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Bollgard II Cotton: Compositional Analysis and Feeding Studies of Cottonseed from Insect-Protected Cotton (*Gossypium hirsutum* L.) Producing the Cry1Ac and Cry2Ab2 Proteins

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Bollgard II cotton event 15985 producing the Cry1Ac and Cry2Ab2 proteins has been developed by genetic modification to broaden the spectrum of insects to which the plant is tolerant and to provide an insect resistance management tool to impede the onset of resistance. The purpose of this study was to evaluate the composition and nutrition of Bollgard II cotton, relative to the use for food and animal feed, compared to that of conventional cotton varieties. Compositional analyses were conducted to measure proximate, fiber, amino acid, fatty acid, gossypol, and mineral contents of cottonseed from a total of 14 U.S. field sites over two years. Compositional analysis results showed that the cottonseed and cottonseed oil from Bollgard II cotton were comparable in their composition to those of the conventional control cotton line and other commercial varieties. The composition data are supported by nutritional safety studies conducted with dairy cows, catfish, and quail. Results from these studies showed that Bollgard II performed similarly to the conventional control cotton varieties. These data demonstrate that Bollgard II cotton is compositionally and nutritionally equivalent to conventional cotton varieties. These data support the conclusion that Bollgard II cotton is as safe and nutritious as conventional cotton for food and feed use.

KEYWORDS: Cotton (*Gossypium hirsutum* L.); genetically modified; composition

INTRODUCTION

Lepidopteran insects are the main insect pest problem in cotton. Each year an average of 2.4 insecticide applications are made for bollworm and budworm control across the United States (*1*). Bollgard insect-protected cotton was introduced commercially in the United States in 1996 and has been adopted broadly by growers because it provides effective protection from the feeding and damage caused by lepidopteran insect pests, such as tobacco budworms, pink bollworms, and cotton bollworms. Bollgard cotton contains the pesticidal protein, Cry1Ac, which effectively controls these insect pests while significantly reducing the amount of insecticide needed for control. This results in reduced environmental impact and greater profitability for growers using Bollgard cotton varieties, as compared to conventional insect control products. In the United States, cotton is grown on approximately 13 million acres annually. In 2000 >30% of the total U.S. cotton acreage was planted to Bollgard cotton varieties (*2*).

Bollgard II cotton event 15985 provides increased control of the major insect pests of cotton such as tobacco budworms, pink bollworms, and cotton bollworms, as well as armyworms, and was produced by the stable insertion of a coding sequence that expresses Cry2Ab2 into the genome of Bollgard cotton. Seed from Bollgard cotton has previously been shown to be compositionally and nutritionally equivalent to that of conventional cotton varieties (*3*). Historical levels of insecticidal proteins in both cotton leaf and seed have been previously reported (*4*). Cry2Ab2 protein is derived from *Bacillus thuringiensis* (*B.t.*), for which there is a history of safe dietary exposure in or on raw agricultural commodities (*5*). Compositional data on Bollgard II cotton was generated for the purpose of safety and nutritional assessment.

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Safety assessment studies conducted on Bollgard II cotton were based on the application of the principle of substantial equivalence, which has been adopted by leading international food and regulatory bodies including the World Health Organization (*6*, *7*), the United Nations Food and Agricultural Organization (*8*), the Organization for Economic Cooperation and Development (*9*−*11*), and the International Life Sciences Institute (*12*). According to this approach, if a new food or feed derived from a genetically modified crop is shown to be substantially equivalent to its conventional counterpart and the introduced proteins pose no safety concerns, then the genetically modified product is considered to be as safe as the food or feed from the conventional plant variety. Insecticidal proteins from *B. thuringiensis* (including Cry1Ac and Cry2Ab proteins) pose no food, feed, or environmental concerns as reviewed by Betz et al. (*13*). The data presented demonstrate the compositional and nutritional equivalence of Bollgard II cotton containing event 15985 to conventional cotton varieties.

MATERIALS AND METHODS

Bollgard II Cotton Containing Event 15985. Bollgard II cotton was produced by transformation of Bollgard cotton Delta and Pine Line 50 (DP50) callus tissue with a vector containing the *cry2Ab2* coding sequence.

Cotton Samples for Compositional Analysis Grown in 1998 Field Trials. Insect-protected cottonseed from Bollgard II was collected from field trials conducted with R_2 seed in 1998. Insect-protected Bollgard II cotton 15985 and control line DP50 were planted in a single block with two 15 ft row plots at Winnsboro, LA; Florence, SC; Starkville, MS; and Corpus Christi, TX; in a single block with one 30 ft row plot at Starkville, MS; and in four replicate blocks at Leland, MS; Loxley, AL; Bossier City, LA; and Maricopa, AZ. Plants were confirmed as positive or negative for the *cry2Ab2* gene by Southern blot analyses. All test and control plots were surrounded by a minimum distance of four rows or 15 ft of conventional cotton. The average distance between successive plots in a replicate was 35 ft, which consisted of a combination of planted conventional cotton buffer and bare ground. Samples of fuzzy cottonseed from Bollgard II and control plots from the eight individual field sites in 1998 were harvested and pooled to produce a composite sample for processing into refined oil and processed cottonseed meal for subsequent analyses. Three reference cottonseed samples grown in the 1998 field season and obtained from Delta and Pine Land Co. were also processed at the same time and so used as additional controls. Cottonseed was processed into refined oil (bleached and deodorized) and into toasted cottonseed meal at the Food Protein Research and Development Center at Texas A&M University (Bryan, TX) under Good Laboratory Practices (GLP) using a solvent extraction (*14*). Cottonseed samples were processed to oil in a fashion designed to simulate industrial practice, with the exception that the samples were processed by batch rather than a continuous commercial operation.

Cotton Samples for Compositional Analysis Grown in 1999 Field Trials. In 1999, field trials were conducted at six locations (Florence, SC; Portland, TX; Leland, MS; Loxley, AL; Bossier City, LA; and Maricopa, AZ), using R_3 seed. Five of the locations were repeat locations of the 1998 field sites. All plots were two 15 ft rows arranged in four replicate blocks at each location. Plants were identified as positive or negative for the *cry2Ab2* gene by event-specific Polymerase Chain Reaction (PCR). The genetic purity of Bollgard II cotton plants was maintained as described for 1998 field trials, by establishing a border of a minimum of four rows or 15 ft of conventional cotton between the plots. In addition to the test and control cotton lines, four commercial cotton varieties were planted in 1999 in single replicate plots at each site as reference lines. These sites provided a variety of environmental conditions representative of regions where Bollgard II cotton lines would be grown as commercial products.

Compositional Analyses. Compositional analyses were conducted to measure proximate (protein, fat, ash, carbohydrate, moisture, and calories), fiber, amino acid, fatty acid, cyclopropenoid fatty acids, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), gossypol, and aflatoxin contents of seed. Compositional analyses of cottonseed oil samples included fatty acid, gossypol levels, and vitamin E. Gossypol levels were assessed in toasted cottonseed meal samples. All compositional analyses were performed at Covance Laboratories, Inc. (Madison, WI), under GLP. The whole seed samples generated in 1998 were shipped to Covance under ambient conditions. In 1999, the seed samples were ground to a fine powder in the presence of dry ice and maintained frozen during shipment and until required for compositional analysis. Brief descriptions of the procedures utilized at Covance are given below.

Proximate Analysis. Protein levels were estimated by determining the total nitrogen content using the Kjeldahl method, as previously described (*15*−*18*). Protein was calculated from total nitrogen using the formula N × 6.25. Fat content of the seed was estimated according to the Soxhlet extraction method (*19*). Ash content was estimated by ignition of a sample in an electric furnace and quantitation of the ash by gravimetric analysis (*20*). Moisture content was determined by loss of weight upon drying in a vacuum oven at 100 °C to a constant weight (*21*, *22*). Carbohydrate levels were estimated by using the fresh weight-derived data and the following equation (*23*):

$$\%\ \text{carbohydrate} = 100\% - (\%\ \text{protein} + \%\ \text{fat} + \%\ \text{ash} + \%\ \text{moisture})$$

Calories were calculated using the Atwater factors with the fresh weight-derived data and the following equation (*24*):

$$\text{calories (kcal/100g)} + (4 \times \%\ \text{protein}) + (9 \times \%\ \text{fat}) + (4 \times \%\ \text{carbohydrates})$$

Fiber Analysis. Crude fiber was quantitated as the loss on ignition of dried residue remaining after digestion of the sample with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions under specific conditions (*25*). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured only on the cottonseed samples generated in 1999. To measure ADF, samples were placed in a fritted vessel and washed with an acidic boiling detergent solution that dissolved the protein, carbohydrate, and ash. An acetone wash removed fats and pigments, and the lignocellulose fraction was collected on the frit and determined gravimetrically (*26*). NDF was measured according to a modified enzymatic method based on a USDA method (*26*) and AACC method 32.20 (*27*). The sample was placed in a fritted vessel and washed with a neutral boiling detergent solution and an acetone wash. Hemicellulose, cellulose, and lignin fractions were collected on the frit and determined gravimetrically.

Minerals. To estimate levels of nine minerals, samples were dried, precharred, and ashed. The ashed samples were treated with hydrochloric acid, then taken to dryness, and dissolved in a 5% hydrochloric acid solution. The amount of each element in these solutions was estimated by an atomic absorption spectrophotometer set at appropriate wavelengths by comparing the signal of the unknown sample with that obtained from standard solutions (*28*−*30*).

Amino Acid Composition. Procedures described in the literature (*31*) were used to estimate the values for 18 amino acids in seed. The procedure for tryptophan required a base hydrolysis with sodium hydroxide. The sulfur-containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct hydrolysis with hydrochloric acid. The individual amino acids were then quantitated using an automated amino acid analyzer.

Fatty Acid and Cyclopropenoid Fatty Acid Composition. The total lipid fraction was extracted from the sample using chloroform and methanol and quantitated gravimetrically. A portion of the lipid fraction was then saponified with a mild alkaline hydrolysis. The free fatty acids were extracted with ethyl ether and hexane. The free fatty acids were then converted to their phenacyl derivatives with 2-bromoacetophenone. The derivatives were quantitated on an HPLC system equipped with an ultraviolet detector (*32*).

Table 1. Fiber and Proximate Composition of Cottonseed from Bollgard II Cotton Event 15985

component	Bollgard II 15985,[a] mean ± SE (range)[e]	control DP50,[a,b] mean ± SE (range)[e]	*p* value[c]	commercial (range)[d] [99% tolerance interval[f]]
ash (% DW)	4.28 ± 0.067 (3.85–4.92)	4.32 ± 0.066 (3.76–5.23)	0.639	(3.87–5.29) [3.29, 5.35]
calories (kcal/100 g of DW)	489.65 ± 5.02 (468.50–520.01)	487.11 ± 5.02 (457.77–501.84)	0.098	(471.34–506.95) [446.86, 524.52]
carbohydrates (% DW)	47.95 ± 1.27 (42.97–52.69)	48.55 ± 1.27 (43.69–52.44)	0.395	(45.28–53.62) [40.12, 55.59]
fat, total (% DW)	21.33 ± 1.00 (17.54–27.42)	20.85 ± 1.00 (15.44–24.29)	0.334	(17.37–25.16) [12.99, 28.53]
fiber, crude (% DW)	16.07 ± 0.83 (13.81–17.95)	16.22 ± 0.83 (13.45–19.31)	0.596	(13.85–17.94) [12.47, 19.93]
fiber, ADF (% DW)[g]	25.68 ± 0.61 (21.40–31.95)	25.26 ± 0.60 (21.10–34.80)	0.540	(21.10–34.80) [17.85, 34.62]
fiber, NDF (% DW)[g]	38.75 ± 0.65 (34.90–46.20)	38.97 ± 0.64 (34.75–43.13)	0.816	(32.92–45.83) [25.35–52.64]
moisture (% FW)	4.86 ± 1.12 (2.32–7.59)	4.88 ± 1.12 (2.91–7.26)	0.850	(2.25–7.49) [0, 10.03]
protein (% DW)	26.26 ± 0.44 (21.45–28.82)	26.12 ± 0.44 (21.76–28.24)	0.409	(24.54–30.83) [20.93, 33.53]

[a] Combined data from 14 U.S. trials (4 replicated and 4 nonreplicated sites in 1998 and 6 replicated sites in 1999). [b] Nontransgenic control in the same varietal background as Bollgard II cotton. [c] Observed significance level from a two-sided *t* test of zero mean difference between Bollgard II and the control line. [d] Range includes data from DP20, DP5415, DP5305, DP5690, DP51, DP2379, DP5409, ST474, SG747, SG125, SG821, FM989, PM1560, Terra292, and Phytogen 952 cotton varieties. [e] Range denotes the lowest and highest individual values across sites and years for each line. [f] 99% tolerance interval to contain 99% of the commercial variety population with 95% confidence. Negative limits were set to zero. [g] Combined data from 8 U.S. trials (4 replicated and 4 nonreplicated sites in 1998).

α-*Tocopherol (Vitamin E).* Refined oil samples were saponified to release the tocopherols, which were then extracted with ethyl ether, followed by quantitation on a high-performance liquid chromatography (HPLC) silica column using fluorescence detection (*33*−*35*).

Aflatoxins. The levels of aflatoxins B_1, B_2, G_1, and G_2 were determined on ground, acid-delinted cottonseed samples. The sample was extracted with a mixture of methanol/water. The extract was diluted with water, and a portion was applied to an antibody affinity column. Aflatoxins were eluted with acetonitrile, and the sample was dried with a stream of nitrogen. The aflatoxins were derivatized with acid to form the more highly fluorescent hemiacetal compound of B_1 and G_2. A portion of the extract was injected on a high-performance liquid chromatography system, and the aflatoxins in the sample were compared with a standard of known concentration (*36*−*39*).

Free and Total Gossypol. For free gossypol, the sample was extracted with aqueous acetone (700 parts of acetone plus 300 parts of distilled water). The solution was then filtered, and the free gossypol was reacted with aniline. For total gossypol analysis, the sample was extracted using a complexing reagent containing acetic acid, 3-amino-1-propanol, and dimethylformamide. The solution was then filtered and the total gossypol reacted with analine (*40*, *41*).

Statistical Analysis of Composition Data. Statistical analyses of the composition data were performed at Certus International, Inc., using the SAS statistical program (*42*). The data from 1998 and 1999 were combined and analyzed using a mixed-model analysis of variance. Least-squares means and ranges for each cottonseed component were computed across all sites for Bollgard II cotton and the control. For a particular component, the difference between the mean of the control and the mean of Bollgard II cotton was considered to be statistically significant if the observed significance level (*p* value) for a two-sided *t* test of zero difference was found to be <0.05. Values determined to be statistically significantly different are identified in **Tables 1**−**3** and **5**.

Data for compositional analysis components from the commercial reference cotton lines were used to develop population tolerance intervals. A tolerance interval is an interval with a specified degree of confidence, $100(1-\alpha)\%$, which contains at least a specified proportion, *p*, of an entire population for the parameter measured. For each compositional analysis component, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the values expressed in the population of commercial varieties. Because negative quantities are not possible, negative calculated lower tolerance bounds were set to zero. No statistical analysis was performed on components measured on the composited, processed oil, and meal samples because there was only one sample per line.

RESULTS

The primary use of cottonseed is as roughage for cattle feed (hulls) and as a high-protein supplement for livestock, swine, poultry, and catfish (meal) (*43*). Refined cottonseed vegetable oil and highly processed cottonseed linters (the fiber remaining after the ginning of seed cotton) are the main cotton products used for food. Linters are cellulose feedstock for many industrial and consumer products. The composition of components important for feed and food uses was assessed for the insect-protected Bollgard II cotton lines and compared to that of the conventional control (DP50), as well as to values reported for other commercial cotton varieties processed and analyzed at the same time.

Proximate and Fiber Composition. Compositional analysis results for cottonseed are presented in **Table 1**. These results show that the levels of proximate components (protein, fat, ash, carbohydrate, calories, and moisture) and fiber in cottonseed of insect-protected Bollgard II cotton were comparable to those in the seed of the control lines. There were no statistically significant differences in proximate levels in the seed between Bollgard II and the control. In addition, these values were within the range determined for commercial varieties analyzed at the same time. The crude fiber and ADF and NDF levels were evaluated in cottonseed. There were no statistically significant differences in any of the fiber values when Bollgard II cotton was compared with the control.

Amino Acid Composition. These results are presented in **Table 2**. The contents of the 18 amino acids in the seed of Bollgard II cotton were comparable to those in the seed of the control line. There were no amino acid results for Bollgard II cotton that were statistically significantly different from the parental control, cotton variety DP50.

Fatty Acid Composition. The fatty acid results, presented in **Table 3**, show that the fatty acid levels in the seed of Bollgard II cotton were comparable to those observed in the seed of the

Table 2. Amino Acid Content of Cottonseed from Bollgard II Cotton Event 15985

amino acid	Bollgard II 15985,[a] mean ± SE (range)[e]	control DP50,[a,b] mean ± SE (range)[e]	*p* value[c]	commercial (range)[d] [99% tolerance interval[f]]
alanine (% total AA)	4.33 ± 0.026 (4.20–4.52)	4.33 ± 0.026 (4.15–5.30)	0.910	(4.16–4.41) [3.98, 4.53]
arginine (% total AA)	11.45 ± 0.13 (10.69–11.95)	11.62 ± 0.13 (10.83–15.18)	0.060	(11.28–12.51) [10.72, 13.13]
aspartic acid (% total AA)	9.94 ± 0.070 (9.65–10.49)	9.93 ± 0.070 (9.63–12.37)	0.832	(9.73–9.99) [9.52, 10.20]
cystine (% total AA)	1.80 ± 0.030 (1.64–2.03)	1.83 ± 0.030 (1.61–2.32)	0.506	(1.60–1.92) [1.51, 2.01]
glutamic acid (% total AA)	20.98 ± 0.16 (20.09–21.76)	21.06 ± 0.16 (20.24–21.48)	0.073	(20.76–21.61) [20.36, 22.06]
glycine (% total AA)	4.59 ± 0.025 (4.50–4.72)	4.61 ± 0.025 (4.46–5.72)	0.686	(4.44–4.58) [4.38, 4.67]
histidine (% total AA)	3.06 ± 0.016 (3.00–3.14)	3.09 ± 0.015 (3.01–3.88)	0.135	(3.00–3.12) [2.95, 3.18]
isoleucine (% total AA)	3.56 ± 0.032 (3.29–3.79)	3.56 ± 0.032 (3.37–4.46)	0.949	(3.10–3.67) [3.00, 3.91]
leucine (% total AA)	6.53 ± 0.046 (6.31–6.86)	6.53 ± 0.046 (6.32–8.11)	0.990	(6.27–6.65) [6.09, 6.75]
lysine (% total AA)	5.17 ± 0.047 (4.81–5.46)	5.18 ± 0.047 (4.86–6.60)	0.686	(4.85–5.37) [4.54, 5.74]
methionine (% total AA)	1.71 ± 0.039 (1.55–1.97)	1.71 ± 0.039 (1.49–2.28)	0.912	(1.46–1.88) [1.40, 1.86]
phenylalanine (% total AA)	5.65 ± 0.037 (5.53–5.79)	5.70 ± 0.037 (5.51–7.23)	0.434	(5.56–5.77) [5.44, 5.88]
proline (% total AA)	4.18 ± 0.042 (3.99–4.46)	4.21 ± 0.042 (3.93–5.30)	0.727	(4.06–4.28) [4.00, 4.39]
serine (% total AA)	4.73 ± 0.061 (4.23–5.04)	4.77 ± 0.060 (4.16–5.87)	0.248	(4.45–4.86) [4.28, 5.13]
threonine (% total AA)	3.48 ± 0.064 (3.29–3.77)	3.51 ± 0.064 (3.33–4.26)	0.582	(3.26–3.59) [3.16, 3.66]
tryptophan (% total AA)	1.06 ± 0.035 (0.95–1.24)	1.06 ± 0.035 (0.94–1.40)	0.482	(0.97–1.21) [0.80, 1.36]
tyrosine (% total AA)	2.80 ± 0.028 (2.68–2.91)	2.82 ± 0.028 (2.71–3.46)	0.661	(2.65–2.92) [2.50, 3.05]
valine (% total AA)	5.01 ± 0.053 (4.72–5.34)	4.99 ± 0.053 (4.72–6.24)	0.751	(4.76–5.14) [4.55, 5.32]

[a] Combined data from 14 U.S. sites (4 replicated and 4 nonreplicated sites in 1998 and 6 replicated sites in 1999). [b] Nontransgenic control in the same varietal background as Bollgard II cotton. [c] Observed significance level from a two-sided *t* test of zero mean difference between Bollgard II and the control line. [d] Range includes data from DP20, DP5415, DP5305, DP5690, DP51, DP2379, DP5409, ST474, SG747, SG125, SG821, FM989, PM1560, Terra292, and Phytogen 952 cotton varieties. [e] Range denotes the lowest and highest individual values across sites and years for each line. [f] 99% tolerance interval to contain 99% of the commercial variety population with 95% confidence. Negative limits were set to zero.

control line. No statistically significant differences in the levels of fatty acids between Bollgard II cotton and control were observed for 6 of the 10 fatty acids analyzed. Statistically significant differences in fatty acid content between Bollgard II cotton and the control were noted for myristic (14:0), stearic (18:0), linolenic and γ-linolenic (18:3), and arachidic (22:0) fatty acids. The contents of myristic, stearic, linolenic and γ-linolenic, and arachidic fatty acids in Bollgard II cotton were 16.8, 14.3, 13.3, and 7.4% higher, respectively, than in the control line in the data combined from 1998 and 1999 field trials. The range of values for stearic and linolenic and γ-linolenic acids were within the 99% tolerance interval for the commercial varieties, suggesting that the two groups are within the same population and confirming that the difference observed is unlikely to be of biological significance. The ranges of values obtained for myristic and arachidic acids were similar to ranges reported in the literature for other commercial cotton varieties: 0.5−2.5% for myristic and 0.5−0.41% for arachidic (*44*, *45*).

The cyclopropenoid fatty acids—sterculic, malvalic, and dihydrosterculic—are unique fatty acids present in cotton. The levels of cyclopropenoid fatty acids are greatly decreased during processing (*44*). There were no statistically significant differences in levels of sterculic or malvalic acids between Bollgard II cotton and the control. Dihydrosterculic levels were statistically significantly different between Bollgard II and the control. The range of values for dihydrosterculic acid was within the 99% tolerance interval for the commercial varieties, suggesting that the two groups are within the same population and confirming that the difference observed is unlikely to be of biological significance.

The refined oil fractions resulted from the processing of a single composite seed sample for each line grown in 1998, and so data were not subjected to statistical analyses. The fatty acid levels in oil derived from Bollgard II cottonseed appear to be similar to levels in refined oil from the control and the commercial lines (**Table 4**).

Mineral Composition. The mineral levels of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were measured in cottonseed. The results, presented in **Table 5**, show that the mineral content in the seed of Bollgard II cotton was comparable to that observed in the seed of the control line. Statistically significant differences in mineral content were noted for copper, iron, and phosphorus between Bollgard II cotton and the control. The contents of copper, iron, and phosphorus in Bollgard II cotton were 4.6, 6, and 5.6% lower, respectively, than in the control line in the data combined from the 1998 and 1999 field trials. However, these small differences are unlikely to be of biological significance as these mean values were within the commercial range, and the ranges of values were within the 99% tolerance interval for the

Table 3. Fatty Acid Composition of Cottonseed from Bollgard II Cotton Event 15985

fatty acid	Bollgard II 15985,[a] mean ± SE (range)[e]	control DP50,[a,b] mean ± SE (range)[e]	*p* value[c]	commercial (range)[d] [99% tolerance interval[f]]
14:0 myristic (% total FA)	1.18 ± 0.054[g] (0.88–2.94)	1.01 ± 0.054 (0.77–2.15)	0.002	(0.55–2.40) [0.0, 2.49]
15:0 pentadecanoic (% total FA)	0.087 ± 0.039 (0.050–0.14)	0.091 ± 0.039 (0.050–0.16)	0.489	(0.050–0.17) [0.0, 0.26]
16:0 palmitic (% total FA)	25.33 ± 0.46 (23.00–27.90)	25.47 ± 0.46 (23.51–28.10)	0.467	(21.23–27.90) [18.96, 30.33]
16:1 palmitoleic (% total FA)	0.64 ± 0.065 (0.33–1.97)	0.67 ± 0.065 (0.43–1.74)	0.454	(0.55–1.16) [0.34, 0.92]
18:0 stearic (% total FA)	2.55 ± 0.096[g] (2.18–3.10)	2.23 ± 0.095 (2.00–2.71)	<0.001	(1.99–3.11) [1.43, 3.20]
18:1 oleic (% total FA)	15.71 ± 0.39 (13.60–18.10)	15.61 ± 0.39 (12.90–17.63)	0.375	(13.90–20.10) [11.03, 21.69]
18:2 linoleic (% total FA)	52.83 ± 0.62 (47.70–55.52)	53.38 ± 0.62 (49.50–57.10)	0.227	(46.00–56.88) [43.47, 62.76]
18:3 linolenic and γ-linolenic (% total FA)	0.17 ± 0.040[g] (0.050–0.30)	0.15 ± 0.040 (0.050–0.32)	0.009	(0.050–0.25) [0.0, 0.40]
20:0 arachidic (% total FA)	0.29 ± 0.0093[g] (0.25–0.43)	0.27 ± 0.0093 (0.24–0.34)	0.049	(0.25–0.33) [0.20, 0.36]
22:0 behenic (% total FA)	0.14 ± 0.0056 (0.12–0.21)	0.14 ± 0.0055 (0.12–0.24)	0.714	(0.13–0.17) [0.093, 0.19]
dihydrosterculic C19 (% total FA)	0.18 ± 0.0072[g] (0.12–0.22)	0.15 ± 0.0072 (0.12–0.19)	0.001	(0.13–0.24) [0.071, 0.31]
malvalic (% total FA)	0.45 ± 0.025 (0.26–0.71)	0.42 ± 0.025 (0.17–0.61)	0.383	(0.33–0.58) [0.24, 0.67]
sterculic (% total FA)	0.28 ± 0.024 (0.19–0.58)	0.24 ± 0.024 (0.13–0.43)	0.433	(0.21–0.56) [0.0, 0.58]

[a] Combined data from 14 U.S. sites (4 replicated and 4 nonreplicated sites in 1998 and 6 replicated sites in 1999). [b] Nontransgenic control in the same varietal background as Bollgard II cotton. [c] Observed significance level from a two-sided *t* test of zero mean difference between Bollgard II and the control line. [d] Range includes data from DP20, DP5415, DP5305, DP5690, DP51, DP2379, DP5409, ST474, SG747, SG125, SG821, FM989, PM1560, Terra292, and Phytogen 952 cotton varieties. [e] Range denotes the lowest and highest individual values across sites and years for each line. [f] 99% tolerance interval to contain 99% of the commercial variety population with 95% confidence. Negative limits were set to zero. [g] Statistically significantly different from the control at the 5% level.

Table 4. Composition of Oil Processed from Bollgard II Event 15985 Cottonseed

component[a]	Bollgard II[b] 15985	control[b,c] DP50	commercial reference range[d]
myristic (14:0)	1.32	1.06	0.923–1.45
pentadecanoic	<0.100	<0.100	<0.100
palmitic (16:0)	23.9	25.3	22.7–26.3
palmitoleic (16:1)	0.832	0.780	0.852–0.954
heptadecanoic (17:0)	<0.100	<0.100	<0.100
stearic (18:0)	2.04	2.04	1.98–2.13
oleic (18:1)	15.1	14.7	15.8–17.8
linoleic (18:2)	55.6	54.9	51.0–54.4
linolenic and γ-linoleic (18:3)	0.171	0.145	0.120–0.136
arachidic (20:0)	0.176	0.178	0.178–0.203
behenic (22.0)	<0.100	<0.100	<0.100
malvalic (C17)	0.378	0.377	0.294–0.405
sterculic (C18)	0.205	0.217	0.216–0.289
dihydrosterculic (C19)	0.165	0.146	0.179–0.202
lignoceric (24:0)	<0.100	<0.100	<0.100
vitamin E	59.8	53.4	46.2–58.5

[a] Fatty acids are expressed as percentage of total fatty acids, and vitamin E is expressed as mg/100 g of fresh weight. [b] Values represent samples pooled from eight U.S. field sites in 1998. [c] Nontransgenic control in the same varietal background as Bollgard II cotton. [d] Range includes data from PM2200RR, DP1266, and ST474 cotton varieties.

commercial varieties, establishing with a 95% confidence level that the two groups for these analytes are within the same population.

Gossypol Composition. Levels of total and free gossypol were measured in cottonseed, toasted meal, and refined oil, and the results, presented in **Table 6**, show that the gossypol content in the seed, meal, and oil of Bollgard II cotton was comparable to that observed in the seed of the control line. The free and total gossypol results for Bollgard II cotton were not statistically significantly different from the control DP50. The toasted meal and refined oil fractions resulted from the processing of a single composite seed sample for each line grown in 1998, and so data were not subjected to statistical analyses. Total gossypol and free gossypol were not detected in the refined oil fractions; in toasted meal the levels in Bollgard II cotton were within the commercial reference range.

Aflatoxin Composition. The levels of the four primary aflatoxins (B_1, B_2, G_1, and G_2) in the Bollgard II cottonseed, control, and reference commercial cottonseeds were shown to be undetectable at a sensitivity of 1.00 ppb.

Vitamin E Composition. The levels of the antioxidant vitamin E were similar in cottonseed oil from Bollgard II cotton and the control (**Table 4**) and similar to levels (136−660 mg/kg) previously reported in the literature (*46*, *47*).

Feeding Studies. The nutritional equivalence of Bollgard II cotton and conventional cotton varieties has been evaluated in dairy cows, catfish, and quail. The dairy study used lactating multiparous Argentinean Holstein cows in a Latin square experimental design. The experiment consisted of four treatment groups that included Bollgard II cotton event 15985 and a parental control variety. Each treatment group was replicated three times and consisted of a total of three cows, and each of the four feeding periods was 4 weeks in duration. The cottonseed used in the feeding study was produced in field trials in Argentina, and raw cottonseed meal was fed to the dairy cows at a dietary concentration of 2.75 kg/cow/day or ∼10% of the total dry matter intake. Results of the study indicated that Bollgard II cotton performed similarly to the control cottonseed and did not affect dry matter intake, milk yield, milk composition, and body condition score of the dairy cows under controlled

Table 5. Mineral Composition of Cottonseed from Bollgard II Cotton Event 15985

mineral	Bollgard II 15985,[a] mean ± SE (range)[e]	control DP50,[a,b] mean ± SE (range)[e]	*p* value[c]	commercial (range)[d] [99% tolerance inteval[f]]
calcium (% DW)	0.14 ± 0.013 (0.11–0.19)	0.14 ± 0.013 (0.10–0.20)	0.678	(0.10–0.33) [0.0, 0.34]
copper (mg/kg of DW)	7.08 ± 0.65[g] (2.94–10.12)	7.42 ± 0.65 (3.33–10.45)	0.001	(3.54–11.14) [0.0, 14.37]
iron (mg/kg of DW)	48.88 ± 2.13[g] (36.07–57.56)	52.00 ± 2.13 (39.27–72.15)	<0.001	(40.58–56.54) [34.22, 63.93]
magnesium (% DW)	0.40 ± 0.0069 (0.36–0.47)	0.41 ± 0.0069 (0.36–0.47)	0.132	(0.37–0.46) [0.32, 0.50]
manganese (mg/kg of DW)	14.03 ± 0.42 (11.96–17.09)	14.20 ± 0.42 (11.64–22.16)	0.569	(11.06–18.31) [7.01, 21.59]
phosphorus (% DW)	0.68 ± 0.025[g] (0.56–0.83)	0.72 ± 0.025 (0.56–0.86)	0.003	(0.60–0.84) [0.55, 0.88]
potassium (% DW)	1.11 ± 0.038 (0.99–1.24)	1.12 ± 0.038 (1.02–1.23)	0.624	(0.98–1.24) [0.84, 1.33]
sodium (% DW)	0.18 ± 0.046 (0.067–0.47)	0.18 ± 0.046 (0.040–0.73)	0.886	(0.0054–0.74) [0.0, 0.57]
zinc (mg/kg DW)	39.69 ± 2.42 (27.70–52.50)	40.19 ± 2.42 (29.73–48.62)	0.327	(30.21–47.75) [18.15, 62.27]

[a] Combined data from 14 U.S. sites (4 replicated and 4 nonreplicated sites in 1998 and 6 replicated sites in 1999). [b] Nontransgenic control in the same varietal background as Bollgard II cotton. [c] Observed significance level from a two-sided *t* test of zero mean difference between Bollgard II and the control line. [d] Range includes data from DP20, DP5415, DP5305, DP5690, DP51, DP2379, DP5409, ST474, SG747, SG125, SG821, FM989, PM1560, Terra292, and Phytogen 952 cotton varieties. [e] Range denotes the lowest and highest individual values across sites and years for each line. [f] 99% tolerance interval to contain 99% of the commercial variety population with 95% confidence. Negative limits were set to zero. [g] Statistically significantly different from the control at the 5% level.

Table 6. Gossypol Composition in Cottonseed, Meal, and Oil from Bollgard II Cotton Event 15985

component	Bollgard II 15985,[a] mean ± SE (range)[e]	control DP50,[a,b] mean ± SE (range)[e]	*p* value[c]	commercial (range)[d] [99% tolerance interval[f]]
seed				
free gossypol (% DW)	0.85 ± 0.036 (0.56–1.04)	0.87 ± 0.036 (0.56–1.07)	0.566	(0.53–1.20) [0.37, 1.22]
total gossypol (% DW)	0.96 ± 0.043 (0.73–1.29)	0.98 ± 0.043 (0.71–1.23)	0.728	(0.57–1.42) [0.41, 1.42]
meal				
free gossypol (% FW)	0.037	0.041	N/A	(0.025–0.068)[g]
total gossypol (% FW)	0.986	1.04	N/A	(0.933–1.43)[g]
oil				
free gossypol (% FW)	<0.005	<0.005	N/A	<0.005[g]
total gossypol (% FW)	<0.005	<0.005	N/A	<0.005[g]

[a] Seed values represent combined data from 14 U.S. sites (4 replicated and 4 nonreplicated sites in 1998 and 6 replicated sites in 1999). Meal and oil values represent samples pooled from 8 U.S. field trials in 1998. [b] Nontransgenic control in the same varietal background as Bollgard II cotton. [c] Observed significance level from a two-sided *t* test of zero mean difference between Bollgard II and the control line. [d] Range includes data from DP20, DP5415, DP5305, DP5690, DP51, DP2379, DP5409, ST474, SG747, SG125, SG821, FM989, PM1560, Terra292, and Phytogen 952 cotton varieties. [e] Range denotes the lowest and highest individual values across sites and years for each line. [f] 99% tolerance interval to contain 99% of the commercial variety population with 95% confidence. Negative limits were set to zero. [g] Range includes data from PM2200RR, DP1266, and ST474 cotton varieties.

feeding conditions (*48*). Results of this study confirm that cottonseed from Bollgard II cotton is as wholesome and nutritious as conventional cottonseed for feed for dairy cows.

A catfish study was conducted with channel catfish fed processed cottonseed meal from either Bollgard II cotton or the parental control variety at a diet incorporation of 20% (w/w), which was based on the typical amount of cottonseed meal added to commercial catfish diets. Cottonseed used in this study was produced in field trials in the United States and was processed to toasted cottonseed meal according to standard commercial processing methods. The cottonseed diet was formulated and fed twice daily to 100 catfish per treatment group (five replicates with 20 catfish each) for a period of 8 weeks. Fish in each aquarium were counted and weighed at study initiation and again at 4 and 8 weeks after study initiation. Mortality and behavior of the fish were recorded daily. At the end of the 8-week study, five fish from each aquarium were collected, pooled, and processed to determine percent of moisture, crude protein, fat, and ash content of the fillets. There were no significant differences in survival, weight gain, feed conversion ratio, or fillet composition in channel catfish fed diets containing Bollgard II cotton compared to the control (**Table 7**). These data demonstrate that processed cottonseed meal from Bollgard II cotton is as safe and as nutritious as conventional cottonseed meal for use in catfish diet.

To assess any potential impact of Bollgard II cottonseed on birds, a quail study was also conducted. Bobwhite quail chicks were fed diet containing 10% raw cottonseed meal from Bollgard II cotton and control cotton. This feeding level of cottonseed approximates consumption of 400 seeds/kg of body weight per bird. Cottonseed used in this study was produced in field trials in the United States. The cottonseed diet was formulated and fed to 20 birds per treatment group (four replicates with five birds each) for a period of 5 days and then switched to basal diet for the remaining 3 days of the study. Feed consumption was measured daily for each replicate of each

Table 7. Mean Feed Consumption, Weight Gain, Feed Conversion Ratio, and Survival of Channel Catfish Fed Cottonseed Meal from Bollgard II Cotton Event 15985[a]

	Bollgard II 15985	control DP50	reference DP1266	reference ST474
feed consumption (g/fish)	67.3	64.6	67.5	68.0
weight gain[b] (g/fish)	47.0	40.2	46.0	47.2
feed conversion ratio[c]	1.44	1.61	1.47	1.45
survival (%)	100	100	100	100
visceral fat (%)	2.77	3.22	2.85	2.65
fillet moisture (%)	77.9	78.1	77.8	78.5
fillet fat (%)	4.00	3.74	3.98	3.66
fillet protein (%)	16.9	17.3	17.3	16.7
fillet ash (%)	1.14	1.16	1.15	1.13

[a] Study conducted at Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, Stoneville, MS. Means in each column were found not to differ ($p > 0.05$, one-way analysis of variance). [b] Mean initial weight was 8.2 g/fish. [c] Feed conversion ratio = weight of feed consumption (based on 90% dry matter)/weight gain of live fish.

Table 8. Mean Feed Consumption and Individual Body Weights of Northern Bobwhite Quail Fed Cottonseed Bollgard II Cotton Event 15985[a]

	feed consumption[b] (mean g/bird/day)					
	exposure period					post exposure period
treatment	days 0–1	days 1–2	days 2–3	days 3–4	days 4–5	days 6–8
Bollgard II 15985	5.8	9.3	11.5	10.5	11.8	9.5
control DP50	6.8	8.5	10.5	11.0	13.5	10.5
reference DP1266	6.5	9.3	11.8	10.5	11.0	9.0
reference ST474	6.8	9.0	12.8	11.8	13.0	9.3

		mean individual body weights[c] (g)			total
treatment	replicate	day 0	day 5	day 8	change
Bollgard II 15985	1	22 (3)	29 (5)	35 (9)	13 (6)
	2	22 (2)	29 (4)	38 (6)	16 (4)
	3	22 (2)	30 (3)	39 (4)	17 (3)
	4	21 (3)	31 (3)	40 (3)	19 (2)
control DP50	1	22 (2)	31 (4)	38 (5)	16 (3)
	2	22 (2)	32 (4)	41 (5)	19 (4)
	3	23 (3)	30 (4)	39 (6)	17 (6)
	4	23 (3)	33 (7)	43 (9)	21 (6)
reference DP1266	1	22 (2)	31 (3)	39 (5)	17 (5)
	2	22 (4)	31 (7)	42 (7)	19 (5)
	3	22 (3)	32 (4)	39 (4)	17 (3)
	4	22 (2)	29 (6)	35 (7)	14 (6)
reference ST474	1	21 (2)	31 (2)	36 (4)	15 (3)
	2	21 (2)	31 (4)	40 (4)	19 (3)
	3	22 (2)	30 (3)	38 (4)	16 (3)
	4	21 (2)	31 (4)	38 (6)	16 (6)

[a] Study conducted at Wildlife International, Ltd., Easton, MD. [b] Mean of four replicate pens each containing five birds. [c] Mean of five birds per replicate. Standard deviation in parentheses.

treatment group. Individual body weights were recorded at study initiation, day 5, and at study termination. There was no mortality or overt signs of toxicity in birds from either the Bollgard II cotton or control treatment groups. There was no difference in the feed consumption or weight gain for chicks eating diet with Bollgard II cottonseed meal versus the control cottonseed meal (**Table 8**). These data, taken together with the dairy cow and catfish feeding studies, as well as compositional data presented in this paper, demonstrate that Bollgard II cotton is as safe and as nutritious as conventional cotton for food and feed use.

DISCUSSION

Compositional analysis results generated from 14 field trials over a period of two years show that the cottonseed of Bollgard II cotton is comparable in composition to that of control cotton lines and to conventional cotton varieties. The incorporation of commercial reference cotton varieties into field trials suggests that the few statistically significant differences observed are most likely due to random chance and are unlikely to be biologically significant. This is supported by the facts that (a) statistically significant differences were not observed in comparisons of Bollgard II cotton with the commercial lines and (b) all component values determined for Bollgard II cotton were either within the 99% tolerance interval for commercial varieties or within the published literature ranges. The compositional analyses, together with the supporting feed performance studies, lead to the conclusion that Bollgard II cotton containing event 15985 is compositionally and nutritionally equivalent to the control cotton line as well as to commercial cotton varieties.

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