Comparison of Broiler Performance and Carcass Yields When Fed Diets Containing Transgenic Maize Grains from Event DP-Ø9814Ø-6 (Optimum GAT), Near-Isogenic Control Maize Grain, or Commercial Reference Maize Grains[1]

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ABSTRACT A genetically modified maize (*Zea mays* L.) line that contains the Optimum GAT trait (event DP-Ø9814Ø-6; 98140) was produced by integration of the *gat4621* and *zm-hra* genes. The expressed GAT4621 and ZM-HRA proteins confer tolerance to the herbicidal active ingredient glyphosate and acetolactate synthase-inhibiting herbicides, respectively. The objective of this study was to compare the nutritional performance of 98140 maize grain to nontransgenic maize grain in a 42-d feeding trial in broiler chickens. Diets were prepared using grain from untreated 98140 plants and from plants treated with an in-field application of herbicides (98140 + Spray). For comparison, additional diets were produced with maize grain obtained from the nontransgenic near-isogenic control (control) and nontransgenic commercial reference Pioneer brand hybrids 33J56, 33P66, and 33R77. Diets were fed to Ross × Cobb broilers (n = 120/group, 50% male and 50% female) in 3 phases: starter, grower, and finisher containing 58.5, 64, and 71.5% maize grain, respectively. No statistically significant differences were observed in mortality, growth performance variables, or carcass and organ yields between broilers consuming diets produced with maize grains from unsprayed or sprayed 98140 and those consuming diets produced with near-isogenic control maize grain. Additionally, all performance and carcass variables from control, 98140, and 98140 + Spray test maize treatment groups were within tolerance intervals constructed using data from reference maize groups. Based on these results, it was concluded that 98140 maize grain (unsprayed or sprayed with a herbicide mixture) was nutritionally equivalent to nontransgenic control maize with comparable genetic background.

Key words: maize, GAT, ZM-HRA, broiler performance, carcass yield

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INTRODUCTION

The original concept for the safety and nutritional assessment of foods and feed fractions obtained from genetically modified (**GM**) crops was based on equivalency between the transgenic crops and nontransgenic counterparts (Organization of Economic Co-operation and Development, 1993). Subsequent guidelines recommended demonstrating equivalence by compositional analysis and comparison of the nutrient composition in whole grains with that of nontransgenic near-isogenic whole grains using a defined list of components with known nutritional or antinutritional effect (Organization of Economic Co-operation and Development, 2001; European Food Safety Authority, 2006a,b). Although the ingredients evaluated in compositional analyses are comprehensive, they do not account for every individual ingredient present within the complex matrices of whole foods, and it is possible that alterations in additional components could potentially affect the nutritional quality of the GM grains. Therefore, feeding trials with whole grains and processed feed fractions obtained from GM crops have also been conducted to compare their wholesomeness to corresponding non-GM grains to determine if particular genetic modifications could have resulted in unintended changes that could affect nutritional quality. Nutritional equivalency studies have been conducted in broiler chickens, because they are a rapidly growing species whose diet typically contains a high concentration of maize grain and because weight gain and mortality are sensitive indicators of changes in the nutritional quality of their diet (International Life Sciences Institute, 2003). Several nutritional equivalence feeding studies that have been conducted in broiler chickens with grains and processed

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feed fractions from insect-resistant, herbicide-tolerant, or stacked-trait grains have reported no differences in nutritional performance and carcass variables between broilers consuming diets formulated with grains from transgenic crops and those fed diets containing nontransgenic grains (Brake et al., 2003, 2005; Taylor et al., 2003a,b,c, 2005, 2007a,b; McNaughton et al., 2007a,b). Overall, these reports indicate that the particular transgenic substances were as wholesome and nutritious as those obtained from nontransgenic sources.

Maize (*Zea mays* L.) plants were modified by integration of the *gat4621* and *zm-hra* genes to produce event DP-Ø9814Ø-6 (98140, Optimum GAT). The *gat4621* gene, isolated from *Bacillus licheniformis*, was functionally improved by a gene-shuffling process to optimize the kinetics of the glyphosate acetyltransferase enzyme to acetylate glyphosate (Castle et al., 2004; Siehl et al., 2005). The GAT4621 protein, encoded by the *gat4621* gene, confers in planta tolerance to the herbicidal active ingredient glyphosate. The ZM-HRA protein, encoded by the *zm-hra* gene, confers tolerance to acetolactate synthase inhibiting herbicides, such as sulfonylurea and imidazolinone herbicides, by preventing the inhibition of acetolactate synthase, an enzyme required in branched-chain amino acid synthesis (Lee et al., 1988). The objective of this study was to assess the nutritional performance of 98140 maize grain with that of nontransgenic maize grain controls by comparing the growth performance (as measured by body weight and feed efficiency) and carcass yields of growing broiler chickens.

MATERIALS AND METHODS

Maize Grains

Test maize grain 98140 was obtained from plants containing the coding sequence for the *gat4621* and *zm-hra* genes. An additional lot of 98140 maize grain was obtained from plants that were treated (98140 + Spray) with a herbicide tank mixture containing glyphosate (Touchdown HiTech, Syngenta, Basel, Switzerland) and nicosulfuron + rimsulfuron (DuPont Steadfast, E.I. du Pont de Nemours and Company, Wilmington, DE). Control maize grain was obtained from nontransgenic plants with a genetic background similar to 98140 maize plants. Reference maize grains (Pioneer hybrids 33J56, 33P66, and 33R77) were commercially available nontransgenic grains. Neither control nor reference grains were treated with herbicides. All maize grains for this trial were planted in isolated plots in May 2006 in a field trial near Richland, Iowa, and harvested in November 2006.

Maize Grain Characterization

Event-specific real-time PCR testing confirmed the presence of the insert from event DP-Ø9814Ø-6 in the test maize grains (98140, 98140 + Spray) and its absence from control and reference maize grains (data not shown). Enzyme-linked immunosorbent assay analysis confirmed the expression of the transgenic GAT4621 (7.4 and 7.7 ng/mg of grain, respectively) and ZM-HRA (0.14 and 0.16 ng/mg of grain, respectively) proteins from the event in the test maize grains (98140, 98140 + Spray) and their absence from control and reference maize grains [GAT4621 lower limit of quantitation (**LLOQ**) = 0.11 ng/mg of grain, ZM-HRA LLOQ = 0.14 ng/mg of grain]. All maize grains (control, test, and references) were evaluated for nutrient proximate composition, calcium, phosphorous, and mycotoxin content at Cumberland Valley Analytical Services (Hagerstown, MD). Dry matter (930.15), protein (990.03), fiber (978.10), ash (942.05), calcium and phosphorus (985.01), and mycotoxin (994.08, 995.15, and 986.17) analyses were performed according to AOAC (2000) methods; fat analysis was performed according to AOAC (1990) method 920.39. Amino acid content of the grains was determined at Eurofins Scientific (Memphis, TN) in accordance with AOAC (2000; methods 988.15, 982.30, and 994.12). Maize grain samples were analyzed for gross energy content with a bomb calorimeter (model 1271, Parr Instruments, Moline, IL) at Pioneer Hi-Bred (Urbandale, IA). The analyzed nutrient content of the corn sources is presented in Table 1.

Birds and Housing

Animal care and use practices during this trial conformed to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999). Seven hundred twenty (50% males and 50% females) commercial broilers (Ross × Ross 308) were obtained at hatch (trial d 0) from a commercial Maryland hatchery; birds were feather-sexed at hatch and transported to AHPharma Inc. (farm 1, Tyaskin, MD) in February 2007. Broilers were evaluated upon receipt for signs of disease or other complications that may have affected the outcome of the study; observations of bird health and actual number of birds received were documented. Broilers were weighed after examination, identified with a wingband, and placed randomly in 0.914 m × 1.219 m (3 ft × 4 ft) floor pens at a density of approximately 0.305 m^2 (1.0 ft^2) of available floor space per broiler; new pine shavings with a minimal amount of saw dust were provided as litter. Pens were separated by a wire partition and did not touch other pens from any side to minimize potential for cross-contamination. Broilers were housed in a room containing forced-air heaters and individual pen heat lamps with a cross-house ventilation system. A continuous 24-h lighting program was followed. Birds were observed 3 times daily for overall health, behavior and evidence of toxicity, and environmental conditions. No type of medication was administered during the entire feeding period. Mortalities were recorded, and complete necropsy examinations were performed on all broilers found dead or moribund. Carcasses of necropsied broil-

Table 1. Analyzed proximate, calcium, phosphorous, gross energy, and amino acid composition[1] of grain from 98140 and 98140 + Spray maize, nontransgenic near-isogenic control maize (control), and commercial reference maize (33J56, 33P66, and 33R77)

Analyzed composition[2]	Control	98140	98140 + Spray	33J56	33P66	33R77
Proximates						
Moisture, %	11.2	12.1	12.1	11.9	12.3	12.1
Protein, %	7.8	7.9	8.0	7.3	7.4	7.5
Fat, %	3.2	3.1	2.9	2.8	3.0	2.7
Fiber, %	0.9	1.0	0.9	1.0	1.0	1.2
Ash, %	1.5	1.3	1.7	1.3	1.5	1.6
Calcium, %	0.01	0.01	0.02	0.02	0.01	0.02
Phosphorus, %	0.24	0.26	0.24	0.22	0.24	0.25
Gross energy, kcal/kg	3,930	3,927	3,927	3,892	3,915	3,910
ME,[3] kcal/kg	3,459	3,456	3,456	3,425	3,446	3,441
Essential amino acids						
Arginine, %	0.35	0.30	0.30	0.31	0.28	0.27
Lysine, %	0.21	0.19	0.18	0.21	0.18	0.17
Histidine, %	0.31	0.21	0.21	0.20	0.18	0.19
Isoleucine, %	0.26	0.23	0.23	0.23	0.21	0.21
Leucine, %	0.97	0.89	0.88	0.91	0.80	0.79
Methionine, %	0.20	0.21	0.20	0.18	0.21	0.19
Phenylalanine, %	0.39	0.35	0.34	0.35	0.31	0.31
Threonine, %	0.28	0.26	0.25	0.25	0.24	0.24
Tryptophan, %	0.07	0.08	0.08	0.07	0.08	0.07
Valine, %	0.38	0.33	0.33	0.33	0.29	0.30
Nonessential amino acids						
Alanine, %	0.62	0.55	0.55	0.58	0.50	0.50
Aspartic acid, %	0.57	0.52	0.51	0.57	0.47	0.47
Cystine, %	0.20	0.20	0.20	0.19	0.20	0.20
Glutamic acid, %	1.55	1.42	1.41	1.44	1.29	1.29
Glycine, %	0.29	0.26	0.26	0.26	0.24	0.25
Proline, %	0.71	0.67	0.66	0.63	0.61	0.64
Serine, %	0.37	0.33	0.34	0.34	0.31	0.31
Tyrosine, %	0.22	0.16	0.15	0.22	0.16	0.16

[1]Proximate and mineral analyses were performed by Cumberland Valley Analytical Services (Hagerstown, MD). Gross energy analysis was performed by Pioneer Hi-Bred (Urbandale, IA). Amino acid analysis was conducted by Eurofins Scientific Inc. (Memphis, TN). Each cell represents the mean of 2 samples.

[2]All values on an as-is basis.

[3]The ME values were calculated for maize grain using conversion factors based upon internal Pioneer Hi-Bred data.

ers were disposed of according to local regulations via composting. Drinking water was provided for ad libitum consumption.

Experimental Design

This study was designed as a randomized complete block with 6 dietary treatments (control, 98140, 98140 + Spray, and 3 commercial references). There were 10 broilers per pen (5 males and 5 females) and 12 pens (replicates) per treatment for a total of 120 broilers per treatment. Broilers were fed their respective dietary treatments from time of hatching (trial d 0) to 42 d of age.

Diets

Diets were fed in 3 phases: starter (d 0 to 21), grower (d 22 to 35), and finisher (d 36 to 42). All diets were offered as a mash feed for ad libitum consumption. Starter, grower, and finisher diets were formulated to meet the nutrient requirements of a typical commercial broiler diet using the NRC Nutrient Requirements of Poultry as a guideline (NRC, 1994). Diets were prepared at the Pioneer Livestock Nutrition Center (Polk City, IA). Before diet preparation, each maize grain source was individually cleaned and milled (375 kg) to an average particle diameter of 650 to 750 microns using a Bliss Experimental hammer mill (Bliss Manufacturing, Ponca City, OK). Grain sources were milled in the following order to minimize the potential for cross-contamination of nontransgenic grain with transgenic grain: control, 33J56, 33P66, 33R77, 98140, and 98140 + Spray. Control, test, or reference maize grains were added to the indicated diets in equal amounts; requirements for protein, lysine, methionine, cystine, calcium, and phosphorus were met by adjusting the concentrations of nonmaize ingredients. Within each phase, all diets were formulated to the same ME level: starter diets, 3,210 kcal of ME/kg; grower diets, 3,250 kcal of ME/kg; and finisher diets, 3,285 kcal of ME/kg. Starter, grower, and finisher diets for each maize grain source were mixed in the same order as followed for grain milling. Mixing equipment was flushed with nontransgenic soy hulls before diet preparation. All diets were prepared using a ribbon mixer (Sudenga M750, Sudenga Industries Inc., George, IA) that was cleaned between each diet (starter, grower, and finisher) using compressed air and vacuum; mixing equipment was flushed with nontransgenic soybean hulls between each maize grain source. Prepared diets were subsampled, and samples were composited for proximate analysis (including calcium and phosphorus), amino acid analysis, and gross energy analysis, as described previously; ingredient and analyzed nutrient compositions of starter, grower, and finisher diets are presented in Tables 2, 3, and 4, respectively. All diets were also evaluated for expression of the GAT4621 and ZM-HRA transgenic proteins using ELISA methods (Pioneer Hi-Bred). Concentrations of these proteins were evaluated in samples collected at the time of diet preparation (beginning, middle, and end of production) to determine if the diets were blended homogeneously and again at the start and end of each diet phase to determine whether the expressed transgenic proteins were stable over the duration of the feeding phase.

Measurements

Body weights and feed weights (including amount of feed added and amount remaining) were determined every 7 d. Body weight gain, feed intake and mortality-corrected feed:gain ratio (feed efficiency) were calculated for d 0 through 42. All surviving birds were killed on study d 42 by cervical dislocation and subjected to a gross necropsy. Carcass and carcass parts yield data were collected from 576 broilers (4 males and 4 females per pen) and included carcass yield (postchilled), thighs, breasts, wings, legs, abdominal fat (including fat around gizzard), kidneys, and whole liver. Combined total mass was recorded for all parts considered as pairs (i.e., legs, thighs, both sides of the breast). Kidney and liver weights were expressed as percentages

Table 2. Ingredient[1] and analyzed nutrient[2] (as-fed basis) compositions of starter diets containing grain from 98140 and 98140 + Spray maize, nontransgenic near-isogenic control maize (control), and commercial reference maize (33J56, 33P66, and 33R77)

Item	Control	98140	98140 + Spray	33J56	33P66	33R77
Ingredient, %						
Maize	58.500	58.500	58.500	58.500	58.500	58.500
Soybean meal	29.359	28.864	28.927	27.923	29.060	28.494
Soybean hulls	4.185	4.500	4.500	4.500	4.067	4.394
Sysco soybean oil	0.050	0.055	0.109	0.050	0.050	0.050
Protein blend	4.041	4.201	4.075	5.247	4.489	4.725
DL-Methionine	0.212	0.212	0.216	0.199	0.193	0.207
L-Lysine-HCL	0.032	0.057	0.067	0.039	0.049	0.067
Limestone	0.808	0.797	0.787	0.716	0.789	0.759
Di-Cal 18[3]	1.746	1.747	1.750	1.760	1.737	1.736
Choline-Cl	0.006	0.007	0.007	0.010	0.007	0.008
NaCl	0.436	0.436	0.436	0.432	0.435	0.434
VTM Premix[4]	0.625	0.625	0.625	0.625	0.625	0.625
Analyzed nutrient and fatty acid compositions						
Proximates						
Moisture, %	12.2	12.6	12.4	12.5	12.5	12.5
Protein, %	23.3	23.7	22.7	23.9	23.2	22.7
Fat, %	2.4	2.3	2.2	2.2	2.0	2.2
Fiber, %	2.7	2.7	3.0	2.8	2.8	3.0
Ash, %	5.0	5.7	5.6	5.0	5.0	5.6
Calcium, %	0.83	0.89	0.84	0.87	0.89	0.81
Phosphorus, %	0.64	0.73	0.70	0.66	0.69	0.66
Gross energy, kcal/kg	3,934	3,912	3,889	3,916	3,908	3,907
Essential amino acids						
Arginine, %	1.49	1.52	1.48	1.52	1.50	1.48
Histidine, %	1.19	1.22	1.25	1.22	1.26	1.25
Isoleucine, %	0.58	0.57	0.56	0.59	0.56	0.56
Leucine, %	0.97	0.97	0.92	0.99	0.95	0.89
Lysine, %	2.00	2.04	1.98	2.03	2.00	1.97
Methionine, %	0.54	0.53	0.56	0.51	0.55	0.51
Phenylalanine, %	1.15	1.17	1.16	1.19	1.16	1.17
Threonine, %	0.90	0.91	0.91	0.93	0.91	0.93
Tryptophan, %	0.31	0.31	0.29	0.32	0.32	0.31
Valine, %	1.15	1.16	1.09	1.21	1.13	1.07
Nonessential amino acids						
Alanine, %	1.19	1.22	1.20	1.22	1.21	1.20
Aspartic acid, %	2.31	2.35	2.32	2.37	2.35	2.34
Cystine, %	0.42	0.42	0.42	0.45	0.44	0.45
Glutamic acid, %	4.11	4.19	4.14	4.13	4.13	4.13
Glycine, %	1.03	1.06	1.03	1.09	1.06	1.07
Proline, %	1.60	1.69	1.62	1.66	1.65	1.68
Serine, %	1.27	1.33	1.32	1.35	1.34	1.39
Tyrosine, %	0.68	0.70	0.70	0.70	0.70	0.71

[1]Diets were formulated to contain the following: ME, 3,210 kcal/kg; protein, 23.00%; lysine, 1.20%; and methionine + cystine, 1.02%.

[2]Proximate and mineral analyses were performed by Cumberland Valley Analytical Services (Hagerstown, MD). Gross energy and fatty acid analyses were performed by Pioneer Hi-Bred International Inc. (Urbandale, IA). Amino acid analysis was conducted by Eurofins Scientific Inc. (Memphis, TN). Each cell represents 1 (n = 1) determination.

[3]Di-Cal 18 was purchased from Agritronics Corporation (Elkhart, IA).

[4]Poultry VTM 88 vitamin and trace mineral premix was purchased from Archer Daniels Midland (Quincy, IL).

of whole live bird weight. Carcass yield was expressed as the percentage of whole live bird weight, and parts yields were expressed as the percentage of postchilled dressed carcass weight. Birds and remaining test feeds were disposed of by composting, conforming to local and state regulations.

Statistical Analysis

The mean value of data from control and 98140 test maize groups was calculated for each variable to test the primary hypothesis that growth performance and carcass yield would be different between broiler chickens fed diets containing test maize grain and those fed diets containing the respective nontransgenic near-isogenic control maize grain. A secondary hypothesis tested was that growth performance and carcass yield of birds fed diets containing test maize grain produced under a herbicide spray regimen (98140 + Spray) would be different from that of birds fed diets containing control maize grain. Thus, comparisons made were control versus 98140 and control versus 98140 + Spray (Table 5). Data were analyzed using a mixed model ANOVA (PROC MIXED, SAS version 9.1 software, SAS Institute Inc., Cary, NC). Statistical analysis of live performance data was determined on a per-pen basis and did not consider sex, whereas analysis of carcass data was determined on a per-animal basis and did consider sex. The model used for live performance data analysis was: $Y_{ij} = U + T_i + B_j + e_{ij}$ where Y_{ij} = observed pen response; U = overall mean; T_i = treatment effect; B_j = random block effect; and e_{ij} = residual error. The

Table 3. Ingredient[1] and analyzed nutrient[2] (as-fed basis) compositions of grower diets containing grain from 98140 and 98140 + Spray maize, nontransgenic near-isogenic control maize (control), and commercial reference maize (33J56, 33P66, and 33R77)

Item	Control	98140	98140 + Spray	33J56	33P66	33R77
Ingredient, %						
Maize	64.000	64.000	64.000	64.000	64.000	64.000
Soybean meal	23.414	23.066	23.134	22.043	23.090	22.499
Soybean hulls	4.289	4.500	4.500	4.500	4.159	4.500
Soybean oil	0.050	0.089	0.149	0.084	0.050	0.054
Protein blend	4.622	4.698	4.560	5.838	5.110	5.354
DL-Methionine	0.146	0.147	0.152	0.134	0.125	0.141
L-Lysine-HCL	0.124	0.149	0.160	0.129	0.144	0.162
Limestone	0.735	0.730	0.719	0.641	0.715	0.683
Di-Cal 18[3]	1.611	1.613	1.616	1.627	1.601	1.600
NaCl	0.384	0.383	0.384	0.379	0.382	0.381
VTM Premix[4]	0.625	0.625	0.625	0.625	0.625	0.625
Analyzed nutrient and fatty acid compositions						
Proximates						
Moisture, %	12.2	12.3	12.3	12.4	12.4	12.4
Protein, %	20.8	21.7	21.6	21.0	21.2	21.3
Fat, %	2.5	2.2	2.5	2.4	2.4	2.1
Fiber, %	2.7	2.6	2.9	3.0	2.5	2.8
Ash, %	5.1	5.0	4.9	4.8	4.9	5.1
Calcium, %	0.82	0.75	0.73	0.81	0.81	0.78
Phosphorus, %	0.67	0.64	0.61	0.60	0.62	0.62
Gross energy, kcal/kg	3,939	3,912	3,922	3,916	3,905	3,914
Essential amino acids						
Arginine, %	1.34	1.36	1.31	1.35	1.37	1.36
Histidine, %	1.13	1.15	1.19	1.15	1.22	1.14
Isoleucine, %	0.54	0.53	0.50	0.51	0.51	0.53
Leucine, %	0.88	0.87	0.81	0.88	0.86	0.89
Lysine, %	1.91	1.91	1.86	1.88	1.90	1.89
Methionine, %	0.47	0.47	0.44	0.42	0.42	0.43
Phenylalanine, %	1.08	1.07	1.06	1.08	1.08	1.07
Threonine, %	0.84	0.85	0.84	0.84	0.87	0.83
Tryptophan, %	0.27	0.28	0.27	0.26	0.28	0.27
Valine, %	1.08	1.08	0.99	1.10	1.06	1.12
Nonessential amino acids						
Alanine, %	1.15	1.15	1.13	1.14	1.16	1.15
Aspartic acid, %	2.08	2.10	2.06	2.07	2.11	2.09
Cystine, %	0.41	0.41	0.42	0.42	0.48	0.41
Glutamic acid, %	3.76	3.80	3.74	3.67	3.78	3.75
Glycine, %	0.98	0.99	0.96	1.00	1.01	1.01
Proline, %	1.55	1.58	1.57	1.61	1.60	1.64
Serine, %	1.23	1.25	1.24	1.26	1.30	1.24
Tyrosine, %	0.64	0.64	0.63	0.64	0.65	0.60

[1]Diets were formulated to contain the following: ME, 3,250 kcal/kg; protein, 21.00%; lysine, 1.12%; and methionine + cystine, 0.92%.

[2]Proximate and mineral analyses were performed by Cumberland Valley Analytical Services (Hagerstown, MD). Gross energy and fatty acid analyses were performed by Pioneer Hi-Bred International Inc. (Urbandale, IA). Amino acid analysis was conducted by Eurofins Scientific Inc. (Memphis, TN). Each cell represents 1 (n = 1) determination.

[3]Di-Cal 18 was purchased from Agritronics Corporation (Elkhart, IA).

[4]Poultry VTM 88 vitamin and trace mineral premix was purchased from Archer Daniels Midland (Quincy, IL).

Table 4. Ingredient[1] and analyzed nutrient[2] (as-fed basis) compositions of finisher diets containing grain from 98140 and 98140 + Spray maize, non-transgenic near-isogenic control maize (control) and commercial reference maize (33J56, 33P66, and 33R77)

Item	Control	98140	98140 + Spray	33J56	33P66	33R77
Ingredient, %						
Maize	71.500	71.500	71.500	71.500	71.500	71.500
Soybean meal	15.492	15.439	15.515	14.297	14.924	14.806
Soybean hulls	4.500	4.500	4.500	4.500	4.500	4.500
Soybean oil	0.090	0.194	0.261	0.188	0.053	0.155
Protein blend	4.764	4.677	4.524	5.951	5.415	5.410
DL-Methionine	0.148	0.152	0.157	0.137	0.122	0.145
L-Lysine-HCL	0.355	0.379	0.391	0.356	0.380	0.394
Limestone	0.689	0.693	0.681	0.594	0.659	0.641
Di-Cal 18[3]	1.505	1.508	1.511	1.523	1.492	1.494
NaCl	0.333	0.333	0.334	0.328	0.330	0.330
VTM Premix[4]	0.625	0.625	0.625	0.625	0.625	0.625
Analyzed nutrient and fatty acid compositions						
Proximates						
Moisture, %	12.0	12.3	12.2	12.3	12.3	12.4
Protein, %	18.6	18.6	17.9	19.1	17.6	18.2
Fat, %	2.2	2.5	2.5	2.6	2.5	2.7
Fiber, %	2.7	2.8	2.8	2.8	3.0	2.6
Ash, %	4.4	5.2	4.7	4.1	4.4	4.6
Calcium, %	0.78	0.74	0.76	0.70	0.73	0.69
Phosphorus, %	0.60	0.56	0.59	0.55	0.55	0.59
Gross energy, kcal/kg	3,921	3,932	3,919	3,932	3,923	3,912
Essential amino acids						
Arginine, %	1.06	1.07	1.07	1.13	1.07	1.06
Histidine, %	1.05	1.03	1.15	1.08	1.08	1.12
Isoleucine, %	0.45	0.44	0.43	0.44	0.42	0.51
Leucine, %	0.70	0.70	0.66	0.75	0.68	0.71
Lysine, %	1.65	1.68	1.67	1.71	1.65	1.63
Methionine, %	0.45	0.44	0.47	0.45	0.38	0.40
Phenylalanine, %	0.88	0.89	0.89	0.94	0.89	0.88
Threonine, %	0.70	0.70	0.71	0.74	0.72	0.69
Tryptophan, %	0.22	0.20	0.22	0.23	0.23	0.21
Valine, %	0.90	0.89	0.84	0.97	0.87	0.92
Nonessential amino acids						
Alanine, %	1.00	1.02	1.02	1.04	1.02	0.99
Aspartic acid, %	1.63	1.66	1.67	1.71	1.65	1.61
Cystine, %	0.40	0.38	0.40	0.38	0.40	0.38
Glutamic acid, %	3.09	3.17	3.18	3.14	3.09	3.01
Glycine, %	0.83	0.82	0.84	0.89	0.85	0.83
Proline, %	1.38	1.40	1.43	1.45	1.32	1.44
Serine, %	1.07	1.06	1.13	1.15	1.12	1.05
Tyrosine, %	0.53	0.53	0.54	0.55	0.54	0.51

[1]Diets were formulated to contain the following: ME, 3,285 kcal/kg; protein, 18.00%; lysine, 1.08%; and methionine + cystine, 0.85%.

[2]Proximate and mineral analyses were performed by Cumberland Valley Analytical Services (Hagerstown, MD). Gross energy and fatty acid analyses were performed by Pioneer Hi-Bred International Inc. (Urbandale, IA). Amino acid analysis was conducted by Eurofins Scientific Inc. (Memphis, TN). Each cell represents 1 (n = 1) determination.

[3]Di-Cal 18 was purchased from Agritronics Corporation (Elkhart, IA).

[4]Poultry VTM 88 vitamin and trace mineral premix was purchased from Archer Daniels Midland (Quincy, IL).

model used for carcass data analysis was: $Y_{ijk} = U + T_i + B_j + TB_{ij} + e_{ijk}$ where Y_{ijk} = observed bird response; U = overall mean; T_i = treatment effect; B_j = random block effect; TB_{ij} = random treatment × block effect (referred to as pen); and e_{ijk} = residual error. The value TB_{ij} was used as the error term for the fixed effect of treatment (T_i), which allowed within-pen variability to become residual error. Estimate statements were used to generate the treatment comparisons for each live performance and carcass trait; differences between means were considered statistically significant at $P < 0.05$.

The chance of falsely declaring a significant difference between 2 treatment means for at least 1 variable across a large number (20 or greater) of response variables is greater than 90% (Milliken and Johnson, 1992). Because of this, false discovery rate (**FDR**) as described by Benjamini and Hochberg (1995) was applied across all response variables analyzed to control the false positive rate in which, due to the number of variables evaluated within this study, effects may be falsely declared significant. The FDR-adjusted P-value was reviewed if statistically significant differences ($P \leq 0.05$) generated from the estimate comparison statement were observed for a trait.

Data generated from broilers fed diets formulated with grain from the reference maize sources (33J56, 33P66, and 33R77) were used in the estimation of experimental variability; least squares means were generated for each reference maize treatment (Table 6), but comparisons between reference maize, control, 98140, and 98140 + Spray treatments were not included in the statistical output. Instead, the reference maize data were used to

Table 5. Comparison of growth performance,[1] prechill organ yields,[2] and postchill carcass and parts yields[3] of broilers fed diets containing grain from 98140 and 98140 + Spray maize and nontransgenic near-isogenic control maize (control)

					Control vs. 98140		Control vs. 98140 + Spray		
Item	Control	98140	98140 + Spray	SEM	FDR P-value[4]	Raw P-value[5]	FDR P-value[4]	Raw P-value[5]	Tolerance interval[6]
Growth performance									
Initial weight (g), d 0	47.0	47.2	47.1	0.2	1.00	0.55	1.00	0.62	44.8 to 49.1
Final weight (kg), d 42	1.80	1.80	1.80	0.02	1.00	0.81	1.00	0.92	1.57 to 2.02
Mortality[7] (%)	1.67	1.67	0.83	0.97	1.00	1.00	1.00	0.54	0.00 to 13.58
Feed:gain (kg/kg),[8] 0 to 42 d	1.858	1.873	1.865	0.015	1.00	0.49	1.00	0.76	1.693 to 2.050
Prechill organ yields									
Kidney (%)									
Overall	2.08	2.14	1.98	0.05	0.99	0.39	0.99	0.16	
Males	2.12	2.12	2.02	0.07	0.99	0.99	0.99	0.27	0.64 to 3.41
Females	2.04	2.15	1.95	0.07	0.99	0.22	0.99	0.37	0.77 to 3.41
Liver (%)									
Overall	3.43	3.56	3.63	0.05	0.99	0.08	0.58	0.0087[9]	
Males	3.51	3.55	3.55	0.08	0.99	0.67	0.99	0.68	1.96 to 5.16
Females	3.34	3.57	3.71	0.08	0.99	0.0354[9]	0.11	0.0008[9]	2.06 to 5.07
Postchill carcass and parts yields									
Carcass (%)									
Overall	71.04	71.06	70.79	0.33	0.99	0.97	0.99	0.60	
Males	70.66	71.14	70.10	0.46	0.99	0.45	0.99	0.39	61.72 to 80.22
Females	71.42	70.97	71.48	0.46	0.99	0.49	0.99	0.93	61.51 to 80.19
Breast (%)									
Overall	26.57	26.59	26.74	0.21	0.99	0.94	0.99	0.57	
Males	26.86	26.73	26.92	0.29	0.99	0.74	0.99	0.89	20.66 to 32.65
Females	26.27	26.46	26.56	0.29	0.99	0.66	0.99	0.49	20.70 to 32.45
Thigh (%)									
Overall	15.87	15.75	15.91	0.14	0.99	0.52	0.99	0.87	
Males	15.92	15.68	16.15	0.20	0.99	0.40	0.99	0.42	12.12 to 19.81
Females	15.83	15.81	15.67	0.20	0.99	0.94	0.99	0.56	11.80 to 20.03
Leg (%)									
Overall	14.41	14.30	14.31	0.12	0.99	0.50	0.99	0.57	
Males	14.45	14.31	14.53	0.17	0.99	0.56	0.99	0.72	11.21 to 17.57
Females	14.37	14.28	14.09	0.17	0.99	0.71	0.99	0.24	11.05 to 17.63
Wing (%)									
Overall	10.49	10.61	10.55	0.08	0.99	0.25	0.99	0.58	
Males	10.53	10.60	10.69	0.11	0.99	0.65	0.99	0.29	8.22 to 12.94
Females	10.45	10.63	10.41	0.11	0.99	0.24	0.99	0.79	8.22 to 12.82
Abdominal fat (%)									
Overall	1.46	1.49	1.52	0.04	0.99	0.59	0.99	0.21	
Males	1.49	1.52	1.55	0.05	0.99	0.62	0.99	0.41	0.36 to 2.53
Females	1.43	1.45	1.50	0.05	0.99	0.79	0.99	0.34	0.39 to 2.50

[1]Control, 98140, and 98140 + Spray treatment growth performance means represent 12 pens per treatment group with 10 birds/pen.

[2]Prechill organ yields were calculated as a percentage of live bird weight. Control, 98140, and 98140 + Spray treatment means represent 12 pens per treatment group with 8 birds/pen.

[3]Carcass yield was calculated as a percentage of live bird weight; parts yield was calculated as a percentage of postchill carcass weight. Control, 98140, and 98140 + Spray treatment means represent 12 pens per treatment group with 8 birds/pen.

[4]P-value was adjusted using false discovery rate (FDR).

[5]Nonadjusted P-value.

[6]Lower and upper limits of a 95% tolerance interval on 99% of the observed performance, organ yield, and postchill carcass and parts yield trait values from birds fed 33J56, 33P66, and 33R77 reference maize diets.

[7]Negative lower limit of tolerance interval set to zero.

[8]Feed:gain calculated as kilograms of feed intake per kilogram of body weight gain.

[9]Statistically significant difference, $P \leq 0.05$.

Table 6. Growth performance,[1] prechill organ yields,[2] and postchill carcass and parts yields[3] of broilers fed diets containing grain from 98140 and 98140 + Spray maize, nontransgenic near-isogenic control maize (control), and commercial reference maize (33J56, 33P66, and 33R77)

				Reference maize grains[4]			
Item	Control	98140	98140 + Spray	33J56	33P66	33R77	SEM
Growth performance							
Initial weight (g), d 0	47.0	47.2	47.1	46.9	47.0	46.9	0.2
Final weight (kg), d 42	1.80	1.80	1.80	1.80	1.79	1.80	0.02
Mortality (%)	1.67	1.67	0.83	0.83	1.67	1.67	0.97
Feed:gain (kg/kg),[5] 0 to 42 d	1.858	1.873	1.865	1.868	1.875	1.871	0.015
Prechill organ yields							
Kidney (%)							
Overall	2.08	2.14	1.98	2.11	2.02	2.04	0.05
Males	2.12	2.12	2.02	2.09	1.97	2.01	0.07
Females	2.04	2.15	1.95	2.13	2.06	2.07	0.07
Liver (%)							
Overall	3.43	3.56	3.63	3.60	3.52	3.57	0.05
Males	3.51	3.55	3.55	3.62	3.53	3.53	0.08
Females	3.34	3.57	3.71	3.57	3.50	3.62	0.08
Postchill carcass and parts yields							
Carcass (%)							
Overall	71.04	71.06	70.79	70.99	71.12	70.63	0.33
Males	70.66	71.14	70.10	71.25	71.00	70.66	0.46
Females	71.42	70.97	71.48	70.73	71.23	70.60	0.46
Breast (%)							
Overall	26.57	26.59	26.74	26.44	26.75	26.65	0.21
Males	26.86	26.73	26.92	26.43	27.14	26.40	0.29
Females	26.27	26.46	26.56	26.46	26.37	26.89	0.29
Thigh (%)							
Overall	15.87	15.75	15.91	15.96	16.06	15.80	0.14
Males	15.92	15.68	16.15	15.91	16.14	15.86	0.20
Females	15.83	15.81	15.67	16.01	15.99	15.74	0.20
Leg (%)							
Overall	14.41	14.30	14.31	14.29	14.35	14.45	0.12
Males	14.45	14.31	14.53	14.24	14.49	14.44	0.17
Females	14.37	14.28	14.09	14.33	14.22	14.46	0.17
Wing (%)							
Overall	10.49	10.61	10.55	10.48	10.57	10.60	0.08
Males	10.53	10.60	10.69	10.33	10.65	10.76	0.11
Females	10.45	10.63	10.41	10.62	10.49	10.44	0.11
Abdominal fat (%)							
Overall	1.46	1.49	1.52	1.45	1.43	1.47	0.04
Males	1.49	1.52	1.55	1.46	1.42	1.46	0.05
Females	1.43	1.45	1.50	1.43	1.43	1.48	0.05

[1]Control, 98140, 98140 + Spray, 33J56, 33P66, and 33R77 treatment growth performance means represent 12 pens per treatment group with 10 birds/pen.

[2]Prechill organ yields were calculated as a percentage of live bird weight. Control, 98140, 98140 + Spray, 33J56, 33P66, and 33R77 treatment means represent 12 pens per treatment group with 8 birds/pen.

[3]Carcass yield was calculated as a percentage of live bird weight; parts yield was calculated as a percentage of postchill carcass weight. Control, 98140, 98140 + Spray, 33J56, 33P66, and 33R77 treatment means represent 12 pens per treatment group with 8 birds/pen.

[4]Commercial reference maize least squares means presented for reference purposes only. The comparisons of interest were control vs. 98140 and control vs. 98140 + Spray.

[5]Feed:gain calculated as kilograms of feed intake per kilogram of body weight gain.

construct a 95% tolerance interval containing 99% of the observed performance and carcass trait values from birds fed typical (nontransgenic commercial) maize diets, as described by Graybill (1976). These tolerance intervals were used to estimate the expected response range of broilers obtained from the same supplier and housed and fed under the same conditions as the broilers fed control, 98140, and 98140 + Spray diets. In the event of a difference still being statistically significant ($P \leq 0.05$) after FDR adjustment, data from control, 98140, and 98140 + Spray groups were evaluated to determine whether or not the observed values were contained within the tolerance interval. If an observed response value for a treatment was contained within the tolerance interval, that value was considered to be similar to the response of broilers fed typical maize grain diets. Tolerance intervals for organ and carcass variables were created by sex due to expected yield differences between male and female broilers.

RESULTS

Maize Grain and Diet Composition

Nutrient profiles were comparable between control, test, and reference maize grains (Table 1). The presence of mycotoxins in the maize grains was limited to fumonsins, which were found at very low (<2 mg/kg) concentrations (data not shown). Fumonisins B_1 and B_2 were detected in all control, test, and reference maize grains, whereas fumonisin B_3 was detected in only reference maize grain 33P66 (data not shown). Starter, grower, and finisher diets were formulated based on the analyzed concentrations of the nutrients (Tables 2, 3, and 4). The analyzed nutrient, gross energy, and amino acid concentrations of the diets produced using grain from control, test, and reference maize sources were all similar in corresponding feeding phases.

An ELISA analysis of diet samples collected for homogeneity evaluation confirmed the absence of GAT4621 and ZM-HRA proteins from diets produced with control and reference maize grains (GAT4621 LLOQ = 0.054 ng/mg of diet, ZM-HRA LLOQ = 0.13 ng/mg of diet). Because the GAT4621 and ZM-HRA proteins were not detected in control and reference maize diet homogeneity samples, the diet samples collected from these treatments for protein stability were not analyzed. An ELISA analysis of diets produced with 98140 and 98140 + Spray test maize grains confirmed the presence of the GAT4621 protein (starter diets 4.0 and 4.1 ng/mg, respectively; grower diets 4.6 and 5.0 ng/mg, respectively; and finisher diets 5.5 and 5.4 ng/mg, respectively) and the ZM-HRA protein (starter diets 0.0 and 0.045 ng/mg, respectively; grower diets 0.023 and 0.093 ng/mg, respectively; and finisher diets 0.15 and 0.15 ng/mg of diet, respectively). The ZM-HRA protein results were more variable due to the detection of the protein at levels below and just slightly above the assay LLOQ. Assay replicate samples at or below the LLOQ were given a value of 0 for calculation purposes, which resulted in a mean value lower than the LLOQ. Results were driven by the dietary corn percentage (58.5 to 71.5% inclusion rate), with values increasing with each successive diet phase. Diets produced with the test maize grains were blended homogeneously, and the protein concentrations were stable for the duration of the respective feeding phases (data not shown).

Performance Response Variables

There were no statistically significant differences in growth performance, mortality, or mortality-adjusted feed efficiency between control and 98140 or 98140 + Spray groups (Table 5). Further, all growth performance measures for broilers fed control and test (98140, 98140 + Spray) diets fell within the tolerance intervals calculated for this study using data obtained from broilers consuming diets produced with nontransgenic commercial maize grains (33J56, 33P66, and 33R77).

Organ and Carcass Yields

Kidney yields (Table 5) were not significantly different between control and 98140 or 98140 + Spray test diet groups; observed values for all groups fell within the tolerance interval calculated for this study using data obtained from broilers consuming diets produced with nontransgenic reference maize grains. Overall liver yields and liver yields for male broilers were not significantly different between control and 98140 test diet groups. Within females, liver yield was greater ($P < 0.05$) for the 98140 test diet group compared with the control diet group; however, this difference was not statistically significant when the P-value was adjusted using FDR ($P = 0.99$). Overall liver yield was greater ($P < 0.05$) for the 98140 + Spray test diet group compared with the control group. This effect was driven by the greater ($P < 0.05$) liver yield for females fed the 98140 + Spray test diet; yields for male broilers were not significantly different. However, both the overall and within-female differences were not statistically significant when the P-values were adjusted using FDR ($P = 0.58$ and $P = 0.11$, respectively). The lack of significance upon adjustment for FDR would indicate that initial occurrences of significant unadjusted P-values were likely due to the increased chance of finding a significant difference given the large number of response variables evaluated in this study. Furthermore, these differences are unlikely to be biologically significant, because all observed values were within the tolerance intervals calculated for this study using data obtained from broilers consuming diets produced with nontransgenic reference maize grains.

No statistically significant differences were observed for carcass or individual parts yields between the respective control and test diet groups (Table 5). Additionally, all observed values fell within the nontransgenic reference maize grain tolerance intervals calculated for this study, indicating that carcass and parts yields of broilers fed control and 98140 or 98140 + Spray maize grain diets were similar to those of broilers fed typical maize grain diets.

DISCUSSION

Biotechnology has been used to produce GM field crops with input traits including herbicide tolerance and insect resistance. The benefits of biotechnology-derived crops include improved yields, reductions in pest management costs and pesticide usage, and an increase in conservation tillage practices (Sankula, 2006). Genetically modified corn varieties have been widely adopted in the United States since their commercial introduction in 1996. Insect-protected, herbicide-tolerant, and stacked gene varieties accounted for 73% of all corn planted in the United States in the 2007 growing season (USDA Economic Research Service, 2007). United States hectares planted to biotech crops in 2006 accounted for 53% of the total global biotech area, and approximately 28% of those hectares were 2- and 3-stacked-trait products (James, 2006).

The current feeding study was conducted to compare the nutritional performance of grain from 98140 maize with grain from nontransgenic near-isogenic control maize, along with that of grain from 3 additional non-GM commercial reference maize sources (33J56, 33P66, and 33R77). Information about maize grain nutrient composition used to formulate broiler chicken diets is limited primarily to nutritional proximates, amino acids, calcium, phosphorous, and gross energy values. No differences in key nutrients were observed between the grains from the maize sources used in this study that would have limited the grain inclusion amount, so the grains from all sources were considered suitable for the production of commercial-type broiler chicken diets (McNaughton et al., 2007a,b, 2008). The presence of fumonisins in the maize sources was not a concern for diet production, because the concentrations were well below the 100 mg/kg of US FDA guideline for total fumonisins in corn (US FDA et al., 2001), and diet production also resulted in further dilution of the concentrations.

The performance of the chickens fed different diets in this study was compared using standard nutritional performance variables, along with organ and carcass yields. No biologically significant differences in body weights, weight gain, or carcass yields were observed between broiler chickens consuming diets prepared with grain from 98140 (unsprayed or sprayed), nontransgenic near-isogenic control, or reference maize sources. These results are consistent with previous broiler feeding trials conducted with grain from GM maize plants (Brake and Vlachos, 1998; Sidhu et al., 2000; Taylor et al., 2003a,b,c, 2005, 2007a,b; Brake et al., 2005; McNaughton et al., 2007a), which demonstrated similar weight gains and feed conversion between groups of broilers fed diets with transgenic grain or nontransgenic near-isogenic controls. The results of the sprayed test maize group (98140 + Spray) are consistent with those of Brake et al. (2003), who found no difference in performance measures or carcass yields between broiler fed diets containing maize grain from nontransgenic near-isogenic control, unsprayed transgenic event Bt11, or transgenic event Bt11 sprayed with Liberty herbicide (Bayer CropScience, Monheim, Germany).

Liver and kidney yields are indicators of broiler health resulting from dietary inadequacies (Velu et al., 1971; Whitehead et al., 1978; Carew et al., 2005) or the presence of antinutritional factors (Ledoux et al., 1992; Edrington et al., 1997; Bailey et al., 2000; Farran et al., 2005). Nutritional performance trials of transgenic grains in other species, such as rodents, routinely include measures of organ weights as overall health indicators (Hammond et al., 2004; MacKenzie et al., 2007; Malley et al., 2007). The organ yield results in this study are consistent with those of previous studies in which no biologically significant differences in organ yields were observed between broilers fed diets prepared with transgenic grain (McNaughton et al., 2007a) or feed fractions (McNaughton et al., 2007b, 2008) and those fed diets with grain or feed fractions from nontransgenic controls.

Statistical analysis of all data in this study resulted in rejection of the hypotheses of expected growth performance and carcass yield differences between birds fed nontransgenic isoline control maize and 98140 or 98140 + Gly-Spray test maize. The results from this study demonstrated that grain obtained from maize plants containing event DP-Ø9814Ø-6 was nutritionally equivalent to grain obtained from nontransgenic near-isogenic control maize plants.

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