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Comparison of the Forage and Grain Composition from Insect-Protected and Glyphosate-Tolerant MON 88017 Corn to Conventional Corn (*Zea mays* L.)

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The next generation of biotechnology-derived products with the combined benefit of herbicide tolerance and insect protection (MON 88017) was developed to withstand feeding damage caused by the coleopteran pest corn rootworm and over-the-top applications of glyphosate, the active ingredient in Roundup herbicides. As a part of a larger safety and characterization assessment, MON 88017 was grown under field conditions at geographically diverse locations within the United States and Argentina during the 2002 and 2003−2004 field seasons, respectively, along with a near-isogenic control and other conventional corn hybrids for compositional assessment. Field trials were conducted using a randomized complete block design with three replication blocks at each site. Corn forage samples were harvested at the late dough/early dent stage, ground, and analyzed for the concentration of proximate constituents, fibers, and minerals. Samples of mature grain were harvested, ground, and analyzed for the concentration of proximate constituents, fiber, minerals, amino acids, fatty acids, vitamins, antinutrients, and secondary metabolites. The results showed that the forage and grain from MON 88017 are compositionally equivalent to forage and grain from control and conventional corn hybrids.

KEYWORDS: Corn (*Zea mays* L.); glyphosate-tolerant; composition; insect-protected

INTRODUCTION

Corn (*Zea mays* L.), or maize, is grown in nearly all areas of the world and ranks third behind rice (*Oryza sativa* L.) and wheat (*Triticum* sp.) in total global production. In the fall of 2004 and spring of 2005, corn was planted globally on 144 M ha with a total production of 708 million metric tons (*1*). In the United States, corn is the largest crop grown in terms of acreage planted and net value. In 2005, corn production covered 81 million acres that yielded 11.2 billion bushels and had a net value of approximately $27 billion (*2*). Corn's high yield makes it one of the most economical sources of metabolizable energy for animal feeds, as well as starch and sugar for food and industrial products. In industrialized countries, corn has two major uses: (i) as animal feed in the form of grain, forage, or silage and (ii) as a raw material for wet- or dry-milled processed products such as high fructose corn syrup, oil, starch, glucose, and dextrose (*3*). These processed products are used as ingredients in many industrial applications and in human food products.

Weed control is essential in corn fields since weeds compete with the crop for sunlight, water, and nutrients. Failure to control weeds results in decreased yields and reduced crop quality. The introduction of Roundup Ready Corn 2 (Roundup Ready, Roundup, Roundup UltraMAX, and YieldGard are registered trademarks of Monsanto Technology LLC), and herbicide-tolerant crops in general, has allowed superior weed control in the field with minimal damage to the crop (*4*). Corn yields are also negatively impacted by a number of insect pests. One of the most pernicious pests in the U.S. corn belt is corn rootworm (CRW; *Diabrotica* spp.). CRW larvae damage corn by feeding on the roots, reducing the ability of the plant to absorb water and nutrients from soil and causing harvesting difficulties due to plant lodging. CRW is the most significant insect pest problem for corn production in the U.S. corn belt from the standpoint of chemical insecticide usage. The introduction of YieldGard Rootworm corn, and insect-protected crops in general, has proved to maintain insect control in the field with minimal insect damage to the crop (*5*).

As in the case of Roundup Ready 2 and YieldGard Rootworm corn, modern biotechnology offers effective solutions to address weed and insect problems in field crop production. Over the past 10 years, a number of biotechnology-derived products have been introduced into the marketplace (*6*, *7*). Prior to its

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availability on the market, each product has undergone a thorough safety evaluation. One component of the safety evaluation is to compare the biotechnology-derived product to a near-isogenic conventional counterpart (control), in an effort to determine substantial equivalence. The substantial equivalence approach has been endorsed by a number of international organizations (*8*−*10*). A critical component of this comparative safety assessment process is determining whether the common nutrients and antinutrients of the biotechnology-derived product are equivalent to the nutrients and antinutrients of a near-isogenic control.

MON 88017, marketed under the trade name YieldGard VT Rootworm/RR2 (YieldGard VT is a trademark of Monsanto Technology LLC), is the next generation of biotechnology-derived products, with the combined benefit of glyphosate tolerance like that found in Roundup Ready Corn 2 and insect protection like that found in YieldGard Rootworm corn. The purpose of this study was to evaluate whether the corn forage and grain collected from MON 88017 is compositionally equivalent to corn forage and grain collected from a near-isogenic control and other conventional commercial corn hybrids.

MATERIALS AND METHODS

Plant Generation. MON 88017 is protected from feeding damage caused by the coleopteran pest CRW and is tolerant to glyphosate, the active ingredient in the Roundup family of agricultural herbicides. MON 88017 was developed by recombinant DNA techniques using *Agrobacterium*-mediated transformation of corn cells with plasmid vector PV-ZMIR39. The plasmid vector contains a 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium* sp. strain CP4 (*cp4 epsps*), in which the protein, CP4 EPSPS, confers tolerance to glyphosate. It also contains a modified *Bacillus thuringiensis* (subspecies *kumamotoensis*) *cry3Bb1* gene, in which the protein, Cry3Bb1, selectively controls CRW species. The transformation of corn cells with one plasmid vector containing multiple genes increases the efficiency of simultaneous introduction of both traits into corn, thereby providing growers with access to a hybrid of elite corn germplasms containing both traits. CP4 EPSPS is structurally similar and functionally identical to endogenous plant EPSPS enzymes but has a much reduced affinity for glyphosate relative to endogenous plant EPSPS (*11*). In conventional plants, glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of EPSP, thereby depriving plants of essential amino acids (*12*, *13*). In plants that are tolerant to Roundup agricultural herbicides, the required aromatic amino acids and other metabolites that are necessary for growth and development are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate (*11*). A comprehensive characterization and safety assessment of CP4 EPSPS has been conducted (*14*). The Cry3Bb1 present in MON 88017 is a member of the Cry3Bb class of proteins and shares >99% amino acid sequence identity with the wild-type Cry3Bb1 contained in the topically applied commercial microbial product Raven (Raven is a registered trademark of Ecogen, Inc.) Oil Flowable Bioinsecticide and to the Cry3Bb1 produced in YieldGard Rootworm corn (*7*). A comprehensive characterization and safety assessment of Cry3Bb1 has been conducted (*15*−*17*).

U.S. and Argentina Field Seasons. Corn seed from MON 88017, a control hybrid (LH198 × LH59), and 12 different conventional hybrids (**Table 1**) were planted in Illinois, Iowa, and Nebraska in the United States during the 2002 field season. Corn seed from MON 88017, a control hybrid (LH198 × LH59), and 16 different conventional hybrids (**Table 1**) were planted in Buenos Aires (two sites), Cordoba, and Santa Fe in Argentina during the 2003−2004 field season. Each field site in each country had a randomized complete block design with three replicates per block and was grown under normal agronomic field conditions for their respective geographic region. All MON 88017 plots received an application of Roundup UltraMAX herbicide according to the label for over-the-top applications in corn. Corn forage samples were harvested at all field sites at the late dough/early dent stage and were stored on dry ice or in a −20 °C freezer until the samples were ground for analysis. Mature corn grain samples were harvested at all field sites and stored at ambient temperature until ground. Once ground, both forage and grain samples were stored in a −20 °C freezer until the samples were shipped for analysis.

Table 1. Conventional Hybrids Grown in Field Trial Locations

hybrid	vendor	field site
	U.S. trials in 2002[a]	
RX708	Asgrow	Iowa
DK579	Dekalb	Iowa
8464 IT	Garst	Iowa
8590 IT	Garst	Iowa
RX690	Asgrow	Illinois
DKC60-15	Dekalb	Illinois
DKC61-24	Dekalb	Illinois
N59-Q9	Northrup King	Illinois
7474	Mycogen	Nebraska
6431	Mycogen	Nebraska
N60-N2	Northrup King	Nebraska
N67-H6	Northrup King	Nebraska
	Argentina trials in 2003/2004[a]	
SPS 2601	SPS	Buenos Aires[b]
SPS 2602	SPS	Buenos Aires[b]
Impacto	KWS	Buenos Aires[b]
Tamden	KWS	Buenos Aires[b]
AX707	Nidera SA	Buenos Aires[c]
AX599	Nidera SA	Buenos Aires[c]
32F82	Pioneer	Buenos Aires[c]
DK615	DEKALB	Buenos Aires[c]
AW140	Asgrow	Córdoba
Indiana	Agar Cross	Córdoba
Albion	Sursem	Córdoba
Portos	Sursem	Córdoba
37P73	Pioneer	Santa Fe
32G62	Pioneer	Santa Fe
M9	Dow AgroSciences	Santa Fe
LT550	La Tijereta	Santa Fe

[a] Each hybrid was grown in a randomized complete block design with three replicates. [b] First site within Buenos Aires. [c] Second site within Buenos Aires.

Compositional Analysis. Ground corn forage samples were analyzed for the levels of proximate constituents (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), and minerals (Ca and P). The carbohydrate concentration was estimated by calculation. Ground corn grain samples were analyzed for the levels of proximate constituents (protein, fat, ash, and moisture), ADF, NDF, total dietary fiber (TDF), amino acids, fatty acids (C8−C22), minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn), vitamins (B_1, B_2, B_6, E, niacin, and folic acid), antinutrients (phytic acid and raffinose), and secondary metabolites (2-furaldehyde, ferulic acid, and *p*-coumaric acid). The carbohydrate concentration was estimated by calculation. The analytes were chosen based on important nutrients and antinutrients of corn food and feed uses as defined by the Organization for Economic Cooperation and Development (*18*). Covance Laboratories Inc. (Madison, WI) conducted all compositional analyses using established methods. Each method utilized a standard or a quality control sample with known analyte content, and each sample was analyzed once. Brief descriptions of the methods, including the limit of quantitation (LOQ), are provided below. All laboratory activities followed Good Laboratory Practices (*19*).

Proximate Constituent Analysis. The ash content was estimated by igniting the sample with an electric furnace and determining the percent ash gravimetrically (*20*). The moisture content was estimated by loss of weight upon drying the samples in an oven at constant temperature (*21*, *22*). The crude protein content was estimated by determining the total nitrogen using the Kjeldahl method, previously described (*23*, *24*). The total fat content was estimated by acid hydrolysis with extraction using diethyl ether followed by hexane (*25*, *26*) for the forage samples and estimated by Soxhlet extraction using pentane (*27*) for the grain samples. The LOQ of ash, moisture, protein,

Table 2. Proximate Constituents, Fiber, and Mineral Composition of Forage from MON 88017, Control, and Conventional Corn Hybrids

	United States			Argentina			
component[a]	MON 88017 mean[d] ± SE (range)[f]	control[b] mean[d] ± SE (range)[f]	conventional[c] tolerance interval[g] (range)[f]	MON 88017 mean[e] ± SE (range)[f]	control[b] mean[e] ± SE (range)[f]	conventional[c] tolerance interval[g] (range)[f]	literature[h] (range) [f]
ash	3.99 ± 0.24	4.04 ± 0.24	0.72, 7.42	5.13 ± 0.22	5.22 ± 0.22	3.54, 6.73	2.0–9.6
	(3.30–5.53)	(3.59–4.67)	(2.62–6.78)	(4.69–5.75)	(4.60–6.94)	(4.27–6.22)	
carbohydrates	86.19 ± 0.62	86.48 ± 0.62	78.70, 93.43	86.44 ± 0.56	86.43 ± 0.56	80.91, 89.90	76.4–91.5
	(83.54–87.88)	(84.43–87.71)	(81.86–89.90)	(84.95–88.60)	(85.08–87.42)	(82.28–88.19)	
fat, total	1.61 ± 0.29	1.65 ± 0.29	0.80, 2.95	1.39 ± 0.33	1.91 ± 0.33	0.073, 3.71	0.37–4.6
	(0.80–3.13)	(0.83–2.97)	(0.69–2.92)	(0.64–2.29)	(1.02–2.36)	(0.76–3.54)	
moisture	70.86 ± 0.66	70.66 ± 0.66	59.37, 80.83	69.02 ± 0.57	69.30 ± 0.57	56.80, 81.85	55.3–80.4
	(68.50–72.70)	(69.10–72.70)	(65.20–78.60)	(67.60–70.80)	(65.70–71.40)	(60.50–74.30)	
protein	8.20 ± 0.31	7.82 ± 0.31	4.17, 11.81	7.04 ± 0.24	6.44 ± 0.24	4.38, 10.73	3.14–11.6
	(7.44–8.97)	(6.79–8.54)	(6.31–9.96)	(5.90–7.68)	(5.78–7.25)	(5.34–9.80)	
ADF	26.54 ± 1.25	25.45 ± 1.25	13.95, 38.96	27.60 ± 0.86	25.91 ± 0.86	19.68, 33.41	16.1–41.9
	(24.29–29.97)	(23.34–28.13)	(19.16–35.55)	(24.48–32.06)	(23.06–29.10)	(21.01–32.95)	
NDF	37.34 ± 1.22	38.33 ± 1.22	23.80, 54.73	38.45 ± 1.00	39.25 ± 1.00	26.28, 50.79	20.3–63.7
	(33.44–45.05)	(35.86–41.18)	(30.27–57.93)	(32.80–43.51)	(36.93–45.33)	(29.80–59.60)	
Ca	0.22 ± 0.014	0.23 ± 0.014	0.11, 0.32	0.14 ± 0.0088	0.13 ± 0.0088	0.071, 0.26	0.097–0.32
	(0.19–0.26)	(0.18–0.31)	(0.13–0.32)	(0.12–0.17)	(0.110–0.14)	(0.12–0.24)	
P	0.25 ± 0.011	0.25 ± 0.011	0.095, 0.38	0.18 ± 0.0074	0.17 ± 0.0074	0.036, 0.39	0.118–0.32
	(0.21–0.30)	(0.20–0.30)	(0.16–0.31)	(0.16–0.22)	(0.15–0.19)	(0.13–0.34)	

[a] All data are expressed as percent dry weight of sample, except moisture, which is percent fresh weight of sample. [b] Conventional control corn (LH198 × LH59) forage samples. [c] Commercial hybrids planted at each field site. [d] The least-squares mean of nine values. [e] The least-squares mean of 12 values. [f] Range denotes the lowest and highest individual values across samples. [g] Tolerance interval is specified to contain 99% of the commercial hybrid population; negative limits are set to zero. [h] ILSI Crop Composition Database, ref *53*.

and total fat was 0.1% fresh weight of sample (fw). The carbohydrate content in ground forage and grain samples was calculated using the following equation (*28*):

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

The LOQ for carbohydrates was 1.0% fw.

Fiber Analysis. The ADF content was determined by boiling the sample with a sulfuric acid solution, rinsing with acetone, and then determining the percent ADF gravimetrically (*29*). The NDF content was determined by boiling the sample with a neutral solution, adding α-amylase, rinsing with acetone, and then determining the percent NDF gravimetrically (*29*, *30*). The TDF content was calculated by determining the protein and ash content in the samples, as previously described (*31*). The LOQ of ADF and NDF was 0.1% fw, and the TDF was 1.0% fw.

Mineral Analysis. The mineral content was determined from ashed samples mixed with a 5% solution of hydrochloric acid (*32*, *33*). The amount of each element was determined at appropriate wavelengths using inductively coupled plasma spectroscopy. The emission of the unknown sample was compared with the emission of standard solutions. The LOQ for K and Na was 100 mg/kg fw; for Ca, Mg, and P, the LOQ was 20 mg/kg fw; for Fe, the LOQ was 2 mg/kg fw; for Cu, the LOQ was 0.5 mg/kg fw; for Zn, the LOQ was 0.4 mg/kg fw; and for Mn, the LOQ was 0.3 mg/kg fw.

Amino Acid Analysis. The individual amino acid content was determined by three methods (*34*). Tryptophan required a base hydrolysis using sodium hydroxide. Sulfur-containing amino acids required an oxidation using performic acid prior to hydrolysis with hydrochloric acid. Analysis of the remaining amino acids was accomplished through direct hydrolysis with hydrochloric acid. All individual amino acids, regardless of the extraction method, were quantitated using an automated amino acid analyzer. The LOQ for each amino acid was 0.1 mg/g fw.

Fatty Acid Analysis. The individual fatty acid content was determined by lipid extraction and saponification with 0.5 N sodium hydroxide in methanol (*35*). The saponification mixture was methylated with 14% boron trifluoride/methanol, and resulting methyl esters were extracted with heptane. The methyl esters of the fatty acids were analyzed by gas chromatography. Tridecanoic methyl ester was used as an internal standard. The LOQ for each fatty acid was 0.003% fw.

Secondary Metabolite Analysis. Ferulic and *p*-coumaric acid contents were determined by extracting the samples with methanol using ultrasonication and then hydrolyzing using 4 N sodium hydroxide (*36*). Extracts were neutralized and filtered prior to quantitation by reversed-phase high-performance liquid chromatography (HPLC) with UV detection (*36*). The 2-furaldehyde content was determined by extracting with 4% trichloroacetic acid, centrifuging, filtering, concentrating, and analyzing by reversed-phase HPLC with UV detection (*37*). The LOQs for ferulic and *p*-coumaric acid were calculated to be approximately 50 μg/g fw, and for 2-furaldehyde, it was calculated to be approximately 0.5 μg/g fw.

Antinutrient Analysis. The phytic acid content was determined by extracting the sample with hydrochloric acid using ultrasonication as described by Lehrfeld (*38*, *39*). Purification and concentration of the extract were conducted using a silica-based anion exchange column followed by quantitation using a polymer HPLC column fitted with a refractive index detector. The raffinose content was determined by two methods (*40*, *41*), in which the grain samples were extracted with deionized water and the extracts were treated with a solution of hydroxylamine hydrochloride in pyridine containing phenyl-α-D-glucoside as an internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoroacetic acid and analyzed by gas chromatography with flame ionization detection. The LOQs for phytic acid and raffinose were 0.133% fw and 0.0358% fw, respectively.

Vitamin Analysis. Folic acid was extracted from the samples using hydrolysis, followed by autoclaving with a chicken pancreas enzyme (*42*, *43*). The amount of folic acid was then estimated by the growth response of the bacteria *Lactobacillus casei* to the samples. Niacin was extracted from the samples using hydrolysis (*44*). The amount of niacin was then estimated by the growth response of the bacteria *Lactobacillus plantarum* to the samples. Vitamin B_1 was extracted from the samples under weak acid conditions, followed by incubation with a buffered enzyme solution (*45*−*47*). The amount of vitamin B_1 was then estimated using a fluorometer and a known standard. Vitamin B_2 was extracted from the samples using dilute hydrochloric acid (*48*). The amount of vitamin B_2 was then estimated by the growth response of the bacteria *L. casei* to the samples. Vitamin B_6 was extracted from the samples

Table 3. Proximate Constituents, Fiber, and Mineral Compositions of Grain from MON 88017, Control, and Conventional Corn Hybrids

	United States			Argentina			
component[a]	MON 88017 mean[d] ± SE (range)[f]	control[b] mean[d] ± SE (range)[f]	conventional[c] tolerance interval[g] (range)[f]	MON 88017 mean[e] ± SE (range)[f]	control[b] mean[e] ± SE (range)[f]	conventional[c] tolerance interval[g] (range)[f]	literature[h] (range)[f]
ash	1.54 ± 0.077 (1.31–1.68)	1.59 ± 0.077 (1.23–1.97)	0.94, 1.73 (1.04–1.86)	1.51 ± 0.11 (1.14–2.05)	1.46 ± 0.11 (1.19–1.82)	0.90, 2.32 (1.21–2.06)	0.62–6.28
carbohydrates	82.32 ± 0.40 (81.61–83.39)	82.33 ± 0.40 (80.67–83.62)	79.39, 89.67 (81.46–86.68)	84.62 ± 0.46 (82.99–86.14)	85.28 ± 0.46 (83.35–87.18)	80.54, 87.24 (81.69–86.02)	77.4–89.5
fat, total	3.64 ± 0.13 (3.44–3.96)	3.79 ± 0.13 (3.53–4.36)	0.74, 6.01 (2.38–4.43)	3.34 ± 0.067 (2.83–3.86)	3.53 ± 0.067 (3.23–3.97)	1.82, 5.17 (2.63–4.39)	1.74–5.56
moisture	11.10 ± 0.99 (9.03–13.20)	11.60 ± 0.99 (9.73–14.20)	4.67, 17.56 (9.15–14.90)	12.23 ± 0.25 (11.40–12.80)	12.42 ± 0.25 (11.70–13.40)	10.40, 13.89 (11.10–13.20)	6.1–26.2
protein	12.51 ± 0.35 (11.63–13.00)	12.28 ± 0.35 (11.22–13.82)	6.20, 15.35 (9.26–13.37)	10.52 ± 0.41 (9.27–11.45)	9.73 ± 0.41 (7.88–11.78)	8.03, 13.99 (9.24–13.45)	6.15–15
ADF	3.77 ± 0.16 (3.31–4.40)	3.54 ± 0.16 (2.97–4.69)	1.89, 5.23 (2.39–4.89)	4.69 ± 0.28 (3.28–6.58)	4.84 ± 0.28 (3.66–5.84)	2.46, 7.89 (3.51–7.70)	1.82–11.3
NDF	12.44 ± 0.62 (10.99–13.58)	11.87 ± 0.62 (10.38–14.29)	3.51, 21.65 (8.41–16.54)	12.40 ± 0.59 (8.90–14.30)	12.79 ± 0.59 (9.08–16.08)	7.39, 17.08 (8.36–19.75)	5.59–22.6
TDF	16.24 ± 0.71 (13.57–18.64)	15.40 ± 0.71 (13.18–17.84)	5.72, 27.10 (11.80–23.04)	18.18 ± 0.99 (12.80–23.31)	18.01 ± 0.99 (14.22–21.97)	6.24, 32.83 (12.26–30.19)	11.8–25.6
Ca	0.0054 ± 0.00035 (0.0047–0.0060)	0.0058 ± 0.00035 (0.0049–0.0069)	0.0017, 0.0062 (0.0032–0.0060)	0.0043 ± 0.00014 (0.0039–0.0048)	0.0045 ± 0.00014 (0.0040–0.0054)	0.0022, 0.0072 (0.0031–0.0064)	0.002–0.021
Cu	1.73 ± 0.086 (1.48–2.05)	1.99 ± 0.086 (1.64–2.63)	0.17, 3.00 (1.01–2.34)	1.76 ± 0.15 (1.34–2.38)	1.71 ± 0.15 (1.38–2.14)	0.33, 3.13 (0.92–3.20)	0.73–5.01
Fe	21.51 ± 0.59 (20.07–22.92)	21.84 ± 0.59 (20.31–23.93)	12.60, 31.26 (16.42–26.03)	20.28[i] ± 0.87 (17.87–22.97)	19.08 ± 0.87 (16.40–23.90)	9.95, 34.09 (17.38–30.97)	10.4–49.1
Mg	0.14 ± 0.0034 (0.13–0.15)	0.14 ± 0.0034 (0.13–0.16)	0.088, 0.16 (0.10–0.14)	0.12[i] ± 0.004 (0.10–0.14)	0.11 ± 0.004 (0.098–0.13)	0.086, 0.16 (0.10–0.15)	0.079–0.161
Mn	9.72 ± 0.38 (9.01–10.76)	9.37 ± 0.38 (7.55–10.44)	2.45, 10.60 (4.96–9.81)	7.77 ± 0.60 (6.74–10.10)	7.48 ± 0.60 (6.03–10.58)	2.17, 12.62 (5.24–10.33)	2.61–11.3
P	0.39 ± 0.01 (0.37–0.41)	0.39 ± 0.01 (0.36–0.43)	0.24, 0.44 (0.28–0.41)	0.32 ± 0.014 (0.28–0.37)	0.31 ± 0.014 (0.26–0.38)	0.25, 0.43 (0.27–0.43)	0.208–0.434
K	0.41 ± 0.012 (0.39–0.44)	0.42 ± 0.012 (0.38–0.47)	0.27, 0.48 (0.29–0.43)	0.39 ± 0.017 (0.35–0.47)	0.39 ± 0.017 (0.36–0.48)	0.23, 0.56 (0.29–0.54)	0.271–0.528
Zn	24.53 ± 0.98 (22.31–27.27)	24.92 ± 0.98 (22.02–27.18)	13.42, 31.37 (17.15–26.18)	18.58 ± 1.69 (12.61–22.50)	17.87 ± 1.69 (13.24–21.98)	5.64, 36.71 (13.70–30.57)	6.5–37.2

[a] All data are expressed as percent dry weight of sample, except moisture, which is percent fresh weight of sample, and copper, iron, manganese, and zinc, which are milligram per kilogram dry weight of sample. [b] Conventional control corn (LH198 × LH59) grain samples. [c] Commercial hybrids planted at each field site. [d] The least-squares mean of nine values. [e] The least-squares mean of 12 values. [f] Range denotes the lowest and highest individual values across samples. [g] Tolerance interval is specified to contain 99% of the commercial hybrid population; negative limits are set to zero. [h] ILSI Crop Composition Database, ref *53*. [i] Statistically different from the control at the 5% level ($p < 0.05$).

using dilute sulfuric acid (*49*). The amount of vitamin B_6 was then estimated by the growth response of the yeast *Saccharromyces carlsbergenesis* to the samples. Vitamin E was extracted from the samples by saponification and extraction with ethyl ether. The amount of vitamin E was then estimated using a HPLC (*50−52*). The LOQ for folic acid was 0.06 μg/g fw, niacin was 0.3 μg/g fw, vitamin B_1 was 0.01 mg/100 g fw, vitamin B_2 was 0.2 μg/g fw, vitamin B_6 was 0.07 μg/g fw, and vitamin E was 0.003 mg/g fw.

Data Reduction and Statistical Analysis. The following 15 analytes with >50% of the individual observations below the LOQ for their respective assays were excluded from statistical analysis from both sets of field trials: sodium, 2-furaldehyde, 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 γ-linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid. Additionally, raffinose measured in the Argentina samples had >50% of the individual observations below the LOQ and, therefore, was excluded from the statistical analyses. Some individual sample values were below the LOQ for 16:1 palmitoleic acid (9% of the total U.S. samples and 21% of the total Argentina samples), 22:0 behenic acid (2% of the total U.S. samples), and vitamin E (2% of the total U.S. samples and 5.6% of the total Argentina samples). These samples were assigned a value equal to half the LOQ prior to statistical analyses. The SAS GLM procedure was applied to all data prior to statistical analysis to detect potential outliers in the data set by screening studentized PRESS residuals. A PRESS residual is the difference between any value and its predicted value from a statistical model that excludes the data point. The studentized version scales these residuals so that the values tend to have a standard normal distibution when outliers are absent. Extreme data points that are outside of the ±6 studentized PRESS residual range were considered for exclusion, as outliers, from the statistical analyses. The only data point considered extreme and removed from the statistical evaluation was a single iron value from the Sante Fe site in Argentina. In addition, all data values in the United States and Argentina were converted to the appropriate units for statistical comparison, with the exception of moisture, which remained percent on a fresh weight basis. Amino acids were converted to percent total amino acid, and fatty acids were converted to percent total fatty acid, while all remaining analytes were converted to their respective units on a dry weight basis.

A total of 62 (nine in forage and 53 in grain) different components were evaluated in samples collected from U.S. field trials, and 61 (nine in forage and 52 in grain) different components were evaluated in samples collected from Argentina field trials. Each field season was analyzed separately. Statistical analyses were conducted using a mixed model analysis of variance for a combination of all three (U.S.) or four (Argentina) field sites using the following equation:

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk}$$

where Y_{ijk} = unique individual observation, U = overall mean, T_i = hybrid effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by hybrid interaction effect, and e_{ijk} = residual error. In these analyses, values from MON 88017

Table 4. Amino Acid Composition of Grain from MON 88017, Control, and Conventional Corn Hybrids

	United States			Argentina			
component[a]	MON 88017 mean[d] ± SE (range)[f]	control[b] mean[d] ± SE (range)[f]	conventional[c] tolerance interval[g] (range)[f]	MON 88017 mean[e] ± SE (range)[f]	control[b] mean[e] ± SE (range)[f]	conventional[c] tolerance interval[g] (range)[f]	literature[h] (range)[f]
Ala	7.55 ± 0.084 (7.29–7.70)	7.55 ± 0.084 (7.34–7.79)	6.66, 8.49 (7.24–8.16)	7.66[i] ± 0.072 (7.37–7.85)	7.57 ± 0.072 (7.28–7.87)	6.90, 8.25 (7.13–7.93)	6.4–9.9
Arg	4.42 ± 0.11 (4.10–4.74)	4.29 ± 0.11 (4.01–4.63)	3.34, 5.67 (3.72–5.08)	4.49 ± 0.11 (4.04–5.12)	4.67 ± 0.11 (4.24–5.09)	3.47, 5.41 (3.82–5.10)	2.9–5.9
Asp	6.22 ± 0.05 (6.09–6.34)	6.25 ± 0.05 (6.04–6.45)	5.77, 7.16 (6.18–6.81)	6.71 ± 0.051 (6.50–6.91)	6.83 ± 0.051 (6.63–7.02)	5.88, 7.28 (6.28–7.10)	5.8–7.2
Cys	2.14 ± 0.054 (1.93–2.26)	2.15 ± 0.054 (1.93–2.30)	1.46, 2.89 (1.82–2.58)	1.97 ± 0.052 (1.77–2.17)	1.99 ± 0.052 (1.82–2.24)	1.32, 2.75 (1.73–2.60)	1.2–1.6
Glu	20.40 ± 0.18 (19.80–20.87)	20.44 ± 0.18 (19.91–20.84)	18.01, 22.15 (19.46–21.57)	19.53[i] ± 0.17 (18.73–20.01)	19.24 ± 0.17 (18.61–19.90)	18.10, 21.32 (18.28–20.50)	12.4–19.6
Gly	3.45 ± 0.063 (3.32–3.62)	3.45 ± 0.063 (3.18–3.61)	2.81, 4.54 (3.29–4.03)	3.79[i] ± 0.067 (3.55–4.06)	3.92 ± 0.067 (3.74–4.18)	2.99, 4.38 (3.22–4.18)	2.6–4.7
His	2.99 ± 0.049 (2.90–3.10)	2.95 ± 0.049 (2.83–3.14)	2.16, 3.60 (2.50–3.12)	2.83 ± 0.051 (2.70–3.05)	2.86 ± 0.051 (2.76–3.05)	2.12, 3.83 (2.65–3.60)	2.0–2.8
Ile	3.59 ± 0.037 (3.43–3.71)	3.57 ± 0.037 (3.45–3.76)	3.30, 3.84 (3.39–3.79)	3.59[i] ± 0.052 (3.42–3.86)	3.53 ± 0.052 (3.34–3.64)	3.19, 3.79 (3.21–3.68)	2.6–4.0
Leu	13.28 ± 0.20 (12.69–13.62)	13.31 ± 0.20 (12.76–14.11)	10.72, 15.18 (12.11–14.35)	13.12[i] ± 0.12 (12.49–13.59)	12.78 ± 0.12 (12.18–13.30)	11.47, 14.93 (11.99–14.29)	7.8–15.2
Lys	2.69 ± 0.058 (2.42–2.87)	2.66 ± 0.058 (2.49–2.82)	2.06, 3.73 (2.44–3.27)	3.13[i] ± 0.069 (2.90–3.38)	3.26 ± 0.069 (3.05–3.62)	2.37, 3.57 (2.51–3.42)	2.0–3.8
Met	1.98 ± 0.059 (1.85–2.05)	2.01 ± 0.059 (1.83–2.20)	1.37, 2.60 (1.70–2.47)	1.98 ± 0.051 (1.75–2.16)	1.95 ± 0.051 (1.71–2.08)	1.42, 2.50 (1.72–2.40)	1.0–2.1
Phe	5.18 ± 0.059 (4.97–5.31)	5.14 ± 0.059 (5.01–5.32)	4.57, 5.71 (4.82–5.39)	5.25 ± 0.022 (5.15–5.31)	5.19 ± 0.022 (5.02–5.35)	4.65, 5.69 (4.94–5.55)	2.9–5.7
Pro	9.39 ± 0.094 (9.02–9.69)	9.34 ± 0.094 (8.85–9.80)	7.60, 10.37 (8.35–9.72)	8.72[i] ± 0.049 (8.45–8.95)	8.58 ± 0.049 (8.45–8.78)	7.89, 10.04 (7.78–9.78)	6.6–10.3
Ser	4.83 ± 0.049 (4.65–5.04)	4.91 ± 0.049 (4.63–5.13)	4.60, 5.43 (4.81–5.23)	5.26 ± 0.095 (5.02–5.51)	5.30 ± 0.095 (5.02–5.74)	4.57, 5.86 (4.89–5.83)	4.2–5.5
Thr	3.22 ± 0.04 (3.10–3.38)	3.25 ± 0.04 (3.06–3.37)	2.89, 3.84 (2.96–3.55)	3.36 ± 0.09 (2.95–3.53)	3.39 ± 0.09 (2.93–3.60)	2.64, 3.97 (2.66–3.60)	2.9–3.9
Trp	0.54 ± 0.027 (0.48–0.60)	0.55 ± 0.027 (0.41–0.68)	0.36, 0.77 (0.44–0.83)	0.59 ± 0.029 (0.48–0.70)	0.61 ± 0.029 (0.49–0.68)	0.43, 0.73 (0.47–0.74)	0.5–1.2
Tyr	3.35 ± 0.16 (2.35–3.66)	3.43 ± 0.16 (2.58–3.66)	2.62, 4.26 (2.26–3.80)	3.29 ± 0.14 (2.31–3.85)	3.60 ± 0.14 (2.45–3.93)	2.25, 4.62 (2.19–3.91)	2.9–4.7
Val	4.79 ± 0.039 (4.60–4.92)	4.74 ± 0.039 (4.60–4.94)	4.22, 5.27 (4.44–5.04)	4.73 ± 0.06 (4.52–4.97)	4.72 ± 0.06 (4.56–4.84)	4.23, 5.19 (4.42–5.03)	2.1–5.2

[a] All data are expressed as a percent total of amino acid. [b] Conventional control corn (LH198 × LH59) grain samples. [c] Commercial hybrids planted at each field site. [d] The least-squares mean of nine values. [e] The least-squares mean of 12 values. [f] Range denotes the lowest and highest individual values across samples. [g] Tolerance interval is specified to contain 99% of the commercial hybrid population; negative limits are set to zero. [h] Ref *54.* [i] Statistically different from the control at the 5% level ($p < 0.05$).

samples were compared to the values from control samples to determine statistical differences at $p < 0.05$. The conventional hybrids were not statistically analyzed for differences; however, a range of observed values from the conventional hybrids was determined for each analytical component. Additionally, the conventional data were used to develop population tolerance intervals using the following equation:

$$\vec{y} \pm ks$$

where y = mean, s = standard deviation, and k = function of the samples size, the degree of confidence, and the coverage. A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each compositional component, 99% tolerance intervals were calculated for each country that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of conventional varieties. Each unique conventional hybrid contributed one value to the calculation of the tolerance interval. This value is generated for a specific hybrid by first averaging over the replicates within a site and then averaging across the sites. Because negative quantities are not possible, negative calculated lower tolerance bounds were set to zero.

RESULTS AND DISCUSSION

Results from the analyses of the combination of all field sites showed that there were no significant differences ($p \geq 0.05$) in the levels observed between the MON 88017 and the control for 59 of the 62 analytes measured in U.S. samples and 47 of the 61 analytes measured in Argentina samples. For the comparisons observed to be statistically different between MON 88017 and control, all MON 88017 values were within the 99% tolerance interval except for isoleucine in the Argentina samples. Below is a summary of results for corn forage and grain composition for each analyte measured. A review of the literature is also presented with the summary of results (**Tables 2−6**).

Proximate, Fiber, and Mineral Composition of Corn Forage. Table 2 contains the proximate, fiber, and mineral composition combined site results of corn forage for all samples in the United States and Argentina. The results show that there were no statistical differences between samples of MON 88017 and the control across field sites in the United States or Argentina. The proximate, fiber, and mineral compositions are also similar to values reported in the literature (**Table 2**) and,

Table 5. Fatty Acid Composition of Grain from MON 88017, Control, and Conventional Corn Hybrids

	United States			Argentina			
component[a]	MON 88017 mean[d] ± SE (range)[f]	control[b] mean[d] ± SE (range)[f]	conventional[c] tolerance interval[g] (range)[f]	MON 88017 mean[e] ± SE (range)[f]	control[b] mean[e] ± SE (range)[f]	conventional[c] tolerance interval[g] (range)[f]	literature[h] (range)[f]
palmitic (16:0)	10.24 ± 0.43	11.27 ± 0.43	6.51, 16.50	11.52 ± 0.081	11.71 ± 0.081	7.03, 14.36	8.51–17.46
	(10.07–10.52)	(10.14–14.57)	(9.29–17.81)	(11.31–11.79)	(11.35–12.58)	(8.29–12.81)	
palmitoleic (16:1)	0.18 ± 0.01	0.18 ± 0.01	0.0017, 0.28	0.10 ± 0.017	0.10 ± 0.017	0.028, 0.21	0.101–0.325
	(0.16–0.21)	(0.16–0.22)	(0.054–0.21)	(0.051–0.17)	(0.055–0.15)	(0.055–0.18)	
stearic (18:0)	2.01 ± 0.073	2.07 ± 0.073	1.41, 2.53	2.05 ± 0.022	2.07 ± 0.022	0.91, 2.95	1.02–2.76
	(1.80–2.19)	(1.76–2.23)	(1.68–2.30)	(2.00–2.11)	(1.95–2.20)	(1.35–2.49)	
oleic (18:1)	22.74 ± 0.23	22.87 ± 0.23	9.25, 44.14	26.61[i] ± 0.46	32.12 ± 0.46	8.21, 45.14	18.6–40.1
	(22.20–23.53)	(21.43–23.51)	(19.79–34.46)	(25.72–27.74)	(30.50–33.97)	(19.73–40.72)	
linoleic (18:2)	62.85[i] ± 0.39	61.52 ± 0.39	41.22, 74.09	57.69[i] ± 0.50	51.97 ± 0.50	40.78, 76.51	43.1–65.6
	(61.86–63.72)	(59.10–63.18)	(51.64–64.12)	(56.22–58.80)	(49.67–53.98)	(45.41–65.50)	
linolenic (18:3)	1.21 ± 0.062	1.32 ± 0.062	0.42, 1.95	1.12[i] ± 0.014	1.08 ± 0.014	0.52, 1.60	0.70–1.92
	(1.15–1.26)	(1.19–1.77)	(0.84–1.91)	(1.08–1.16)	(0.92–1.14)	(0.73–1.30)	
arachidic (20:0)	0.37[i] ± 0.01	0.38 ± 0.01	0.31, 0.49	0.43 ± 0.0073	0.45 ± 0.0073	0.21, 0.61	0.279–0.720
	(0.35–0.39)	(0.35–0.41)	(0.36–0.45)	(0.41–0.44)	(0.42–0.49)	(0.30–0.53)	
eicosenoic (20:1)	0.24 ± 0.0056	0.25 ± 0.0056	0.18, 0.40	0.30[i] ± 0.007	0.33 ± 0.007	0.13, 0.47	0.170–1.917
	(0.23–0.26)	(0.24–0.26)	(0.24–0.36)	(0.29–0.31)	(0.30–0.40)	(0.21–0.42)	
behenic (22:0)	0.15 ± 0.0027	0.15 ± 0.0027	0.071, 0.25	0.17 ± 0.004	0.17 ± 0.004	0.10, 0.24	0.110–0.349
	(0.14–0.16)	(0.14–0.17)	(0.074–0.24)	(0.15–0.19)	(0.15–0.20)	(0.12–0.21)	

[a] All data are expressed as a percent total of fatty acid. [b] Conventional control corn (LH198 × LH59) grain samples. [c] Commercial hybrids planted at each field site. [d] The least-squares mean of nine values. [e] The least-squares mean of 12 values. [f] Range denotes the lowest and highest individual values across samples. [g] Tolerance interval is specified to contain 99% of the commercial hybrid population; negative limits are set to zero. [h] ILSI Crop Composition Database, ref *53*. [i] Statistically different from the control at the 5% level ($p < 0.05$).

Table 6. Phytic Acid, Secondary Metabolite, and Vitamin Composition of Grain from MON 88017, Control, and Conventional Corn Hybrids

	United States			Argentina			
component	MON 88017 mean[d] ± SE (range)[f]	control[b] mean[d] ± SE (range)[f]	conventional[c] tolerance interval[g] (range)[f]	MON 88017 mean[e] ± SE (range)[f]	control[b] mean[e] ± SE (range)[f]	conventional[c] tolerance interval[g] (range)[f]	literature[h] (range)[f]
ferulic acid	2175.34 ± 46.31	2121.05 ± 46.31	1415.19, 3173.90	1841.12 ± 29.8	1782.07 ± 29.8	820.14, 3209.76	1340–3725
	(1986.75–2275.48)	(1927.55–2339.71)	(1717.17–2687.57)	(1642.13–2018.14)	(1628.70–1895.57)	(1371.88–2711.86)	
p-coumaric acid	169.26 ± 7.26	154.83 ± 7.26	43.13, 384.34	157.44 ± 6.99	176.95 ± 6.99	45.63, 370.04	90.7–576
	(148.45–215.25)	(141.41–173.24)	(152.30–319.15)	(133.79–182.44)	(150.96–210.59)	(143.18–297.18)	
phytic acid	0.95 ± 0.043	0.89 ± 0.043	0.28, 1.12	0.68 ± 0.041	0.70 ± 0.041	0.24, 1.12	0.29–1.29
	(0.83–1.05)	(0.72–1.03)	(0.45–1.00)	(0.62–0.78)	(0.44–0.91)	(0.18–1.00)	
raffinose	0.17 ± 0.013	0.17 ± 0.013	0, 0.32	n/a	n/a	n/a	0.04–0.29
	(0.14–0.20)	(0.14–0.23)	(0.073–0.22)				
folic acid	0.48 ± 0.021	0.48 ± 0.021	0.12, 0.77	0.77[i] ± 0.037	0.68 ± 0.037	0.48, 0.99	0.147–1.209
	(0.38–0.60)	(0.42–0.59)	(0.28–0.61)	(0.54–0.86)	(0.42–0.84)	(0.52–0.97)	
niacin	20.94 ± 1.20	21.75 ± 1.20	3.19, 34.49	22.26 ± 0.42	21.33 ± 0.42	10.15, 37.38	14.11–36.28
	(17.04–24.14)	(19.08–23.92)	(14.11–27.77)	(20.30–23.80)	(19.59–24.13)	(17.26–34.43)	
vitamin B_1	2.47[i] ± 0.14	3.24 ± 0.14	1.96, 4.38	3.53 ± 0.29	4.17 ± 0.29	2.16, 6.13	1.26–8.54
	(2.30–2.69)	(2.99–3.60)	(2.69–3.73)	(2.72–4.79)	(3.11–4.58)	(2.94–5.37)	
vitamin B_2	1.10 ± 0.041	1.13 ± 0.041	0.67, 1.51	1.35 ± 0.035	1.28 ± 0.035	0.85, 1.82	0.70–1.93
	(0.98–1.22)	(0.99–1.33)	(0.88–1.32)	(1.24–1.68)	(1.03–1.38)	(0.82–1.80)	
vitamin B_6	7.16 ± 0.22	7.10 ± 0.22	4.29, 7.84	5.72 ± 0.21	5.88 ± 0.21	3.83, 9.30	4.57–7.32
	(6.57–8.06)	(5.65–8.54)	(4.93–7.24)	(5.12–6.58)	(4.85–6.66)	(5.13–8.06)	
vitamin E	14.15 ± 1.70	14.07 ± 1.70	0, 29.69	19.26 ± 1.5	17.63 ± 1.5	0, 26.50	1.5–68.7
	(6.08–16.93)	(1.74–17.77)	(8.09–21.97)	(16.84–33.03)	(1.71–21.48)	(1.71–19.43)	

[a] All data are expressed as milligram per kilogram dry weight of sample, except for ferulic acid and *p*-coumaric acid, which are expressed as microgram per gram dry weight of sample, and phytic acid and raffinose, which are expressed as percent dry weight of sample. [b] Conventional control corn (LH198 × LH59) grain samples. [c] Commercial hybrids planted at each field site. [d] The least-squares mean of nine values. [e] The least-squares mean of 12 values. [f] Range denotes the lowest and highest individual values across samples. [g] Tolerance interval is specified to contain 99% of the commercial hybrid population; negative limits are set to zero. [h] Ref *53*. [i] Statistically different from the control at the 5% level ($p < 0.05$).

with the results herein, considered to fall within the population of corn forage currently available in the market.

Proximate, Fiber, and Mineral Composition of Corn Grain. Table 3 contains the proximate, fiber, and mineral composition combined site results of corn grain for all samples in the United States and Argentina. The results show that either there were no statistical differences between MON 88017 and control, or when there was a statistical difference, all MON 88017 values were found to be within the 99% tolerance interval (calculated for each country from the conventional commercial hybrids). The proximate, fiber, and mineral compositions are also similar to values reported in the literature (**Table 3**) and, with the results herein, considered to fall within the population of corn grain currently available in the market.

Amino Acid Composition of Corn Grain. Table 4 contains the amino acid composition combined site results of corn grain for all samples in the United States and Argentina. The results show that either there were no statistical differences between MON 88017 and control, or when there was a statistical difference, all MON 88017 values were found to be within the 99% tolerance interval, with the exception of isoleucine in Argentina. At one site in Argentina, the isoleucine value from one sample of MON 88017 was <2% greater than the upper end of the 99% tolerance interval, while all other samples with statistical differences were within the interval. Notably, the magnitude of the difference between the means of MON 88017 and control samples for isoleucine was small (<2%), and all MON 88017 isoleucine values were found to be within reported literature ranges. In the U.S. samples, isoleucine was not found to be statistically different from the control, further indicating that the difference observed in the Argentina sample is not likely to be biologically significant. The similarity in levels of aromatic amino acids between MON 88017, control, and conventional corn hybrids as well as reported literature values indicates that the presence of the CP4 EPSPS enzyme in MON 88017 had no effect on the normal distribution of these amino acids.

Fatty Acid Composition of Corn Grain. Table 5 contains the fatty acid composition combined site results of corn grain for all samples in the United States and Argentina. The results show that either there were no statistical differences between MON 88017 and control, or when there was a statistical difference, all MON 88017 values were found to be within the 99% tolerance interval. The fatty acid composition is also similar to values reported in the literature(**Table 5**) and, with the results herein, considered to fall-within the population of corn grain currently available in the market.

Secondary Metabolite, Antinutrient, and Vitamin Composition of Corn Grain. Table 6 contains the secondary metabolite, antinutrient, and vitamin composition combined site results of corn grain for all samples in the United States and Argentina. The results show that either there were no statistical difference between MON 88017 and control, or when there was a statistical difference, all MON 88017 values were found to be within the 99% tolerance interval. The secondary metabolite, antinutrient, and vitamin compositions are also similar to values reported in the literature(**Table 6**) and, with the results herein, considered to fall within the population of corn grain currently available in the market.

In conclusion, the results of compositional analyses generated from seven field sites in two countries over a 2 year period demonstrate that the corn forage and grain of MON 88017 are comparable with a near-isogenic control and conventional corn hybrids. There were no statistical differences ($p \geq 0.05$) observed between MON 88017 and control or all MON 88017 values were within the 99% tolerance interval for all analytes measured in the U.S. samples and for 60 of the 61 analytes measured in Argentina samples.

The results of this study demonstrate that the composition of MON 88017 is equivalent to the composition of the control and conventional corn hybrids and representative of the composition of corn within the population of corn hybrids currently available. Along with the safety evaluations previously concluded on the proteins (*14*, *17*) and products containing those proteins (*6*, *7*), this study further demonstrates MON 88017 is as safe as conventional hybrids of corn on the market today.

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