Evaluation of Transgenic Event 176 "Bt" Corn in Broiler Chickens[1]

J. BRAKE*,[2] and D. VLACHOS†

*Department of Poultry Science, North Carolina State University, Raleigh, North Carolina 27695-7608, and †Novartis Seeds Biotechnology Research Unit, Research Triangle Park, North Carolina 27709-2257

ABSTRACT A 38-d feeding study evaluated whether standard broiler diets prepared with transgenic Event 176-derived "Bt" corn (maize) grain had any adverse effects on male or female broiler chickens as compared to diets prepared with nontransgenic (isogenic) control corn grain. No statistically significant differences in survival or BW were observed between birds reared on mash or pelleted diets prepared with transgenic corn and similar diets prepared using control corn. Broilers raised on diets prepared from the transgenic corn exhibited significantly better feed conversion ratios and improved yield of the *Pectoralis minor* breast muscle. Although it is not clear whether this enhanced performance was attributable to the transgenic corn *per se*, or due to possible slight differences in overall composition of the formulated diets, it was clear that the transgenic corn had no deleterious effects in this study.

(*Key words*: Bt corn, transgenic corn, Bt maize, transgenic maize, broiler)

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INTRODUCTION

In recent years, agricultural biotechnology has produced several new varieties of crop plants with enhanced features such as protection against pests and improved quality or processing traits. In 1997, U.S. growers planted approximately 30 million acres of transgenic crops, including insect-resistant or herbicide-tolerant varieties of corn (maize), soybean, potato, and cotton. The first commercial-scale plantings of insect-protected field corn hybrids, commonly referred to as "Bt" corn, occurred in 1996, following regulatory review by U.S. and Canadian authorities.[3] These first field corn hybrids, derived from a genetic modification designated "Event 176", were developed for control of the European corn borer (ECB; *Ostrinia nubilalis* Hübner) (Koziel *et al.*, 1993). The ECB is a major lepidopteran (caterpillar) pest in the U.S., Canada, and Europe, and has proven difficult to control by conventional means. The annual cost of ECB damage to U.S. corn growers exceeds $1 billion, as measured in yield loss and control expenditures (Mason *et al.*, 1996). Corn hybrids derived from Event 176 express a gene that enables the plants to produce an insecticidal protein, Cry1Ab, similar to that produced in nature by certain subspecies of the common soil bacterium *Bacillus thuringiensis* (*B.t.*). Cry1Ab is a member of a class of insecticidal proteins produced by *B.t.* as parasporal crystals (hence, the name "Cry") (reviewed by Peferoen, 1997). Upon ingestion, the Cry1Ab protein is selectively toxic to the larvae of ECB and certain other lepidopteran pests, whereas other species are unaffected. The basis of this selectivity is the presence of receptor-like sites, with affinity for the Cry1Ab protein, in midgut membranes of susceptible lepidopteran larvae. After ingesting Cry1Ab, the ECB midgut membrane is disrupted, and the larvae cease feeding and soon die. Commercial formulations of *B.t.* have been used as topical insecticides since 1938, and such *B.t.*-based products have been registered for use on food crops in the U.S. since 1961. These products have had a long history of safe use, owing to the lack of Cry protein toxicity to nontarget organisms, including mammals, birds, beneficial insects, and other wildlife (U.S. Environmental Protection Agency, 1986).

Expression of the Cry1Ab insecticidal protein in corn plant tissues allows for effective, targeted control of ECB in this crop. Through the use of tissue-specific plant gene promoters, Event 176-derived corn hybrids produce Cry1Ab protein primarily in green plant tissues and pollen, the principal corn tissues fed upon by young ECB larvae (Koziel *et al.*, 1993). Other plant tissues produce only trace quantities of the insecticidal protein;

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[1]The use of trade names in this publication does not imply endorsement of the products named nor criticism of similar products not mentioned.

[2]To whom correspondence should be addressed: jbrake@ncsu.edu

[3]The USDA, U.S. Environmental Protection Agency, U.S. Food and Drug Administration, Agriculture and Agri-Food Canada, and Health Canada.

Abbreviation Key: B.t. = *Bacillus thuringiensis*; ECB = European corn borer; FSY = feather-sexable yield.

Cry1Ab levels in grain, for example, are less than 5 ppb (Fearing *et al.*, 1997). As part of the regulatory and safety reviews (e.g., US Food and Drug Administration, 1992) that preceded commercialization of Event 176-derived corn, it was demonstrated that the process of genetic modification introduced no detectable, unintended effects on food or feed quality or nutrient composition of the grain. Representative hybrids were shown to be equivalent to their conventional counterparts, as measured by proximate analyses (including total fat, protein, and fiber) and other parameters (including total carbohydrate, amino acid composition, fatty acid composition, and carotenoid content) (Novartis Seeds, unpublished data). Nevertheless, because corn represents a significant portion of the diet of broiler chickens, it was of interest to confirm that there would be no unexpected dietary effects of Event 176-derived corn on broiler performance, either as a direct effect of Cry1Ab protein in the diet or as a result of any unintended compositional changes in the grain.

To determine whether transgenic Event 176-derived corn[4] has an effect on broiler chicken performance, this study compared male and female chickens receiving feed made with field corn from a transgenic hybrid vs feed made with the corresponding nontransgenic version of the same hybrid. The feed was prepared in two forms, mash and pellet. Although better performance was expected from the pelleted form (Lanson and Smyth, 1955), mash feed was also prepared because it would more likely reveal any differences that might result from the presence of the Cry1Ab protein. These differences may occur because the preparation of mash feeds does not subject the ingredients to extreme conditions (Bayley *et al.*, 1968) that serve to degrade proteins in pelleted feeds. The performance endpoints measured in this study included mortality, BW, feed efficiency, and carcass yield.

MATERIALS AND METHODS

Test and Control Corn

The transgenic corn grain used was from Event 176-derived hybrid number 5506BTX. This hybrid corn was field-grown in Bloomington, IL in 1994. The presence of CryIA(b) protein in the grain at the expected level of less than 5 ppb was confirmed by ELISA in April 1996. The nontransgenic control corn used in this study was from hybrid number G4665, the isogenic version of the transgenic hybrid, and was grown, processed, and stored concurrently with the transgenic corn under the same environmental conditions. Isolation procedures ensured that intermixing of grain genotypes did not occur.

[4]Novartis Seeds Biotechnology Research Unit, Research Triangle Park, NC 27709-2257.

[5]Woodson-Tenent Laboratories, Goldston, NC 27252.

Experimental Design

The experimental design consisted of diets made from two types of corn (transgenic or nontransgenic) by two forms of feed (mash or pelleted) by two sexes, with four replicate pens for each three-way interaction. Pens were assigned in a randomized complete block design to compensate for known position effects in the experimental facility.

The broiler chicks were from a commercial strain of Arbor Acres Yieldmaster males and feather-sexable yield (FSY) females. The broiler chicks were feather-sexed at hatch and weighed individually to determine the BW range distribution. Based on this distribution, very large and small birds were excluded. A total of 1,280 birds were randomly distributed into 32 pens of a curtain-sided house at 1 d of age. Each pen contained 40 birds of the same sex.

Corn Analyses

Samples of corn of each genotype were sent to an independent laboratory[5] for proximate analyses (Table 1). Novartis Seeds (Bloomington, IL 61704) measured the density of the transgenic 5506BTX corn as 63.9 lb / bu (830 kg / m^3) and the density of the nontransgenic G4665 corn as 63.3 lb / bu (822 kg / m^3). Amino acid analyses showed very similar amino acid patterns for samples of transgenic and nontransgenic corn (Table 1). Initial routine mycotoxin determinations showed extremely low contamination for both corn samples (Table 1).

Diet Formulation

To compensate for the difference in percentage crude protein and moisture between the two corn types, 95.5% as much nontransgenic corn was used as transgenic corn, and an inert filler of the same density was added to volume (Table 2). Diets were pelleted in a small commercial style pellet mill immediately after mixing. Pelleted starter feed was crumbled. Mash feeds were directly bagged without pelleting.

Access to Feed and Water

Birds were provided continuous access to feed and water from two tube feeders and one automatic bell waterer in each pen. Supplemental waterers and feeders were used during the 1st wk. The feeders were manually agitated as needed to maintain the flow of feed from the tube into the feeder pan from which the birds fed. Birds had access to 1 kg of starter diet per bird during the first 14 d of the study. This was followed by grower diet, which was added to any starter diet that remained in the feeders. Access to feed was discontinued approximately 16 h before slaughter.

Housing and Environmental Conditions

Birds were housed in 1.2 m × 3.7 m pens with pine shavings as litter. House temperature was initially 32 C

TABLE 1. Analyses of corn samples

Analyses	Corn 5506BTX	Corn G4665
Proximate analyses,[1] %		
Moisture	12.13	11.62
Fat	3.19	3.00
Protein	8.43	8.87
Fiber	2.20	2.10
Ash	1.02	0.93
Amino acids,[2] %		
Taurine	0.12	0.12
Hydroxyproline	0.02	0.02
Aspartic acid	0.55	0.55
Threonine	0.31	0.31
Serine	0.40	0.40
Glutamic acid	1.65	1.66
Proline	0.84	0.85
Lanthionine	0.00	0.00
Glycine	0.34	0.33
Alanine	0.69	0.70
Cysteine	0.23	0.23
Valine	0.42	0.41
Methionine	0.21	0.21
Isoleucine	0.29	0.29
Leucine	1.14	1.15
Tyrosine	0.29	0.27
Phenylalanine	0.45	0.45
Hydroxylysine	0.00	0.00
Histidine	0.27	0.27
Ornithine	0.02	0.02
Lysine	0.26	0.25
Arginine	0.39	0.38
Tryptophan	0.05	0.06
Mycotoxin analyses[3]		
Aflatoxins, ppb	<2	4
Deoxynivalenol,[4] ppb	ND	30
Fumonisin B_1, ppm	<1	<1

[1]Woodson-Tenent Laboratories, Goldston, NC 27252. Reported on dry weight basis except for percentage moisture.

[2]Experiment Station Chemical Laboratory, University of Missouri, Columbia, MO 75211.

[3]Mycotoxin Laboratory, North Carolina State University, Raleigh, NC 27695-7608.

[4]Vomitoxin, one of the trichothecenes.

and decreased approximately 3 C/wk until 24 C was reached. Space heaters and circulating fans were used to maintain an even temperature distribution. The birds received 23 h of incandescent light/d until 29 d of age when the supplemental lighting was turned off and the curtains were opened as an aid to preventing heat-related mortality.

Data Collection and Statistical Analyses

Total pen weight data were collected at hatch (1 d), 14, 28, and 38 d of age. At the latter three time-points, feed consumption per pen was determined in order to calculate adjusted feed conversion ratios. At 41 d, a random sample of six birds from each pen was processed in order to determine carcass yield. These birds were killed by exsanguination, scalded, picked, eviscerated, and deboned as previously described (Brake *et al.*, 1993).

Feed conversion, BW, survival, and carcass processing data were analyzed in order to determine statistical differences between corn diets, feed form, and sex. Statistical analyses were performed using the General Linear Models (GLM) procedure with sex, corn diet, and feed form as main effects in a multi-way analysis of variance, with random error (between-pen variation) as the error term (SAS Institute, 1993).

RESULTS AND DISCUSSION

Diet Analyses

The proximate analysis data of the corn are shown in Table 1. The percentage crude protein, moisture, fat, fiber, and ash of the formulated diets, as determined by proximate analyses, are shown in Table 2. The observed minor differences in proximate values may have been related to differences in corn density (test weight) or moisture content, which are known to occur across hybrids and growing conditions, and may be related to the general health of the plant (e.g., higher test weight of the transgenic grain as a result of improved insect control by Event 176-derived hybrid plants). The variability in percentage crude protein was within the normally expected range, and variation between diets from the two corn sources did not exceed the variation between the feed forms (mash vs pellet) of the same corn source (data not shown). There appeared to be little difference in final diet moisture even though the transgenic corn initially contained higher moisture. The nontransgenic diets appeared to be slightly higher in fiber and ash. This difference can be accounted for by the cardboard (fiber) and sand (ash) filler. These differences were not substantial, but could have had small effects on growth and feed conversion. Trace amounts (< 5 ppb) of the Cry1Ab protein were detectable by ELISA in both the mash and pelleted diets formulated with the transgenic corn (data not shown).

Body Weight Data

There were no statistically significant differences in BW at any time (14, 28, or 38 d) between birds that received the transgenic corn diets and those that received the nontransgenic corn diets (Table 3). There were no statistically significant interactions between corn diet and other main effects (feed form or sex). As expected, broilers receiving the pelleted diets grew faster than broilers fed the mash diets (Lanson and Smyth, 1955; Auckland and Fulton, 1972) and males grew faster than females (Brake *et al.*, 1993).

Feed Conversion

Table 3 shows the adjusted feed conversion ratios at 14, 28, and 38 d. The birds that received diets made with transgenic corn exhibited improved adjusted feed conversion ratios at 28 and 38 d of age. Although efforts were made in this study to adjust the diets for observed small differences in corn source with respect to total protein (Tables 1 and 2), it cannot be stated that the diets made from transgenic and nontransgenic corn were identical (Table 2). Given the small differences in percentage moisture and total protein of the corn sources and the efforts to adjust for the difference in total protein in formulating the diets, it was not feasible to formulate the corresponding transgenic and nontransgenic corn diets so that they would be identical with respect to all other measured components. Therefore, the improved feed conversion ratios cannot necessarily be attributed to the corn source *per se*, but these data do show absence of any deleterious effects associated with the diets made from transgenic corn when compared to diets made from nontransgenic corn.

Birds receiving the pelleted diets exhibited improved performance at 14 d of age, but the differences were not statistically significant at 28 or 38 d of age. Pelleted diets are known to elicit improved feed efficiency (Lanson and Smyth, 1955). Males had significantly better adjusted feed conversion ratios than the females at 14, 28, and 38 d of age as has been reported by Marks (1985). There were no statistically significant interactions between corn diet and other main effects.

Survival

There were no statistically significant differences in percentage survivors between birds that received the transgenic corn diets and those that received the nontransgenic corn diets at any age (Table 3). There were also no significant differences due to feed form (pellet vs mash) or sex, and no statistically significant interactions.

Carcass and Parts Yield

The dressed carcass parts yield as a percentage of live body weight is displayed in Table 4. Birds that received the transgenic corn diets exhibited a significant increase in breast skin and *Pectoralis minor* yield. Although this cannot necessarily be attributed to the transgenic corn *per se*, it does indicate an absence of detrimental effects. There were no statistically significant interactions between corn diet and other main effects.

For feed form and sex, birds consuming the pelleted feed and males had significantly greater BW. The pelleted feed produced a significantly higher percentage of fat pad, breast skin, and *Pectoralis major* than the mash feed. The males yielded a higher percentage of legs, whereas the

TABLE 2. Diets and analyses

Ingredients and analyses	5506BTX		G4665	
	Starter	Grower	Starter	Grower
	(% by weight)			
Corn	61.37	67.42	58.61	64.39
Soybean meal	25.01	14.72	25.01	14.72
Poultry by-product meal	7.82	11.96	7.82	11.96
Poultry fat	2.00	2.67	2.00	2.67
Dicalcium phosphate	1.78	1.88	1.78	1.88
Calcium carbonate	0.70	0.00	0.70	0.00
Salt	0.35	0.30	0.35	0.30
D,L-methionine	0.35	0.35	0.35	0.35
Mineral premix[1]	0.20	0.20	0.20	0.20
Vitamin premix[2]	0.05	0.05	0.05	0.05
Coccidiostat	0.07	0.07	0.07	0.07
Selenium premix[3]	0.15	0.15	0.15	0.15
Lysine HCl	0.15	0.23	0.15	0.23
Filler of corn density[4]	0.00	0.00	2.76	3.03
Calculated analyses				
Metabolizable energy, kcal/kg	3,058	3,190	3,058	3,190
Crude protein, %	22.00	20.00	22.00	20.00
Actual analyses[5]				
Crude protein, %	22.10	20.40	22.70	20.55
Moisture, %	11.35	11.10	11.15	10.40
Fat, %	5.00	6.55	5.17	6.04
Fiber, %	2.65	2.25	3.10	2.60
Ash, %	5.76	5.39	6.39	6.19

[1]Trace mineral premix provided in milligrams per kilogram of diet: manganese, 120; zinc, 120; iron, 80; copper, 10; iodine, 2.5; cobalt 1.0.

[2]Vitamin premix provided per kilogram of diet: vitamin A, 6,600 IU; cholecalciferol, 2,000 IU; vitamin E, 33 IU; vitamin B_{12}, 19.8 μg; riboflavin, 6.6 mg; niacin, 55 mg; pantothenic acid, 11 mg; Vitamin K, 2 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; and biotin, 126 mg.

[3]Selenium premix provided 0.15 mg selenium/kg of diet.

[4]Combination of cardboard (Solka Floc) and washed builders sand blended to the density of corn. Solka Floc was obtained from Fibre Sales and Development Corporation, Checkerboard Square, St. Louis, MO 63164.

[5]Average of mash and pelleted forms shown.

TABLE 3. Effect of corn diet, feed form, and sex on body weight, feed conversion ratio, and percentage birds alive at 14, 28, and 38 d of age

Main effects[1]	14 d BW	14 d FCR[2]	14 d Alive[3]	28 d BW	28 d FCR[2]	28 d Alive[3]	38 d BW	38 d FCR[2]	38 d Alive[3]
	(g)	(g:g)	(%)	(g)	(g:g)	(%)	(g)	(g:g)	(%)
Corn diet									
5506BTX	375	1.17	99.4	1,213	1.50[b]	97.0	1,825	1.72[b]	96.1
G4665	372	1.19	99.2	1,199	1.54[a]	98.3	1,802	1.75[a]	97.8
SE	4	0.01	0.4	24	0.01	0.6	44	0.01	0.8
Feed form									
Pellet	383[a]	1.15[b]	99.4	1,255[a]	1.51	97.2	1,880[a]	1.72	95.9
Mash	364[b]	1.21[a]	99.2	1,157[b]	1.53	98.1	1,747[b]	1.74	98.0
SE	3	0.01	0.3	21	0.01	0.6	41	0.01	0.8
Sex									
Male	385[a]	1.17[b]	99.5	1,283[a]	1.50[b]	97.3	1,968[a]	1.71[b]	96.4
Female	362[b]	1.19[a]	99.1	1,130[b]	1.54[a]	98.0	1,659[b]	1.76[a]	97.5
SE	3	0.01	0.3	14	0.01	0.6	19	0.01	0.9

[a,b]Means in a column within corn diet, feed form, or sex with no common superscript differ significantly ($P \leq 0.05$).

[1]No significant interactions were found.

[2]Adjusted feed conversion ratio = feed consumed to age/(Live BW + BW of dead birds).

[3]Percentage birds remaining alive at the respective ages.

females had a higher percentage of fat pad, breast skin, and *P. minor* in agreement with a previous report (Brake *et al.*, 1993).

General

The experiment was designed so that if the transgenic corn was to have deleterious effects, they should have been more pronounced in the mash feed. The lack of statistical interaction suggests that pelleting had no differential effect on the response of birds to diets made from the transgenic corn other than the typical growth promoting effect of pelleting. In other words, there appeared to be no deleterious factor(s) present in the diets made with the transgenic corn that may have been destroyed by the pelleting process.

To our knowledge, this is the first published large-scale feeding study of transgenic corn. We have demonstrated that broiler chickens receiving diets prepared with transgenic corn perform at least as well as those receiving diets prepared with the corresponding conventional corn. Although the primary benefits of insect-protected corn are evident to the corn grower as increased yield and harvestability, better plant health, and decreased reliance on chemical insecticides, significant secondary benefits on grain and feed quality can be potentially realized. The ECB damage has long been associated with secondary infestations of fungi that cause ear and stalk rot diseases of corn (Christensen and Schneider, 1950). Under conditions of ECB pressure, recent studies of Bt corn hybrids have demonstrated significantly reduced incidence and severity of *Fusarium* ear rots compared to the correspond-

TABLE 4. The mean live body weight and parts yield as a percentage of live body weight at 41 d of age as affected by corn diet, feed form, and sex

Main effect[1]	Live BW	Neck	Fat pad	Legs	Thighs	Wings	Breast skin	*Pectoralis major*	*Pectoralis minor*	Ribs and back
	(g)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Corn diet										
5506BTX	1,905	5.74	1.42	10.50	12.52	8.19	2.08[a]	13.82	3.39[a]	16.7
G4665	1,893	5.67	1.36	10.59	12.36	8.24	1.89[b]	13.56	3.27[b]	17.0
SE	± 22	± 0.05	± 0.05	± 0.06	± 0.10	± 0.04	± 0.04	± 0.11	± 0.03	± 0.24
Feed form										
Pellet	1,972[a]	5.75	1.46[a]	10.51	12.50	8.20	2.06[a]	13.96[a]	3.34	16.7
Mash	1,823[b]	5.66	1.32[b]	10.59	12.37	8.24	1.91[b]	13.41[b]	3.31	17.1
SE	± 21	± 0.05	± 0.05	± 0.06	± 0.10	± 0.04	± 0.04	± 0.11	± 0.03	± 0.24
Sex										
Male	2,053[a]	5.66	1.32[b]	10.79[a]	12.45	8.21	1.89[b]	13.73	3.21[b]	16.7
Female	1,738[b]	5.75	1.47[a]	10.29[b]	12.42	8.22	2.09[a]	13.65	3.44[a]	17.0
SE	± 15	± 0.05	± 0.05	± 0.05	± 0.10	± 0.04	± 0.04	± 0.11	± 0.03	± 0.24

[a,b]Means in a column within corn diet, feed form, or sex with no common superscript differ significantly ($P \leq 0.05$).

[1]No significant interactions were found.

ing conventional corn hybrids (Munkvold *et al.*, 1997). Additional studies can determine whether such observations will translate into reduced incidence of mycotoxin contamination in feeds prepared with Bt corn.

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