Glyphosate-Tolerant Corn: The Composition and Feeding Value of Grain from Glyphosate-Tolerant Corn Is Equivalent to That of Conventional Corn (*Zea mays* L.)

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Glyphosate-tolerant (Roundup Ready) corn line GA21 has been developed by genetic modification to tolerate glyphosate, the active ingredient in Roundup herbicide. The purpose of this study was to evaluate the compositional and nutritional safety of corn line GA21 compared to that of conventional corn. Compositional analyses were conducted to measure proximate, fiber, amino acid, fatty acid, and mineral contents of grain and proximate, fiber, and mineral contents of forage collected from 16 field sites over two growing seasons. The nutritional safety of corn line GA21 was evaluated in a poultry feeding study conducted with 2-day old, rapidly growing broiler chickens, at a dietary concentration of 50−60% w/w. Compositional analysis results showed that, except for a few minor differences that are unlikely to be of biological significance, the grain and forage of GA21 corn were comparable in their composition to that of the control corn line and to conventional corn. Results from the poultry feeding study showed that there were no differences in growth, feed efficiency, adjusted feed efficiency, and fat pad weights between chickens fed with GA21 grain or with parental control grain. These data taken together demonstrate that Roundup Ready corn is as safe and nutritious as conventional corn for food and feed use.

Keywords: *Corn (Zea mays L.); genetically modified; glyphosate tolerant; Roundup*

INTRODUCTION

Genetically modified crops offer a wide variety of benefits to growers. Recognition of these benefits has been demonstrated by the planting of 100 million acres (or 40 million hectares) of genetically modified crops globally in 1999 (James, 1999). Between 1998 and 1999, the global area planted to genetically modified crops increased by 12 million hectares, or 44%, with seven different genetically modified crops being grown commercially in 12 different countries. Herbicide tolerance has been introduced, through genetic modification, into a number of crops including corn. Glyphosate, which is the active ingredient in the herbicide Roundup, is one of the most widely used herbicides in the world. Since 1996, glyphosate-tolerant or Roundup Ready crop varieties have been developed and commercialized for soybean (*Glycine max*) (1996), canola (*Brassica napus*) (1996), cotton (*Gossypium hirsutum*) (1997), and corn (*Zea mays* L.) (1998).

The introduction of Roundup Ready (trademark of Monsanto Co.) corn in the United States in 1998, and in Canada and other countries in 1999, has provided growers with a valuable weed control option that offers significant advantages including superior weed control, agronomic benefits (e.g., fit with reduced-tillage systems), environmental benefits (e.g., resistance to leaching), and extremely low toxicity to mammals, birds, and fish (Malik et al., 1989).

Roundup Ready corn line GA21 was produced by the stable insertion of a gene that expresses a glyphosate-tolerant, modified corn 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS) protein in corn that is 99.3% identical in its amino acid sequence to the wild-type corn EPSPS enzyme (LeBrun et al., 1997). Glyphosate acts by inhibition of EPSPS, an enzyme involved in the shikimic acid pathway for aromatic acid biosynthesis in plants and microorganisms (Steinruken and Amrhein, 1980). EPSPS is present in plants, bacteria, and fungi but not in animals (Levin and Sprinson, 1964). In plants, EPSPS is localized in the chloroplasts or plastids (della-Cioppa et al., 1986). Expression of mEPSPS fused to an optimized transit peptide enables targeting of this protein to the chloroplast, thereby conferring glyphosate tolerance to the corn plant while meeting the plant's needs for the production of aromatic amino acids.

Safety assessment studies conducted on corn line GA21 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 (WHO, 1991, 1995), the United Nations Food and Agricultural Organization (FAO, 1996), the Organization for Economic Cooperation and Development (OECD, 1993, 1996, 1997), and the International Life Sciences Institute (ILSI, 1997). According to this principle, if a new food or feed derived from a genetically modified crop is shown to be substantially equivalent to its conventional counterpart, then the genetically modified product is considered as safe as the food or feed from the conventional plant

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variety. Government authorities in Japan (MHW 1996), Canada (Health Protection Branch, 1994), the United States (FDA, 1992), the United Kingdom (ACNFP, 1991), the European Union (EC, 1997), and many other countries have adopted substantial equivalence as an integral part of the basis for the safety assessment of food derived from crops developed through biotechnology and have approved numerous products based on this approach.

The food and feed safety assessment of corn line GA21 consists of three main components: (1) composition equivalence demonstrated for corn grain and forage; (2) nutritional equivalence as confirmed in animal feeding studies; and (3) safety assessment of the introduced protein (mEPSPS). This paper describes the compositional analysis of GA21 grain and forage and the evaluation of the nutritional equivalence of GA21 grain in a poultry feeding study. The characterization of mEPSPS, the introduced protein, will be the subject of a future publication. The data presented demonstrate the compositional and nutritional equivalence of Roundup Ready corn line GA21 to conventional corn varieties.

MATERIALS AND METHODS

Roundup Ready Corn Line GA21. This line was produced by transformation of corn callus tissue with a vector containing the mEPSPS gene (LeBrun et al., 1997).

Corn Samples for Compositional Analysis. Grain and forage samples were collected from field trials conducted in 1996 and 1997. In the 1996 field trials, corn plants were grown at five sites in the United States (Dekalb, IL; Jerseyville, IL; Monmouth, IL; Mystic, CT; and Thomasboro, IL). A population of positive segregant plants of Roundup Ready corn line GA21 (i.e., those containing the mEPSPS gene) was utilized. A population of negative segregant plants of corn line GA21 (i.e., those lacking the mEPSPS gene) was utilized as the control. Plants were identified as positive or negative segregants by Polymerase Chain Reaction (PCR) analysis. Roundup Ready corn line GA21 was planted in two plots at each site, except at the Mystic site: one plot was treated with Roundup herbicide and the other was untreated. The genetic purity of transgenic plants was maintained by bagging the tassels and ear shoots at anthesis and self-pollinating each plant by hand. However, at the Thomasboro, IL, and Jerseyville, IL, sites, the genetic purity of the grain samples from the treated plots was compromised due to the lack of self-pollination. Consequently, these were not used for compositional analysis. As limited data from treated plots were available, only data from the untreated grain samples are reported in this paper for the 1996 field trials. Forage was collected at the late dough/early dent stage and grain at normal kernel maturity. Forage and grain samples were ground to a fine powder in the presence of dry ice and maintained frozen until required for compositional analysis.

In 1997, grain and forage samples were collected from three field studies: a U.S. single-site replicated trial conducted at Clarence, MO; U.S. multisite nonreplicated trials conducted at Jerseyville, IL; Noblesville, IN; Richland, IA; Webster City, IA; Andale, KS; and York, NE; and, EU multisite trials conducted at Paderno, Italy; Comasso, Italy; Mallen, Spain; and Alagon, Spain. Roundup Ready corn line GA21 was the test line, and the parental line, DK626, was the control line in these trials. In addition to the test and control corn lines, five to six conventional commercial lines were planted at each site as reference lines. The U.S. single-site replicated trial was based on a randomized complete block design to allow for a within-site statistical evaluation of composition data. Roundup Ultra herbicide was applied to plots containing Roundup Ready plants. The genetic purity of plants was maintained, and forage and grain samples were collected as described for the 1996 field trials.

Compositional Analyses. Compositional analyses were conducted to measure proximate (protein, fat, ash, carbohydrate, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), amino acid, fatty acid, calcium, and phosphorus contents of grain; and proximate, ADF, NDF, calcium, and phosphorus contents of forage. All compositional analyses were performed at Covance, Laboratories, Inc. (Madison, WI). Brief descriptions of the procedures utilized are described below.

Proximate Analysis. Protein levels were estimated by determining the total nitrogen content using the Kjeldahl method, as previously described (AOAC, 1995a,b; Bradstreet, 1965; Kalthoff and Sandell, 1948). Protein was calculated from total nitrogen using the formula N × 6.25. Fat content of the grain was estimated by using the Soxlet extraction method (AOAC, 1995c). Fat content of forage was determined by fat-acid hydrolysis followed by extraction with ether and hexane (AOAC, 1995d,e).

Ash content was estimated by ignition of a sample in an electric furnace and quantitation of the ash by gravimetric analysis (AOAC, 1995f). Moisture content was determined by loss of weight upon drying in a vacuum oven at 100 °C to a constant weight (AOAC, 1995g,h). Carbohydrate levels were estimated by using the fresh weight-derived data and the following equation (USDA, 1973):

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

Fiber Analysis. ADF was estimated by treating the sample with an acidic boiling detergent solution to dissolve the protein, carbohydrate, and ash. An acetone wash removed the fats and pigments. The lignocellulose fraction was collected and determined gravimetrically (USDA, 1970). NDF was estimated by treating the sample with a neutral boiling detergent solution to dissolve the protein, enzymes, carbohydrate, and ash. An acetone wash removed the fats and pigments. Hemicellulose, cellulose, and lignin fractions were collected and determined gravimetrically (USDA, 1970; AACC, 1983).

Minerals. To estimate calcium levels, samples were dried, precharred, and ashed. The ashed samples were treated with nitric acid and then taken to dryness, reashed, and dissolved in a 4% hydrochloric acid solution. The amount of calcium in these solutions was estimated by an atomic absorption spectrophotometer at a wavelength of 422.7 nm by comparing the signal of the unknown sample with that obtained from standard solutions containing 1% lanthanum and 5% hydrochloric acid (AOAC, 1995i−k). To estimate phosphorus levels, samples were dried, precharred, and ashed. The ashed samples were treated with nitric acid, reashed, and dissolved in hydrochloric acid to yield a solution of 4% hydrochloric acid. The amount of phosphorus was estimated colorimetrically at a wavelength of 420 nm by comparing aliquots of the samples, each reacted with a molybdovanadate solution, to standards prepared in the same manner (AOAC, 1995i,j,l).

Amino Acid Composition. Three procedures described in the literature (AOAC, 1995m) were used to estimate the values for 18 amino acids in grain. 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 Composition. The lipid in the grain samples was extracted and saponified with 0.5 N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride/methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation (AOCS, 1981).

Statistical Analysis of Composition Data. Statistical analyses of the composition data were performed using the SAS statisical program (SAS Institute, 1990). For the statistical analysis of the 1996 data, least squares means and ranges for each combination of tissue, component, and sample type (i.e., Roundup Ready corn line GA21 or the control) were computed across all sites. For a particular component/tissue combination, the difference between the mean of the control and the mean of GA21 was considered to be statistically significant at the 5% level if the p value was found to be <0.05 (the p value is the observed significance level for a two-sided t test of zero difference). The lower and upper 95% confidence intervals for the mean difference of GA21 from control corn were also calculated. Statistical analysis data are not included in this paper; however, values determined to be statistically significantly different are identified as such in Tables 1−4.

Statistical analysis of the 1997 data was conducted separately for the three studies as well as combined. Separate, detailed statistical analyses of the three 1997 studies were conducted to assess the effects of geography and within-site replication. The results of these analyses, which were consistent with the conclusions reached in this paper, are not described for reasons of brevity. For the analysis of all three studies combined, the U.S. single-site replicated study was treated as an additional nonreplicated site with only the line means being used. The sites for all three studies were then simply treated as a single composite study containing 11 sites ($1 + 6 + 4$). For comparison of GA21 with the conventional control, all lines were treated as fixed effects. The comparison to the control was by means of a simple pooled-variance t statistic. For each compositional measure, the p value for a test of GA21 equal to the control, the observed difference of GA21 from the control, and lower and upper 95% confidence intervals for the mean difference of GA21 from the control were calculated. For comparing GA21 with the population of commercial reference lines, the analysis was similar to that described above. In this case, however, all conventional lines (control and reference) were treated as another level of random effects in the mixed linear model. In this case, the t statistic compares the means of the two populations of lines from which (1) GA21 and (2) the other commercial lines are considered samples. The p values and 95% confidence intervals for this comparison were also computed in the mixed linear model procedure of SAS.

Corn Samples for Poultry Feeding Study. Corn grain from Roundup Ready line GA21/DK580 and its parental control line, DK580, were obtained from plants grown in Kihei, HI, during 1997. Two plots were established for corn line GA21: in one plot, plants at the V4−V6 stage were treated with Roundup herbicide while plants in the other plot were left untreated. Only GA21 grain from the untreated plots was used for the poultry feeding study. Grain from five conventional commercial lines, obtained from plots grown in Illinois during 1997, were used as the reference lines in this feeding study. The grain was formulated into test diets at Colorado Quality Research (CQR) Inc., Wellington, CO. The dietary corn concentrations (50−60% w/w) of the resulting test diets were within the range used by commercial poultry growers in the United States.

Feeding Study. The feeding study was conducted at CQR, Wellington, CO. Approximately 560, 2-day-old rapidly growing broiler chickens (Longeneckers Hatchery, Elizabethtown, PA) were sexed and placed into pens (8 birds/pen; 5 pens/sex). Treatment groups corresponded to the seven corn lines evaluated in the study. Treatments were assigned to pens using a complete randomized block design. The only source of dietary protein used in the study was from the different test lines of corn and commercial soybean meal; supplemental methionine and lysine were also added. All diets were formulated at the CQR feed mill and designed to conform with industry and current nutritional standards. No growth promotants were added to the test diets. Feed and water were provided ad libitum.

Chickens were weighed by pen at study start (day 0) and study termination (day 38 for males and day 40 for females). The termination dates for the males and females differed due to the limited quantities of the control line available. The average body weight/pen and for each treatment group by sex was calculated. The average feed conversion was calculated for the entire duration of the study by using the total feed consumption during the study by pen divided by the total body weight of the surviving chickens/pen. This was averaged for each group by sex. Adjusted feed conversion was calculated by using the total feed consumption/pen divided by the total body weight of the surviving chickens and body weight of chickens that died or were removed from the pen. At study termination, fat pads were collected from each chicken. The fat pads from all chickens within a pen were combined and weighed.

Statistical Analysis of Feeding Study Data. Statistical analyses were performed on body weight, feed efficiency, adjusted feed efficiency, and fat pad weight. Because the pens were set up as a randomized complete block experimental design with seven diet treatments (based on seven corn varieties) in each of five replicated blocks of pens, the standard randomized block analysis of variance (ANOVA) statistical model was used to analyze the data. Means were compared to each other at the 5% level of significance. An additional analysis was done to compare the fit of GA21 to the population of responses from commercial varieties to verify whether the GA21 group was consistent with the expected variation of responses of animals fed the nontransgenic commercial corn varieties. This analysis was carried out using a linear mixed model procedure in SAS, and comparisons were made at the 5% level of significance.

RESULTS

Proximate, Fiber, and Mineral Composition. Compositional analysis results for corn grain and corn forage are presented in Tables l and 2, respectively. These results show that the levels of proximate components (protein, fat, ash, carbohydrate, and moisture), fiber (ADF and NDF), and phosphorus in the grain and forage of Roundup Ready corn line GA21 were comparable to those in the grain and forage of the control line. In addition, these values were either within published literature ranges, within the range determined for commercial varieties evaluated in the 1997 field trials, or within the range of historical conventional control values determined from previous studies.

The contents of calcium in the grain of Roundup Ready corn line GA21 and the control line were ∼2−4-fold lower than the values reported in the literature. This may be attributed to differences in analytical methods with older procedures subject to interferences from elements such as phosphorus. The validity of the atomic absorption method used for the estimation of calcium content of grain was established by multiple analyses of a corn grain standard obtained from the National Institute of Standards and Technology, Washington, DC. The range of values found for calcium content was approximately the same as those reported for this reference material. No statistically significant differences were observed for the content of calcium in forage in data from either 1996 or 1997 field trials. Similarly, the content of calcium in the grain of corn line GA21 was not statistically significantly different from the control line in data from 1996 field trials. However, the content of calcium in the grain of corn line GA21 was statistically significantly lower (∼9%) than the control line in data from 1997 field trials. This small difference is unlikely to be of biological significance as there were no statistically significant differences observed for the content of calcium in grain in data from 1996 field trials and in comparisons of corn line GA21 with commercial lines in data from 1997 field trials.

Table 1. Fiber, Mineral, and Proximate Composition of Grain from Roundup Ready Corn Line GA21

	1996[a]		1997[b]				
component[c]	GA21 mean (range)[h]	control[d] mean (range)[h]	GA21 mean (range)[h]	control[e] mean (range)[h]	comm lines[f] mean (range)[h]	literature (range)[h]	historical[g] (range)[h]
protein	10.05	10.05	11.05	10.54	10.87	(6.0−12.0)[k]	(9.0−13.6)
	(9.39−11.00)	(9.17−11.19)	(9.48−14.06)	(9.70−12.92)	(7.8−14.20)	(9.7−16.1)[l]	
total fat	3.51	3.55	3.90	3.98	3.69	(3.1−5.7)[k]	(2.4−4.2)
	(2.94−3.72)	(2.76−3.93)	(3.04−4.63)	(3.30−4.81)	(2.48−4.81)	(2.9−6.1)[l]	
ash	1.27	1.27	1.38	1.56	1.79		
	(1.06−1.45)	(1.21−1.40)	(1.06−1.80)	(1.07−3.09)	(0.89−6.28)	(1.1−3.9)[k]	(1.2−1.8)
ADF[i]	3.73	3.72	6.35	6.35	6.06		
	(3.35−3.99)	(3.52−4.05)	(2.73−9.47)	(3.00−9.33)	(2.75−11.34)	(3.3−4.3)[k]	(3.1−5.3)
NDF[i]	10.82	11.70	9.33	9.8	10.12		
	(10.06−11.88)	(9.40−13.58)	(7.51−11.57)	(8.03−11.58)	(7.58−15.91)	(8.3−11.9)[k]	(9.6−15.3)
carbohydrates	85.15	85.15	83.66	83.79	83.68	not reported	(81.7−86.3)
	(84.00−86.11)	(83.71−86.14)	(80.57−84.97)	(81.69−85.26)	(77.41−87.16)	in this form	
calcium	0.0026	0.0027	0.0039[j]	0.0043	0.0040		
	(0.0020−0.0031)	(0.0024−0.0033)	(0.0027−0.0056)	(0.0033−0.0058)	(0.0022−0.0208)	(0.01−0.1)[k]	(0.0029−0.006)
phosphorus	0.299	0.299	0.326	0.326	0.330		
	(0.28−0.32)	(0.28−0.31)	(0.303−0.350)	(0.292−0.349)	(0.208−0.411)	(0.26−0.75)[k]	(0.288−0.363)
moisture	14.15	14.40	16.86	16.21	16.30		
	(7.44−22.60)	(7.24−23.00)	(9.57−23.10)	(8.67−24.70)	(8.18−26.20)	(7−23)[k]	(9.4−15.8)

[a] Data from five U.S. sites; GA21 grain harvested from plants not treated with Roundup herbicide. [b] Combined data from four nonreplicated E.U. sites, six U.S. nonreplicated sites, and one U.S. replicated site; GA21 grain harvested from plants treated with Roundup herbicide. [c] Percent dry weight of sample, except for moisture. [d] Nontransgenic negative segregant. [e] Parental control line. [f] Commercial lines; local hybrids planted at each site. [g] Range for control lines planted in Monsanto Co. field trials conducted between 1993 and 1995. [h] Range denotes the lowest and highest individual values across sites for each line. [i] ADF, acid detergent fiber; NDF, neutral detergent fiber. [j] Statistically significantly different from the control at the 5% level ($p < 0.05$). [k] Watson (1987). [l] Jugenheimer (1976).

Table 2. Fiber, Mineral, and Proximate Composition of Forage from Roundup Ready Corn Line GA21

	1996[a]		1997[b]			
component[c]	GA21 mean (range)[h]	control[d] mean (range)[h]	GA21 mean (range)[h]	control[e] mean (range)[h]	comm lines[f] mean (range)[h]	historical[g] (range)
protein	7.91	7.58	7.49	7.45	7.20	
	(5.70−10.37)	(6.11−8.61)	(6.40−8.67)	(5.88−8.76)	(5.11−10.27)	(4.8−8.4)
ash	4.22	3.85	4.29	4.26	4.19	
	(3.20−4.67)	(2.64−5.28)	(2.12−5.29)	(2.94−5.91)	(2.00−6.60)	(2.9−5.1)
ADF[i]	25.04	25.89	23.85	25.55	25.56	
	(23.06−27.96)	(22.72−28.62)	(20.08−30.21)	(21.13−34.20)	(18.32−40.99)	(21.4−29.2)
NDF[i]	39.47	40.85	37.91	38.92	39.54	
	(35.94−44.48)	(36.97−44.31)	(31.47−46.29)	(33.99−49.28)	(26.37−54.45)	(39.9−46.6)
total fat	1.73	1.50	1.88	2.21	2.04	
	(1.27−2.30)	(1.24−1.93)	(0.71−2.98)	(1.16−3.22)	(0.35−3.62)	(1.4−2.1)
carbohydrates	86.14	87.04	86.35	86.06	86.62	
	(82.94−89.57)	(84.83−89.88)	(85.06−89.96)	(83.58−87.85)	(83.16−91.55)	(84.6−89.1)
calcium	0.1934	0.1766	0.2304	0.2177	0.1948	
	(0.0965−0.2488)	(0.0866−0.2172)	(0.1420−0.3173)	(0.1515−0.2754)	(0.0969−0.3184)	(not available)
phosphorus	0.2288	0.2124	0.2178	0.2179	0.1992	
	(0.1822−0.2622)	(0.2016−0.2365)	(0.1419−0.3475)	(0.1602−0.2914)	(0.1367−0.2914)	(not available)
moisture	72.30	65.52	68.83	68.73	68.31	
	(69.5−77.0)	(42.0−75.3)	(62.20−74.10)	(64.60−73.80)	(55.30−75.30)	(68.7−73.5)

[a] Data from five U.S. sites; GA21 forage harvested from plants not treated with Roundup herbicide. [b] Combined data from four nonreplicated E.U. sites, six U.S. nonreplicated sites, and one U.S. replicated site; GA21 forage harvested from plants treated with Roundup herbicide. [c] Percent dry weight of sample, except for moisture. [d] Nontransgenic negative segregant. [e] Parental control line. [f] Commercial lines; local hybrids grown at each site. [g] Range for control lines planted in Monsanto Co. field trials conducted between 1993 and 1995. [h] Range denotes the lowest and highest individual values across sites for each line. [i] ADF, acid detergent fiber; NDF, neutral detergent fiber.

Amino Acid Composition. These results are presented in Table 3. The contents of the 18 amino acids in the grain of Roundup Ready corn line GA21 were comparable to those in the grain of the control line. In addition, these values were either within published literature ranges, within the range determined for commercial varieties evaluated in 1997 field trials, or within the range of historical conventional control values determined from previous studies. Statistically significant differences between GA21 and control grain were noted for serine and tyrosine in the data from 1996 field trials; however, there were no statistically significant differences in amino acid content between GA21 and control grain in the data from 1997 field trials. The contents of serine and tyrosine in corn line GA21 were 1.1% higher and 3.5% lower, respectively, than the control line in the data from 1996 field trials. However, these small differences are unlikely to be of biological significance as these statistically significant differences were not observed in data from 1997 field trials.

Because EPSPS catalyzes a step in the aromatic amino acid biosynthetic pathway, it was important to assess if expression of mEPSPS influenced the levels of the aromatic amino acids in corn line GA21. EPSPS is not the rate-limiting step in aromatic amino acid biosynthesis (Herrmann, 1983; Weiss and Edwards, 1980). Therefore, increased EPSPS activity would not be expected to increase the levels of aromatic compounds in plants. The data from corn line GA21 grown at 16 sites over two years establish that there were no

Table 3. Amino Acid Composition of Corn Grain from Roundup Ready Corn Line GA21

	1996[b]		1997[c]				
amino acid[a]	GA21 mean (range)[i]	control[d] mean (range)[i]	GA21 mean (range)[i]	control[e] mean (range)[i]	comm lines[f] mean (range)[i]	literature[g] (range)[i]	historical[h] (range)[i]
alanine	7.62 (7.34−7.81)	7.64 (7.45−7.84)	7.64 (7.49−7.86)	7.62 (7.50−7.97)	7.78 (7.44−8.98)	(6.4−9.9)	(7.2−8.8)
arginine	4.13 (3.72−4.34)	4.30 (4.05−4.51)	4.48 (3.74−4.93)	4.51 (4.11−4.90)	4.36 (3.67−5.34)	(2.9−5.9)	(3.5−5.0)
aspartic acid	6.71 (6.46−6.87)	6.78 (6.35−6.83)	6.63 (6.17−7.05)	6.65 (6.22−7.08)	6.57 (6.14−7.35)	(5.8−7.2)	(6.3−7.5)
cystine	2.10 (1.85−2.36)	2.11 (1.91−2.24)	2.22 (1.73−2.49)	2.28 (2.06−2.57)	2.19 (1.63−2.62)	(1.2−1.6)	(1.8−2.7)
glutamic acid	19.27 (18.70−19.71)	19.06 (18.61−19.64)	18.78 (18.12−19.45)	18.70 (18.04−19.43)	19.17 (17.83−20.53)	(12.4−19.6)	(18.6−22.8)
glycine	3.72 (3.44−3.95)	3.78 (3.48−3.96)	3.83 (3.44−4.27)	3.89 (3.52−4.14)	3.71 (3.05−4.29)	(2.6−4.7)	(3.2−4.2)
histidine	2.81 (2.72−2.99)	2.84 (2.75−2.93)	2.67 (2.36−2.87)	2.74 (2.46−2.86)	2.80 (2.36−3.20)	(2.0−2.8)	(2.8−3.4)
isoleucine	3.60 (3.48−3.66)	3.58 (3.44−3.70)	3.53 (3.06−3.85)	3.57 (3.13−3.92)	3.75 (3.13−4.14)	(2.6−4.0)	(3.2−4.3)
leucine	13.11 (12.32−13.71)	12.90 (12.37−13.49)	12.98 (12.33−13.96)	12.87 (12.26−13.69)	13.32 (11.99−15.19)	(7.8−15.2)	(12.0−15.8)
lysine	3.02 (2.68−3.30)	3.09 (2.69−3.27)	3.11 (2.59−4.04)	3.02 (2.66−3.33)	2.96 (2.20−3.50)	(2.0−3.8)	(2.6−3.5)
methionine	1.98 (1.78−2.24)	2.03 (1.85−2.28)	2.16 (1.80−2.34)	2.17 (1.67−2.44)	2.02 (1.53−2.44)	(1.0−2.1)	(1.3−2.6)
phenylalanine	5.15 (4.88−5.31)	5.17 (4.98−5.30)	5.31 (5.03−5.63)	5.33 (4.96−5.76)	5.36 (4.88−6.10)	(2.9−5.7)	(4.9−6.1)
proline	8.69 (8.41−8.92)	8.69 (8.49−9.10)	8.98 (8.22−9.38)	9.00 (8.62−9.23)	9.16 (8.08−9.94)	(6.6−10.3)	(8.7−10.1)
serine	5.33[j] (5.25−5.49)	5.27 (5.17−5.43)	5.17 (4.43−5.60)	5.03 (3.82−5.63)	4.64 (2.87−5.63)	(4.2−5.5)	(4.9−6.0)
threonine	3.77 (3.64−3.88)	3.73 (3.58−3.85)	3.59 (3.33−3.74)	3.54 (3.08−3.71)	3.43 (2.61−3.89)	(2.9−3.9)	(3.3−4.2)
tryptophan	0.62 (0.55−0.66)	0.57 (0.53−0.61)	0.61 (0.52−0.75)	0.61 (0.43−1.04)	0.59 (0.41−1.04)	(0.5−1.2)	(0.4−1.0)
tyrosine	3.81[j] (3.68−3.99)	3.95 (3.88−4.10)	3.73 (3.06−4.20)	3.77 (2.78−4.32)	3.48 (2.37−4.32)	(2.9−4.7)	(3.7−4.3)
valine	4.58 (4.40−4.74)	4.64 (4.45−4.73)	4.57 (4.15−5.18)	4.62 (4.00−5.00)	4.79 (3.93−5.40)	(2.1−5.2)	(4.2−5.3)

[a] Values expressed as percent of total amino acids for statistical comparisons. These values are slightly higher when expressed as percent of total protein, e.g., alanine = 7.8% for GA21 (1996). [b] Data from five U.S. sites; GA21 grain harvested from plants not treated with Roundup herbicide. [c] Combined data from four nonreplicated E.U. sites, six U.S. nonreplicated sites, and one U.S. replicated site; GA21 grain harvested from plants treated with Roundup herbicide. [d] Nontransgenic negative segregant. [e] Parental control line. [f] Commercial lines; local hybrids planted at each site. [g] Watson (1982). Values are percent of total protein [10.1% total protein (N × 6.25)]. [h] Range for control lines planted in Monsanto Co. field trials conducted between 1993 and 1995; values are percent of total protein. [i] Range denotes the lowest and highest individual values across sites. [j] Value statistically significantly different than the control at the 5% level ($p < 0.05$).

statistically significant differences in the levels of the aromatic amino acids phenylalanine and tryptophan in comparisons of corn line GA21 with the control line. The statistically significant decrease in tyrosine levels in the grain of corn line GA21 versus the control line in data from the 1996 field trials is unlikely to be of biological significance as no statistically significant difference in tyrosine levels was observed in data from 1997 field trials. This conclusion is supported by the results of the poultry feeding study, which showed no differences in growth parameters between corn line GA21 and the control line (vide infra). Furthermore, it is known that the tyrosine requirements of chickens can be met by their ability to convert phenylalanine to tyrosine (Sasse and Baker, 1972).

Fatty Acid Composition. These results, presented in Table 4, show that the contents of the fatty acids in the grain of Roundup Ready corn line GA21 were comparable to those observed in the grain of the control line. In addition, these values were either within published literature ranges, within the range determined for commercial varieties evaluated in 1997 field trials, or within the range of historical conventional control values determined from previous studies. No statistically significant differences in the levels of fatty acids between corn line GA21 and the control line were observed in the data from either the 1996 or 1997 field trials. Also, no statistically significant differences were observed in comparisons of corn line GA21 with commercial lines in the data from 1997 field trials.

Feeding Study. The results of this study are summarized in Table 5. Male broiler chickens gained an average of ~2 kg of body weight during the 38-day study period, and female broiler chickens gained an average of ~1.9 kg of body weight during their 40-day study period. The chickens' rapid weight gain enabled the evaluation of the nutritional equivalence of GA21 compared to its parental line and the five commercial varieties. The chickens remained in good health during the study, and their survivability (day 5 to the end of the study) averaged 98% and was similar across all groups.

No statistically significant differences were observed for average body weight, feed efficiency, adjusted (for mortality) feed efficiency, and fat pad weight between corn line GA21 and the parental control line. The linear mixed model used to assess the fit of corn line GA21 to the population of responses from commercial varieties

Table 4. Fatty Acid Composition of Corn Grain from Roundup Ready Corn Line GA21

	1996[b]		1997[c]				
fatty acid[a]	GA21 mean (range)[i]	control[d] mean (range)[i]	GA21 mean (range)[i]	control[e] mean (range)[i]	comm lines[f] mean (range)[i]	literature[g] (range)[i]	historical[h] (range)[i]
arachidic	0.40	0.41	0.37	0.36	0.40		
(20:0)	(0.36−0.48)	(0.39−0.46)	(0.32−0.44)	(0.33−0.41)	(0.31−0.57)	(0.1−2)	(0.3−0.5)
behenic	0.16	0.17	0.16	0.15	0.18		
(22:0)	(0.14−0.18)	(0.16−0.18)	(0.12−0.24)	(0.13−0.16)	(0.13−0.24)	(not reported)	(0.1−0.3)
eicosenoic	0.28	0.29	0.30	0.30	0.30		
(20:1)	(0.27−0.31)	(0.28−0.30)	(0.28−0.34)	(0.28−0.36)	(0.19−0.45)	(not reported)	(0.2−0.3)
linoleic	58.56	58.72	61.40	61.51	59.18		
(18:2)	(54.20−64.70)	(53.40−65.60)	(58.2−63.4)	(59.7−63.0)	(46.9−64.3)	(35−70)	(55.9−66.1)
linolenic	1.10	1.08	1.14	1.14	1.11		
(18:3)	(1.07−1.13)	(0.98−1.16)	(0.92−1.24)	(1.04−1.20)	(0.77−1.55)	(0.8−2)	(0.8−1.1)
oleic	27.50	27.40	24.2	24.1	26.2		
(18:1)	(22.10−31.30)	(21.40−32.40)	(22.4−26.0)	(22.9−26.0)	(21.3−39.2)	(20−46)	(20.6−27.5)
palmitic	9.94	9.92	10.70	10.72	10.58		
(16:0)	(9.59−10.40)	(9.60−10.40)	(10.30−11.40)	(10.40−11.40)	(8.75−13.30)	(7−19)	(9.9−12.0)
stearic	1.87	1.86	1.68	1.67	1.88		
(18:0)	(1.52−2.11)	(1.46−2.11)	(1.44−2.04)	(1.59−1.86)	(1.36−2.65)	(1−3)	(1.4−2.2)

[a] Value of fatty acids expressed as percent of total fatty acid. The method included the analysis of the following fatty acids, which were not detected in the majority of samples analyzed: caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), gamma linolenic (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), and arachidonic acid (20:4). Palmitoleic acid (16:1) was observed at levels of $\sim$0.17% of total fatty acids in grain samples collected in 1996 but was not detected in the majority of grain samples collected in 1997. [b] Data from five U.S. sites; GA21 grain harvested from plants not treated with Roundup herbicide. [c] Combined data from four nonreplicated E.U. sites, six U.S. nonreplicated sites, and one U.S. replicated site; GA21 grain harvested from plants treated with Roundup herbicide. [d] Nontransgenic negative segregant. [e] Parental control line. [f] Commercial lines; local hybrids planted at each site. [g] Watson (1982). Values expressed as percent of total fat except for palmitic acid (16:1), which is expressed as percent of triglyceride fatty acids. [h] Range for control lines planted in Monsanto Co. field trials conducted between 1993 and 1995; values are expressed as percent of total fatty acids. [i] Range denotes the lowest and highest individual values across sites.

Table 5. Feeding Study Comparison of Roundup Ready Corn Line GA21, the Parental Control Line, and Commercial Lines

test group	mean terminal body wt (kg)	feed efficiency	feed efficiency (adj)[a]	% total fat pad wt
males				
GA21	2.013	1.750	1.646	1.775
parental	1.945	1.662	1.629	1.638
population[b]	1.947	1.699	1.650	1.625
females				
GA21	1.885	1.781	1.749	2.085
parental	1.859	1.779	1.721	2.114
population	1.874	1.789	1.744	2.119

LSD[c]	male	female
terminal body wt	0.1063	0.1272
feed efficiency	0.0934	0.1127
adj feed efficiency	0.071	0.0766
% total fat pad wt	0.2579	0.1968

[a] Total feed consumption (per pen) divided by the total weight of surviving chickens and the weight of chickens that died or were removed from pens. [b] Mean of commercial population (5 commercial lines tested separately). [c] Mean differences exceeding least significant difference values are statistically significant at $p < 0.05$ level. There were no statistically significant differences observed.

showed that none of the means of the measured parameters of corn line GA21 differed from the commercial population mean. The final body weights and fat pad weights were within the expected range for chickens of this age, and feed conversion was within normal limits. Both the feed efficiency and body weights were similar to the U.S. industry average.

DISCUSSION

Compositional analysis results generated from 16 field sites over a period of two years show that the grain and forage of GA21 corn are comparable in their composition to those of the control corn line and conventional corn. Furthermore, because the results from 1996 field trials (untreated) were similar to those observed in 1997 field trials (treated), the composition of corn line GA21 is not affected by treatment with Roundup Ready herbicide. At the 5% level of significance, 1 of 20 comparisons between corn line GA21 and the control line is expected to be statistically significantly different by chance alone. The use of multiyear data and incorporation of reference corn lines into field trials suggests that the few statistically significant differences observed are most likely due to random chance and unlikely to be of biological significance. This is supported by the facts that (a) the statistically significant differences were not consistently observed over the two years of data collected, (b) statistically significant differences were not observed in comparisons of corn line GA21 with the commercial lines in 1997, and (c) all component values determined for corn line GA21 were either within published literature ranges, within the range determined for commercial varieties evaluated in 1997 field trials, or within the range of historical nontransgenic control values determined from previous studies. On the basis of the data presented for grain and forage, it is concluded that Roundup Ready corn line GA21 is substantially equivalent in composition to the control corn line as well as to commercial corn varieties.

The results of the chicken feeding study show that there were no differences in growth, feed efficiency, adjusted feed efficiency, and fat pad weights between chickens fed grain of corn line GA21 or grain of parental control line. Corn line GA21 was not different from the population mean of other commercial varieties tested for growth (body weight), feed efficiency, adjusted feed efficiency, and fat pad weight. Although the grain of corn line GA21 was harvested from plants not treated with Roundup herbicide, similar results would be expected from the use of treated grain because the composition of corn line GA21 is unaffected by treatment with Roundup Ready herbicide (vide supra).

The chicken is an excellent experimental model for nutrition studies and has been widely used by many laboratories over the years to investigate nutritional requirements and metabolism of various nutrients (Scott et al., 1982). Growth studies with young chickens are used to assess the bioavailability of essential amino acids, protein quality, and interrelationships of vitamins and essential trace minerals to support growth (Baker et al., 1981; Lewis et al., 1995). Because young chickens have a rapid weight gain during the first six weeks of life (~50-fold), deficiencies or reduced bioavailabilty of key nutrients in the diet are readily manifested by reduced growth during this period (Baker et al., 1977). The finding of no significant differences in this sensitive experimental model leads to the conclusion that Roundup Ready line GA21 is nutritionally equivalent to conventional corn.

The compositional analyses and poultry feeding studies taken together demonstrate that the composition and feeding value of Roundup Ready corn line GA21 are equivalent to those of conventional corn. Together with safety data of the introduced protein, mEPSPS, it is concluded that Roundup Ready corn is as safe and nutritious as conventional corn for food and feed use.

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