene and diet were compared.

RESULTS AND DISCUSSION

Corn and Dietary Analysis

Evaluation of the analytical characteristics of the transgenic and isogenic corn (Table 1) shows numerical differences for CP and EE among corn samples. The highest contents of CP and EE were in Bt corn produced on farm B. Variability of percentages for CP, EE, crude fiber, ash, and starch were, however, within the normally accepted range. According to the expected value, the starter and grower-finisher diets were isonitrogenous and isoenergetic (Tables 2 and 3).

The mycotoxins content of feed sampled directly from the feeders had the same content of mycotoxins as the feed sampled at the start of the second growing period. There were not clear differences between isogenic and Bt corn for deoxynivalenol content.

The fumonisin B$_1$ content of the Bt corn was lower than that of the isogenic corn. A similar reduction was reported by Clements et al. (2003), who found that fumonisin concentration is affected by hybrid genotype and the predominant insect species. Similar results were reported from field trials after experimental infestation of corn with $Ostrinia$ $nubilalis$ (Munkvold et al., 1999). The reduction in our study of fumonisin contamination of Bt corn was approximately of 90% at farm A, 85% at farm B, and 54% at farm C with a mean value of 76%.

Munkvold et al. (1997) reported less mycotoxin contamination, particularly fumonisin, in transgenic corn than in isogenic corn as a consequence of improved resistance to insect pests. Given the negative impact of fumonisin on the production performance of many animals, especially birds (Weibking et al., 1993; Ledoux et al., 1996; Kubena et al., 1997; Prathapkumar et al., 1997), the use of genetically modified corn varieties to increase their resistance to parasites may prove to be economically beneficial, especially in the presence of adverse environmental conditions, which may promote the development of molds in storage rooms. The aflatoxin B$_1$ concentration was below the sensitivity of the analytical method with the exception for the isogenic corn produced on farm C, which had low contamination as reported by Brake and Vlachos (1998) and Brake et al. (2003). In contrast to the study by Ledoux et al. (1992) in which the fumonisin B$_1$ effects were dose-dependent we did not observe any adverse effect of myco-

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[6]Qiagen, GmbH, Hilden, Germany.
[7]Eppendorf AG, Hamburg, Germany.

TABLE 4. Performance of broilers fed isogenic (Iso) or transgenic corn (Bt) in diets

	Corn			Statistical analysis		
	Iso	Bt	Corn effect P	Province effect P	Interaction corn × province P	Mean square error
Live weight						
21 d, g	688.3	684.3	0.83	0.05	0.51	2,037.89
42 d, g	2,641.8	2,707.1	0.17	0.02	0.50	12,283.60
Feed Intake						
From 1 to 21 d, g	59.0	54.9	0.24	0.22	0.27	65.82
From 22 to 42 d, g	168.5	174.3	0.30	0.54	0.64	180.37
From 1 to 42 d, g	126.0	129.4	0.70	0.92	0.95	452.24
Average daily gain						
From 1 to 21 d, g	30.6	30.5	0.86	0.05	0.67	4.55
From 22 to 42 d, g	93.0	96.3	0.15	0.06	0.52	17.64
From 1 to 42 d, g	61.8	63.4	0.16	0.02	0.51	6.98
Feed:gain ratio						
From 1 to 21 d	1.95	1.81	0.34	0.68	0.23	0.10
From 22 to 42 d	2.09	2.09	0.99	0.67	0.41	0.04
From 1 to 42 d	2.02	1.95	0.46	0.58	0.44	0.05

toxin contamination on bird performance. Nor did Henry et al. (2000), supplementing a diet with 80 mg/kg of purified fumonisin B_1, find negative effects on live weight, feed efficiency, or water consumption, whereas Kubena et al. (1995) and Kubena et al. (1997) reported that in turkey poults and broilers, respectively, detrimental effects of fumonisin contamination on live weight, feed intake, and FCR were observed. The absence of a negative effect of mycotoxin is probably due to the lower level used in our trial. In the experiment of Ledoux et al. (1992) and Kubena et al. (1997), in fact, animals were fed at least 75 mg/kg of fumonisin B_1, a value much higher than 16 mg/kg found in our diet.

Performance

Field trials comparing the nutritional value and effects on the animal productive performance of GM corn as opposed to conventional corn are not yet exhaustive (Brake and Vlachos, 1998; Masoero et al., 1999; Barrière et al., 2001; Brake et al., 2003). Nevertheless, available findings seem to rule out negative effects coming from the use of GM corn to replace conventional corn.

Our results on bird performance show no statistical differences between Bt and isogenic corn for live weight, ADG, feed intake, or FCR (Table 4). Furthermore no statistical interaction between site of production and type of corn was observed.

The provinces of corn (Bt and its isogenic form) production had as significant effect on feed intake as on bird live weight. The final weight was greater for birds fed diets with Bt vs. isogenic corn coming from the provinces of Lodi (2,796 vs. 2,707 g; $P < 0.05$; +3.3%) and Venezia (2,619 vs. 2,506 g; $P < 0.01$; +4.5%). The correlation analysis between growth parameters and mycotoxins content in GM and isogenic corn produced in the 3 provinces showed no significant relationship (Table 5). According to Aulrich et al. (2001) is possible to suggest a substantial equivalence for isogenic and transgenic corn hybrids in animal nutrition.

DNA in the Intestinal Tract and Blood

The DNA isolation from crop, gizzard, and cecum contents gave good yield and quality. Genomic DNA from jejunum gave lower yield and had partial degradation. Two different methods were used for DNA isolation because PCR reactions on jejunum and cecum samples were strongly inhibited, as confirmed by the unsuccessful amplification of purified *Zein* DNA fragment added to the samples.

Inhibition problems on these 2 distal sectors were successfully removed with the QIAamp Stool Mini Kit.[6] No difference in genomic DNA isolation was observed between samples for each gastrointestinal sector of birds fed isogenic and Bt corn. The genomic DNA isolated from the samples was used as template in PCR reactions. Three target sequences were selected for identification through PCR amplification. The *Zein* gene, a high copy number maize gene (Woo et al., 2001), was used as positive control to identify maize DNA. The *Cry*1A(b) gene, the synthetic single copy gene inserted into the maize line MON 810 (Armstrong, 1995) was used to monitor transgenic DNA. The *Sh*-2 gene, a maize single copy gene was used as control in a 1:1 ratio with *Cry*1A(b). Amplification of a 3,500-bp fragment from *Cry*1A(b) and *Sh*-2, corresponding to the full-length *Cry*1A(b) gene, was unreliable on gut contents, and results were difficult to obtain, even from the maize meal. Genomic DNA isolated from raw

TABLE 5. Pearson correlation coefficients (r) between mycotoxins content of diets and performance of broilers

Mycotoxin	Average daily gain	Feed conversion ratio	Feed intake
Deoxynivalenol			
Pearson correlation coefficient	−0.66	0.38	−0.42
P-value	0.15	0.45	0.41
Fumonisin B_1			
Pearson correlation coefficient	−0.44	0.10	−0.50
P-value	0.39	0.85	0.31

TABLE 6. Number of positive samples for the presence of plant (*Zein*, *Sh*-2) and transgenic (*Cry*1A(b))
DNA in gut content and blood of animal fed isogenic or Bt corn[1]

	Zein			*Cry*1A(b)			*Sh*-2		
	Iso	Bt	*P*-value[2]	Iso	Bt	*P*-value[2]	Iso	Bt	*P*-value[2]
Crop	28	29	NS	—	21	NS[3]	21	23	NS
Gizzard	25	26	NS	—	22	NS[3]	18	19	NS
Jejunum	17	19	NS	—	ND[4]	—	ND	ND	—
Cecum	9	11	NS	—	ND	—	ND	ND	—
Blood	9	9	NS	—	ND	—	ND	ND	—

[1]n = 10 birds per diet with 3 replicates each for a total of 30.
[2]Chi-square test.
[3]Comparison of positive sample for *Cry*1A(b) and *Sh*-2 in birds fed Bt corn.
[4]Not detectable.

material such as maize meal is probably too degraded for an efficient and reproducible amplification of a 3,500 bp sequence.

To increase reliability to the experimental level required for the analysis of DNA traceability, we decided to focus on amplification of smaller regions and chose to monitor the presence of an 1,800-bp fragment coding for the minimal functional unit, a smaller sequence able to codify a fully toxic protein (Hofte and Whiteley, 1989). The comparison between the degradation profiles of *Cry*1A(b) and *Sh*-2 was used to investigate whether the DNA degradability of a genetically modified plant showed any difference compared with an unmodified plant. The *Zein* fragment was recovered with high efficiency and was detected in all of the gastrointestinal sectors. PCR data showed that *Zein* recovery, calculated as percentage of positives over all the samples, was 90% from the crop, 80% from the gizzard, 60% from the jejunum, and 35% from the cecum. Statistical analysis did not evidence any significant difference in DNA detection between samples derived from broilers fed isogenic corn and broilers fed Bt corn (Table 6).

It was not possible to amplify the 1,800-bp fragment of both *Cry*1A(b) and *Sh*-2 genes in the jejunum and in the cecum. DNA fragmentation in these tracts is probably too high to allow the detection of fragments of such length. The *Cry*1A(b) fragment was recovered with high efficiency from gut contents of the crop and gizzard. The average number of positives in the PCR trials was about 70%, showing a good equivalence between the 2 gastrointestinal areas. Recovery of *Cry*1A(b) and *Sh*-2 was comparable with the last detected also in the animals fed isogenic corn. The transgenic fragment was not detected in samples derived from the birds fed isogenic corn. In the jejunum and cecum, the genetic material was probably too degraded to allow amplification of such size sequences. Detection of large fragments of feed-derived DNA has been reported (Schubbert et al., 1994; Hohlweg and Doerfler, 2001; Duggan et al., 2003) even though our results suggest that survival of DNA fragments longer than 500 bp in distal zones of the gut is unlikely to happen. Reuter and Aulrich (2002) reported the detection of a maize-specific 500-bp fragment in intestinal digesta of pigs, but they did not find Bt fragments greater than 200 bp.
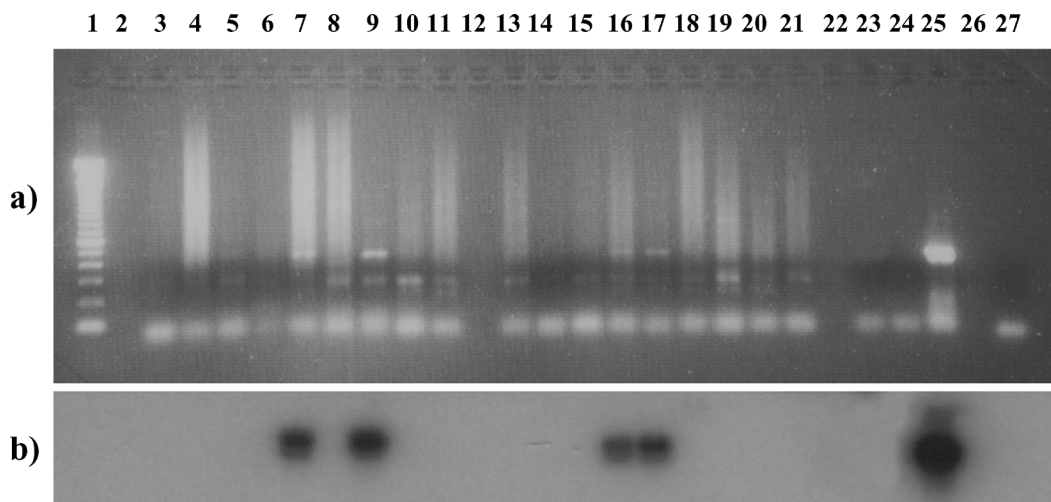

FIGURE 1. (a) Example results of PCR reactions on genomic DNA from total blood samples with primers for *Zein* and (b) relative hybridization with specific radioactive probe. Samples in the lanes are as follows: lane 1 is the 100-bp marker; lanes 3 to 11 and 23 are samples from control group (broilers fed isogenic corn); lanes 13 to 21 and 24 are samples from the test group (broilers fed Bt-corn); lane 25 is the PCR positive control (sample with corn genomic DNA as template); lane 27 is the PCR negative control; lanes 2, 12, 22, and 26 are blank. The signals that can be observed both in the agarose gel (a) and in the Southern blot panel (b) correspond to the *Zein* expected amplicon size (439 bp).

Moreover DNA traceability was tested on genomic DNA from total blood. The samples were positive to a PCR assays for the chicken specific ovalbumin gene, assuring the good quality and amplifiability of DNA. The presence of the *Zein* gene fragment was investigated and was detected with low frequency (Figure 1a). To confirm the presence of plant DNA in blood, a Southern blot analysis was performed on PCR products that hybridized with the specific probe (Figure1b). So far the 1,800-bp *Cry*1A(b) and *Sh*-2 fragments could not be detected in blood samples. No significant difference in *Zein* detection was observed between birds fed isogenic and Bt corn (Table 6). These results are in contrast with those reported by Einspanier et al. (2001), who detected only a 200-bp fragment of plant DNA in organs of broilers but not in the blood. Detection of the maize-specific 439-bp *Zein* fragment in blood samples suggests that residual DNA in the digestive apparatus might be absorbed by the organism and detected in the blood.

No significant difference was observed in plant DNA detection frequency between transgenic and conventional feeds, indicating that genetic modification does not result in increased resistance to DNA degradation. It is, therefore, reasonable to assume that genetic material derived from GM organisms and unmodified plants undergoes the same fate.

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