Comparison of Broiler Performance When Fed Diets Containing Event DP-356Ø43-5 (Optimum GAT[1]), Nontransgenic Near-Isoline Control, or Commercial Reference Soybean Meal, Hulls, and Oil

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ABSTRACT Event DP-356Ø43-5 (356043; Optimum GAT) is a genetically modified soybean (*Glycine max*) that was produced by insertion of the *gat4601* and *gm-hra* genes. The expression products of these genes are the glyphosate acetyltransferase 4601 and acetolactate synthase proteins, respectively. Expression of the glyphosate acetyltransferase 4601 protein confers tolerance in planta to the herbicidal active ingredient glyphosate, whereas expression of the acetolactate synthase protein confers tolerance to sulfonylurea and imidazolinone herbicides. The objective of this study was to compare the nutritional equivalence of 356043 soybeans to nontransgenic soybeans in a 42-d feeding trial in broiler chickens. Diets were prepared using processed fractions (meal, hulls, and oil) from untreated 356043 soybean plants or from soybean plants treated with a mixture of glyphosate, chlorimuron, and thifensulfuron (356043 + Gly/SU). For comparison, additional diets were produced with soybean fractions obtained from a nontransgenic near-isoline (control; 091) and nontransgenic commercial Pioneer varieties (93B86, 93B15, and 93M40). Diets were fed to Ross × Cobb broilers (n = 120/group, 50% male and 50% female) in 3 phases. Starter diets contained 30% soybean meal, grower diets 26% soybean meal, and finisher diets 21.5% soybean meal. Soybean hulls and oil were added at 1.0 and 0.5%, respectively, across all diets in each phase. No statistically significant differences were observed in mortality, growth performance variables, or carcass and organ yields between broilers consuming diets produced with 356043 or 356043 + Gly/SU soybean fractions and those consuming diets produced with near-isoline control soybean fractions. Additionally, all performance and carcass variables from control, 356043, and 356043 + Gly/SU soybean treatment groups fell within the tolerance intervals constructed using data from reference soybean groups. Based on the results from this study, it was concluded that 356043 soybean was nutritionally equivalent to nontransgenic control soybean with a comparable genetic background.

Key words: glyphosate acetyltransferase 4601, acetolactate synthase, glyphosate, broiler performance, carcass yield

2007 Poultry Science 86:2569–2581
doi:10.3382/ps.2007-00140

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INTRODUCTION

Event DP-356Ø43-5 (hereafter called 356043; Optimum GAT) is a genetically modified soybean (*Glycine max*) that was produced by the insertion of the *gat4601* and *gm-hra* genes. The expression products of these genes are the glyphosate acetyltransferase 4601 (**GAT**) and acetolactate synthase (**GM-HRA**) proteins, respectively. Expression of the GAT protein confers tolerance *in planta* to the herbicidal active ingredient glyphosate, whereas expression of the GM-HRA protein 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 *gat4601* gene is the product of a *gat* gene isolated from *Bacillus licheniformis* that was functionally improved through multiple rounds of gene shuffling to produce an enzyme that more efficiently acetylates glyphosate than the native GAT protein (Castle et al., 2004). The acetolactate synthase gene (*gm-hra*) was produced by isolating the herbicide-sensitive *gm-als* gene from soybean and changing 2 specific amino acids (Mazur and Falco, 1989; Green, 2007). The objective of this study was to evaluate the nutritional value of 356043 soybeans by comparing the growth performance and carcass yields of broiler chickens fed diets containing

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Received April 3, 2007.
Accepted August 27, 2007.
[1]Optimum GAT is a registered trademark of Pioneer Hi-Bred International Inc.
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Table 1. Proximate (%), Ca (%), P (%), gross energy (kcal/kg), and amino acid (%) composition[1] of Optimum GAT meal from untreated (356043) and glyphosate/sulfonylurea-treated (356043 + Gly/SU) soybean plants, nontransgenic near-isoline control soybean meal, and meal from commercial reference soybeans (93B86, 93B15, and 93M40)

Analyzed composition[2]	Control	356043	356043 + Gly/SU	93B86	93B15	93M40
Proximates, %						
Moisture	6.9	6.5	7.2	7.2	6.6	6.0
Protein	47.6	46.3	49.0	44.7	47.0	45.2
Fat	1.2	0.7	0.9	1.1	1.2	0.9
Fiber	2.8	3.1	2.6	3.0	2.7	2.9
Ash	6.0	6.3	5.9	5.8	5.5	7.2
Ca, %	0.25	0.25	0.24	0.25	0.23	0.26
P, %	0.87	0.89	0.80	0.84	0.81	0.98
Gross energy, kcal/kg	4,365	4,362	4,391	4,331	4,378	4,343
Essential amino acids, %						
Arg	3.59	3.39	3.79	3.31	3.53	3.25
Lys	2.93	2.84	2.95	2.86	2.99	2.84
His	1.31	1.27	1.34	1.19	1.26	1.22
Ile	2.30	2.16	2.24	2.11	2.22	2.14
Leu	3.85	3.73	3.92	3.60	3.79	3.61
Met	0.75	0.75	0.77	0.71	0.74	0.75
Phe	2.53	2.43	2.60	2.39	2.51	2.37
Thr	1.98	1.96	2.05	1.86	1.96	1.90
Trp	0.70	0.73	0.74	0.72	0.73	0.73
Val	2.43	2.29	2.39	2.20	2.31	2.24
Nonessential amino acids, %						
Ala	2.24	2.21	2.32	2.12	2.21	2.16
Asp	5.88	5.70	6.09	5.53	5.83	5.56
Cystine	0.71	0.72	0.72	0.74	0.74	0.77
Glu	9.36	8.94	9.64	9.00	9.60	8.92
Gly	2.13	2.06	2.17	2.00	2.08	2.00
Pro	2.50	2.55	2.61	2.33	2.54	2.45
Ser	2.46	2.48	2.64	2.41	2.52	2.40
Tyr	1.57	1.58	1.65	1.52	1.52	1.47

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

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

356043 soybean fractions (meal, hulls, and oil) with those fed diets composed of nontransgenic control (comparable genetic background) soybean fractions or diets composed of nontransgenic commercial soybean fractions.

MATERIALS AND METHODS

Soybeans

Control soybeans (line 091) were nontransgenic with a genetic background comparable to 356043 soybeans that do not contain the coding sequences for the GAT and GM-HRA proteins (i.e., a near-isoline of 356043 soybean). Optimum GAT soybeans (356043) were obtained from plants containing the coding sequences for the GAT and GM-HRA proteins. The 356043 + Gly/SU soybeans were obtained from plants containing the coding sequences for the GAT and GM-HRA proteins that were treated with 2 sequential broadcast applications of a herbicide tank mix containing the active ingredients glyphosate (Touchdown IQ, Syngenta, Greensboro, NC), chlorimuron (Spin, DuPont, Wilmington, DE), and thifensulfuron (Refine, DuPont). Reference soybeans (93B86, 93B15, 93M40) were commercially available nontransgenic Pioneer soybeans that were not treated with herbicides. Soybean seeds for this trial were planted in isolated plots at least 3 m from soybean plants not in the same trial in November of 2005 in a field trial near Santiago, Chile, and harvested in April 2006.

Soybean Fraction Production and Characterization

Soybeans from control, reference, and 356043 plants were processed into meal, hull, and oil fractions under similar conditions at GLP Technologies (Navasota, TX). Identity preservation procedures were followed throughout the processing and inventory systems to maintain the identity of each soybean source and the resulting processed fractions. Event-specific real-time PCR testing confirmed the presence of the insert from event DP-35Ø643-5 in 356043 and 356043 + Gly/SU test soybean meal and soy hull fractions and its absence in control and reference soybean meals and soy hull fractions (data not shown). All soybean meals and soy hulls (control, test, and references) were evaluated for nutrient proximate composition, Ca, and P content at Cumberland Valley Analytical Services (Hagerstown, MD). Analytical determinations were conducted according to AOAC (1990, 2000) methods for CM (AOAC,

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Table 2. Proximate (%), Ca (%), P (%), gross energy (kcal/kg), and amino acid (%) composition[1] of Optimum GAT hulls from untreated (356043) and glyphosate/sulfonylurea-treated (356043 + Gly/SU) soybean plants, nontransgenic near-isoline control soybean hulls, and hulls from commercial reference soybeans (93B86, 93B15, and 93M40)

Analyzed composition[2]	Control	356043	356043 + Gly/SU	93B86	93B15	93M40
Proximates, %						
Moisture	6.9	6.8	5.6	5.5	6.1	6.6
Protein	22.8	22.0	24.2	24.3	23.1	22.9
Fat	10.1	11.4	11.1	10.8	10.9	11.8
Fiber	19.4	19.3	18.3	17.6	17.9	18.3
Ash	5.1	5.3	4.6	4.8	4.7	5.3
Ca, %	0.32	0.31	0.31	0.32	0.31	0.34
P, %	0.39	0.41	0.39	0.41	0.37	0.44
Gross energy, kcal/kg	4,572	4,679	4,688	4,713	4,668	4,760
Essential amino acids, %						
Arg	1.57	1.54	1.71	1.69	1.65	1.53
His	0.64	0.63	0.68	0.66	0.65	0.63
Ile	1.01	1.02	1.11	1.11	1.06	1.03
Leu	1.75	1.73	1.89	1.91	1.82	1.75
Lys	1.43	1.42	1.49	1.58	1.54	1.44
Met	0.37	0.35	0.36	0.40	0.38	0.37
Phe	1.12	1.10	1.20	1.24	1.18	1.12
Thr	0.94	0.94	1.00	1.02	0.98	0.96
Trp	0.36	0.37	0.37	0.35	0.39	0.37
Val	1.09	1.10	1.21	1.19	1.13	1.10
Nonessential amino acids, %						
Ala	1.09	1.08	1.16	1.17	1.13	1.10
Asp	2.67	2.75	3.02	2.91	2.80	2.67
Cystine	0.41	0.39	0.38	0.43	0.41	0.43
Glu	3.96	3.82	4.27	4.46	4.31	4.01
Gly	1.22	1.22	1.31	1.25	1.22	1.19
Pro	1.12	1.07	1.23	1.21	1.26	1.04
Ser	1.26	1.21	1.31	1.35	1.30	1.23
Tyr	0.80	0.78	0.41	0.43	0.79	0.39

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

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

2000; method 930.15), protein (AOAC, 2000; method 990.03), fat (AOAC, 1990; method 920.39), fiber (AOAC, 2000; method 978.10), ash (AOAC, 2000; method 942.05), Ca, and P (AOAC, 2000; method 985.01). Amino acid content and concentrations of mycotoxins of these fractions were determined at Eurofins Scientific (Des Moines, IA). Amino acid concentrations were determined in accordance with AOAC methods (AOAC, 2000; methods 988.15, 982.30, and 994.12). Concentrations of mycotoxins were determined in accordance with AOAC methods [mycotoxins (method 994.08), fumonisins (method 995.15), and vomitoxin (986.17); AOAC, 2000]. Soy fractions and diet samples were analyzed for gross energy content with a bomb calorimeter (Parr Instruments model 1271, Parr Instruments, Moline, IL) at Pioneer Hi-Bred (Urbandale, IA).

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). Commercial broilers (Ross × Cobb) were obtained at hatch (trial d 0) from a commercial Maryland hatchery and transported to Solution BioSciences Inc. (farm 1, Tyaskin, MD). Broilers were evaluated upon receipt for signs of disease or other complications that may have affected the outcome of the study. Bird health and actual number of birds received were documented upon receipt. Following examination, broilers were weighed, identified with a wing band, and placed randomly in pens. Broilers were housed at a density of 10 broilers per pen (12 pens per treatment group) 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. Broilers were placed in 3 ft × 4 ft (0.914 m × 1.219 m) floor pens at a density of approximately 1.0 ft^2 (0.305 m^2) of available floor space per broiler. Pens were separated by a wire partition and did not touch other pens from any side to minimize potential for cross-contamination. 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 broilers were disposed of according to local regulations via composting. Drinking water was provided for ad libitum consumption. Body weights and feed weights (including

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Table 3. Ingredient composition of diets containing Optimum GAT meal; hulls and oil from untreated (356043) and glyphosate/sulfonylurea-treated (356043 + Gly/SU) soybean plants; nontransgenic near-isoline control soybean meal, hulls, and oil; and meal, hulls, and oil from commercial reference soybeans (93B86, 93B15, and 93M40)

Ingredient (%)	Control	356043	356043 + Gly/SU	93B86	93B15	93M40
Starter[1]						
Maize	58.403	57.893	59.037	57.167	58.219	57.516
Soybean meal	30.00	30.00	30.00	30.00	30.00	30.00
Soybean hulls	1.00	1.00	1.00	1.00	1.00	1.00
Soybean oil	0.50	0.50	0.50	0.50	0.50	0.50
Commercial soybean oil	2.576	2.596	2.46	2.726	2.515	2.615
Protein blend	3.292	3.809	2.695	4.468	3.569	4.266
DL-Met	0.353	0.346	0.350	0.345	0.344	0.326
L-Lys-HCl	0.104	0.124	0.112	0.097	0.072	0.111
Limestone	0.997	0.973	1.006	0.922	0.981	0.971
Dicalcium 21	1.654	1.641	1.719	1.657	1.681	1.578
Choline-Cl	0.039	0.040	0.037	0.042	0.039	0.041
NaCl	0.456	0.454	0.458	0.451	0.455	0.452
VTM Premix[2]	0.625	0.625	0.625	0.625	0.625	0.625
Grower[1]						
Maize	63.987	63.546	64.543	62.919	63.831	63.224
Soybean meal	26.00	26.00	26.00	26.00	26.00	26.00
Soybean hulls	1.00	1.00	1.00	1.00	1.00	1.00
Soybean oil	0.50	0.50	0.50	0.50	0.50	0.50
Commercial soybean oil	2.005	2.019	1.900	2.133	1.948	2.033
Protein blend	2.629	3.078	2.109	3.645	2.868	3.472
DL-Met	0.301	0.295	0.298	0.294	0.293	0.277
L-Lys-HCl	0.154	0.171	0.160	0.147	0.126	0.160
Limestone	0.967	0.946	0.975	0.902	0.953	0.944
Dicalcium 21	1.548	1.535	1.604	1.550	1.570	1.481
Choline-Cl	0.005	0.006	0.003	0.007	0.005	0.007
NaCl	0.406	0.404	0.408	0.402	0.405	0.403
VTM Premix	0.500	0.500	0.500	0.500	0.500	0.500
Finisher[1]						
Maize	69.741	69.380	70.207	68.867	69.618	69.121
Soybean meal	21.50	21.50	21.50	21.50	21.50	21.50
Soybean hulls	1.00	1.00	1.00	1.00	1.00	1.00
Soybean oil	0.50	0.50	0.50	0.50	0.50	0.50
Commercial soybean oil	1.419	1.427	1.328	1.522	1.368	1.434
Protein blend	2.143	2.515	1.709	2.979	2.340	2.839
DL-Met	0.284	0.279	0.281	0.278	0.277	0.264
L-Lys-HCl	0.267	0.282	0.273	0.262	0.244	0.272
Limestone	0.849	0.831	0.856	0.795	0.837	0.830
Dicalcium 21	1.443	1.433	1.489	1.445	1.462	1.388
NaCl	0.355	0.354	0.357	0.352	0.354	0.352
VTM Premix	0.500	0.500	0.500	0.500	0.500	0.500

[1]Diets were formulated to contain 3,124 kcal of ME/kg (starter), 3,151 kcal of ME/kg (grower), and 3,175 kcal of ME/kg (finisher), respectively.

[2]Poultry VTM 88 Vitamin and Trace Mineral Premix from Archer Daniels Midland (Quincy, IL).

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. A growth curve was prepared from weekly BW.

Experimental Design

Sufficient numbers of broilers were obtained before study initiation to ensure availability of 720 healthy chicks (50% males and 50% females) for the conduct of the study. There were 10 broilers per pen (5 males and 5 females) with 12 pens (replicates) per treatment of 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 as a guideline (NRC, 1994). Diets were prepared at the Pioneer Livestock Nutrition facility (Polk City, IA). Control, test, or reference soy fractions were added to the indicated diets in equal amounts; requirements for protein, Lys, Met, cystine, Ca, and P were met by adjusting the concentrations of nonsoy ingredients. Within each phase, all diets were formulated to the same ME level: starter diets, 3,124 kcal of ME/kg; grower diets, 3,151 kcal of ME/kg; and

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Table 4. Nutrient composition[1] of starter diets containing Optimum GAT meal; hulls and oil from untreated (356043) and glyphosate-sulfonylurea-treated (356043 + Gly/SU) soybean plants; nontransgenic near-isoline control soybean meal, hulls, and oil; and meal, hulls, and oil from commercial reference soybeans (93B86, 93B15, and 93M40)

Analyzed composition[2]	Control	356043	356043 + Gly/SU	93B86	93B15	93M40
Proximates, %						
Moisture	10.2	10.1	10.2	10.1	10.2	10.0
Protein	20.8	21.8	22.1	22.2	21.9	21.8
Fat	5.1	5.5	5.2	5.4	5.5	6.0
Fiber	2.1	2.0	1.9	2.0	1.8	1.9
Ash	5.2	5.3	5.6	4.7	5.7	5.5
Ca, %	0.80	0.72	0.86	0.80	0.80	0.78
P, %	0.68	0.63	0.70	0.69	0.64	0.72
Gross energy, kcal/kg	4,110	4,123	4,125	4,134	4,101	4,127
Essential amino acids, %						
Arg	1.36	1.46	1.55	1.41	1.38	1.38
His	0.57	0.56	0.60	0.54	0.55	0.55
Ile	0.92	0.92	0.94	0.90	0.94	0.92
Leu	1.93	1.90	1.96	1.92	1.93	1.89
Lys	1.20	1.15	1.24	1.18	1.14	1.17
Met	0.68	0.66	0.66	0.64	0.67	0.65
Phe	1.12	1.06	1.12	1.08	1.08	1.07
Thr	0.92	0.87	0.90	0.89	0.88	0.89
Trp	0.29	0.28	0.29	0.29	0.29	0.30
Val	1.16	1.12	1.11	1.14	1.13	1.11
Nonessential amino acids, %						
Ala	1.15	1.21	1.24	1.20	1.24	1.24
Asp	2.41	2.19	2.39	2.25	2.26	2.22
Cystine	0.39	0.42	0.38	0.43	0.41	0.46
Glu	4.06	3.98	4.16	4.08	4.13	4.07
Gly	1.00	1.04	1.01	1.00	1.01	1.02
Pro	1.37	1.09	1.48	1.36	1.11	1.08
Ser	1.24	1.24	1.26	1.26	1.25	1.28
Tyr	0.61	0.62	0.62	0.65	0.55	0.63

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

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

finisher diets, 3,175 kcal of ME/kg. Starter, grower, and finisher diets for each soy source were mixed in the following order to minimize the potential for cross-contamination of nontransgenic soy with transgenic soy: control, 93B86, 93B15, 93M40, 356043, and 356043 + Gly/SU. 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 soy source, and flush material was disposed of by composting. Prepared diets were subsampled, and samples were composited for proximate analysis (including Ca and P), amino acid analysis, and gross energy analysis. Homogeneity and stability analyses of the GAT and GM-HRA proteins in the diets were not performed due to the inability to detect the transgenic proteins by ELISA in the toasted soybean meal and soy hull fractions used to prepare the test diets (356043 and 356043 + Gly/SU; data not shown). The inability to detect the GAT and GM-HRA proteins was likely due to the denaturing effect of heat on the proteins during the toasting process.

Measurements

All surviving birds were euthanized 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); yield data 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 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 the 356043 soybean group was calculated for each variable to test the primary hypothesis that growth performance and carcass

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Table 5. Nutrient composition[1] of grower diets containing Optimum GAT meal; hulls and oil from untreated (356043) and glyphosate/sulfonylurea-treated (356043 + Gly/SU) soybean plants; nontransgenic near-isoline control soybean meal, hulls, and oil; and meal, hulls, and oil from commercial reference soybeans (93B86, 93B15, and 93M40)

Analyzed composition[2]	Control	356043	356043 + Gly/SU	93B86	93B15	93M40
Proximates, %						
Moisture	10.4	10.2	10.5	10.4	10.3	10.1
Protein	19.5	20.9	19.5	19.8	20.2	19.2
Fat	5.1	4.9	4.9	5.2	5.1	5.3
Fiber	1.8	1.7	1.7	1.9	1.7	1.6
Ash	5.0	5.3	5.9	3.9	4.5	5.8
Ca, %	0.79	0.76	0.77	0.75	0.74	0.76
P, %	0.65	0.65	0.67	0.62	0.64	0.68
Gross energy, kcal/kg	4,077	4,090	4,079	4,082	4,075	4,087
Essential amino acids, %						
Arg	1.18	1.18	1.32	1.23	1.28	1.26
His	0.50	0.53	0.52	0.52	0.51	0.51
Ile	0.80	0.82	0.80	0.84	0.80	0.82
Leu	1.71	1.81	1.77	1.77	1.76	1.75
Lys	1.02	1.09	1.08	1.08	1.10	1.10
Met	0.60	0.60	0.60	0.58	0.68	0.61
Phe	0.96	0.99	0.99	1.01	0.97	0.99
Thr	0.73	0.81	0.83	0.79	0.77	0.82
Trp	0.25	0.26	0.26	0.25	0.27	0.27
Val	0.97	1.04	0.97	1.05	1.00	1.03
Nonessential amino acids, %						
Ala	1.08	1.15	1.11	1.14	1.14	1.15
Asp	1.90	2.03	2.13	1.99	2.00	2.04
Cystine	0.36	0.39	0.36	0.40	0.39	0.34
Glu	3.53	3.71	3.73	3.66	3.83	3.67
Gly	0.85	0.93	0.88	0.92	0.89	0.93
Pro	1.21	1.04	1.25	1.00	1.02	1.01
Ser	1.03	1.12	1.12	1.11	1.11	1.14
Tyr	0.58	0.53	0.57	0.57	0.57	0.58

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

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

yield would be different between broiler chickens fed diets containing test soy fractions with the processed fractions from Optimum GAT soybeans and those fed diets containing nontransgenic near-isoline control soy fractions. A secondary hypothesis tested was that growth performance and carcass yield of birds fed diets containing 356043 soy fractions produced under a herbicide spray regimen (356043 + Gly/SU) would differ from that of birds fed diets containing nontransgenic near-isoline control soy fractions. Data generated from control, 356043, and 356043 + Gly/SU soy treatment groups were analyzed using a mixed model ANOVA (PROC MIXED, SAS version 9.1 software, SAS Inst. Inc., Cary NC). 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 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 (called pen); and e_{ijk} = residual error. The error term used for the fixed effect of treatment (T_i) was TB_{ij}, which allowed within-pen variability to become residual error. 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-bird basis and did consider sex. Estimate statements were used to generate the treatment comparisons for each live performance and carcass trait. The observed P-values generated from the estimate comparison statement determined whether 1) the mean of the 356043 test soy group was statistically different from the mean of the control soy group and 2) the mean of the 356043 + Gly/SU test soy group was statistically different from the control soy mean; differences between means were considered significant at $P \le 0.05$. False discovery rate, as described by Benjamini and Hochberg (1995), was applied across all traits analyzed to control the false positive rate. Data generated from reference soy (93B86, 93B15, and 93M40) treatment groups were used in the estimation of experimental variability but were not included in the statistical output; instead, these data were used to construct a 95% tolerance interval containing 99% of the observed performance and carcass trait values from birds fed typical (nontransgenic commercial) soy diets, as described by Graybill (1976). Tolerance intervals were used as estimates of expected ranges of response variables within reference control groups obtained from the same source and housed and fed under the same

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Table 6. Nutrient composition[1] of finisher diets containing Optimum GAT meal; hulls and oil from untreated (356043) and glyphosate/sulfonylurea-treated (356043 + Gly/SU) soybean plants; nontransgenic near-isoline control soybean meal, hulls, and oil; and meal, hulls, and oil from commercial reference soybeans (93B86, 93B15, and 93M40)

Analyzed composition[2]	Control	356043	356043 + Gly/SU	93B86	93B15	93M40
Proximates, %						
Moisture	10.8	10.5	10.8	10.7	10.6	10.5
Protein	16.9	17.3	17.3	17.4	17.9	17.5
Fat	4.8	4.5	4.5	4.8	4.6	4.9
Fiber	1.8	1.7	1.8	1.7	1.7	1.6
Ash	4.1	4.7	4.5	4.4	3.8	5.0
Ca, %	0.72	0.70	0.66	0.73	0.68	0.68
P, %	0.63	0.63	0.64	0.62	0.64	0.62
Gross energy, kcal/kg	4,015	4,029	4,037	4,042	4,033	4,044
Essential amino acids, %						
Arg	1.06	1.15	1.19	1.09	1.13	1.06
His	0.47	0.46	0.46	0.47	0.48	0.46
Ile	0.75	0.72	0.68	0.74	0.75	0.72
Leu	1.61	1.61	1.62	1.65	1.65	1.61
Lys	1.01	1.02	1.05	1.02	1.04	1.04
Met	0.54	0.55	0.55	0.55	0.56	0.62
Phe	0.87	0.86	0.89	0.87	0.88	0.86
Thr	0.66	0.71	0.69	0.70	0.69	0.68
Trp	0.22	0.23	0.22	0.22	0.24	0.22
Val	0.91	0.86	0.83	0.93	0.91	0.89
Nonessential amino acids, %						
Ala	1.02	1.02	1.04	1.07	1.07	1.04
Asp	1.74	1.76	1.80	1.76	1.77	1.69
Cystine	0.32	0.35	0.32	0.35	0.34	0.31
Glu	3.33	3.20	3.33	3.32	3.44	3.26
Gly	0.78	0.77	0.73	0.81	0.81	0.78
Pro	1.07	1.09	1.15	0.92	0.94	0.93
Ser	0.93	0.98	0.99	0.99	0.99	0.98
Tyr	0.52	0.51	0.50	0.51	0.51	0.50

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

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

conditions as the experimental and control broiler chickens within this study. If after false discovery rate adjustments there were still statistically significant differences among response variables in control, 356043, and 356043 + Gly/SU treatment groups, they were evaluated graphically to determine whether the observed values were contained within this interval. If individual observed values for a treatment group were contained within the tolerance interval, the treatment response was considered to be similar to feeding typical soy fractions. Tolerance intervals for organ and carcass variables were created by sex due to expected yield differences between male and female broilers.

RESULTS

Soybean Fractions and Diet Composition

Nutrient profiles of the control, test, and reference soybean meals were comparable for most analytes (Table 1). Slight variations in concentrations of protein were noted among control, test, and reference meals. The nutrient compositions of control, test, and reference soy hulls were similar (Table 2). Protein, fat, and energy concentrations in all soy hull sources were higher than typically observed in commercial soy hulls, indicating that the hulls contained a higher amount of bean meat due to poor separation between the hull and bean. As a result, the quantity of soy hulls added was limited to 1.0% across all diets. None of the soybean meals or soy hulls contained measurable concentrations of mycotoxins (data not shown). Lower gross energy content of all soy oils reflected the fact that the oils were only degummed and not further refined (data not shown); because of the lower nutritional quality, quantities of the soy oils were limited to 0.5% across all diets.

The compositions of the starter, grower, and finisher diets were formulated based on the determined concentrations of the nutrients (Table 3). The nutrient, gross energy, and amino acid concentrations of the diets produced using processed fractions from the control, test, and reference soybeans were all similar in corresponding feeding phases (Tables 4, 5, and 6).

Performance Response Variables

There was no observable difference in the growth performance measures between birds consuming diets produced with the control and 356043 or 356043 + Gly/SU soybean fractions as observed by evaluating the weekly

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Table 7. Growth performance of broilers fed diets containing Optimum GAT meal; hulls and oil from untreated (356043) and glyphosate/sulfonylurea-treated (356043 + Gly/SU) soybean plants; nontransgenic near-isoline control soybean meal, hulls, and oil; and meal, hulls, and oil from commercial reference soybeans (93B86, 93B15, and 93M40)

Item	Control	356043	356043 + Gly/SU	Tolerance interval[1]
Initial weight, g				
Mean[2]	50.9	50.4	51.1	48.8 to 53.4[3]
SEM	0.2	0.2	0.2	
FDR[4]		0.90	0.90	
P-value[5]		0.12	0.37	
Final weight, g				
Mean	1,905.6	1,916.3	1,924.2	1,671.5 to 2,163.5
SEM	21.2	21.2	21.2	
FDR		0.90	0.90	
P-value		0.72	0.54	
Mortality,[6] %				
Mean	0.83	1.67	0.83	0.00 to 13.48
SEM	0.99	0.99	0.99	
FDR		0.90	1.00	
P-value		0.55	1.00	
Feed:gain[7]				
Mean	1.878	1.860	1.881	1.694 to 2.036
SEM	0.015	0.015	0.015	
FDR		0.90	0.99	
P-value		0.36	0.89	

[1]Lower and upper limits of a 95% tolerance interval on 99% of the observed performance trait values from birds fed 93B86, 93B15, and 93M40 reference soybean diets.

[2]Control, 356043, and 356043 + Gly/SU treatment means (n = 12 pens/treatment group with 10 birds/pen).

[3]Range denotes the lowest and highest individual values observed in the treatment group.

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

[5]Nonadjusted *P*-value.

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

[7]Feed:gain calculated as grams of feed intake per gram of BW gain.

growth curve or final BW (Table 7). Additionally, there were no statistically significant differences among growth performance variables (BW and gain, mortality, and mortality-adjusted feed efficiency) of broilers consuming control and 356043 or between control and 356043 + Gly/SU test diets (Table 7). Further, all growth performance measures for broilers fed control, 356043, and 356043 + Gly/SU test diets fell within the tolerance intervals calculated for this study using data obtained from broilers consuming diets produced with nontransgenic commercial reference soybean fractions.

Organ and Carcass Yields

Kidney yields were not significantly different between control and 356043 or 356043 + Gly/SU test diet groups, and values for all groups fell within the tolerance interval calculated for this study using data obtained from broilers consuming diets produced with nontransgenic reference soy fractions (Table 8). Overall liver yields and liver yields for female broilers were not significantly different between control and 356043 or 356043 + Gly/SU test diet groups. Within males, liver yield was higher ($P < 0.05$) for the 356043 + Gly/SU test diet group as compared with the control diet group. However, this difference was not significant when the *P*-value was adjusted using false discovery rate, and all values were within the tolerance intervals calculated for this study.

No statistically significant differences were observed for carcass or individual parts yields between the control and 356043 test diet group, or between the control and 356043 + Gly/SU test diet group (Tables 9 and 10). Additionally, all values for control, 356043, and 356043 + Gly/SU diet groups fell within the tolerance intervals calculated for this study using data obtained from broilers consuming diets produced with nontransgenic reference soy fractions.

DISCUSSION

Biotechnology has been used to producex, 2003; EFSA, 2006a,b). These guidelines are based on the concept of demonstrating that genetically modified (**GM**) crops are not substantially different from those obtained from non-GM crops. These guidelines include comparison of the compositional analysis of nutrients in whole grains or processed feed fractions obtained from GM crops with those obtained from the closest genetic comparator (e.g., near-isoline control) non-GM crop. The compositional analysis for field crops such as soybeans is defined by a list of components with known nutritional or antinutritional effect (OECD, 2001). Although the list of ingredi-

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Table 8. Prechill organ yields[1] of broilers fed diets containing Optimum GAT meal; hulls and oil from untreated (356043) and glyphosate/sulfonylurea-treated (356043 + Gly/SU) soybean plants; nontransgenic near-isoline control soybean meal, hulls, and oil; and meal, hulls, and oil from commercial reference soybeans (93B86, 93B15, and 93M40)

Item	Control	356043	356043 + Gly/SU	Tolerance interval[2]
Kidney (%)—overall				
Mean[3]	2.08	2.09	2.08	
SEM	0.05	0.05	0.05	
FDR[4]		0.98	0.98	
P-value[5]		0.94	0.97	
Kidney (%)—males				
Mean	2.13	2.08	2.10	0.77 to 3.48
SEM	0.07	0.07	0.07	
FDR		0.89	0.89	
P-value		0.62	0.71	
Kidney (%)—females				
Mean	2.03	2.09	2.06	0.69 to 3.50
SEM	0.07	0.07	0.07	
FDR		0.89	0.89	
P-value		0.55	0.76	
Liver (%)—overall				
Mean	3.47	3.40	3.59	
SEM	0.06	0.06	0.06	
FDR		0.89	0.89	
P-value		0.47	0.14	
Liver (%)—males				
Mean	3.37	3.44	3.64	1.95 to 5.13
SEM	0.08	0.08	0.08	
FDR		0.89	0.82	
P-value		0.49	0.0171[6]	
Liver (%)—females				
Mean	3.56	3.37	3.55	1.90 to 5.11
SEM	0.08	0.08	0.08	
FDR		0.89	0.98	
P-value		0.08	0.88	

[1]Calculated as percentage of live bird weight.

[2]Lower and upper limits of a 95% tolerance interval on 99% of the observed organ yield trait values from birds fed 93B86, 93B15, and 93M40 reference soybean diets.

[3]Control, 356043, and 356043 + Gly/SU treatment means (n = 12 pens/treatment group with 10 birds/pen).

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

[5]Nonadjusted *P*-value.

[6]Statistically significant difference, nonadjusted *P*-value < 0.05.

ents within these documents is comprehensive, it is not exhaustive, and it is possible that additional components could affect the nutritional quality of the whole grains or processed feed fractions obtained from GM crops. For this reason, animal feeding trials have been conducted to determine if particular genetic modification could have resulted in unintended changes that could affect the nutritional quality of whole grains or processed feed fractions obtained from GM crops. Broiler chickens are a rapidly growing species of commercial importance that are sensitive to nutritional deficiencies (ILSI, 2003). They have been used extensively in feeding trials to compare the nutritional quality of whole grains and processed fractions obtained from GM crops with those from non-GM crops (Brake and Vlachos, 1998; Brake et al., 2003, 2005; Taylor et al., 2003a,b,c, 2004, 2005; McNaughton et al., 2007). These studies have reported that the nutritional performance and carcass variables of broilers consuming diets formulated with grains and processed fractions from GM crops were not different from those observed in nontransgenic maize (*Zea mays* L.) grains. Overall, these reports indicate that the particular GM substances that were evaluated were as wholesome and nutritious as those obtained from non-GM sources.

The current study was conducted to assess the nutritional performance of processed fractions obtained from a newly developed GM soybean (Optimum GAT; 356043) with the processed fractions obtained from its near-isoline control. Optimum GAT soybeans were produced by insertion of the *gat4601* and *gm-hra* genes. The expression products of these genes, GAT and GM-HRA proteins, respectively, confer in planta tolerance to the herbicidal active ingredients glyphosate and acetolactate synthase-inhibiting herbicides such as sulfonylurea and imidazolinone (Lee et al., 1988; Castle et al., 2004).

The nutritional composition of the soybean meal and hull fractions from 356043 soybeans treated with in-field application of glyphosate and sulfonylurea herbicides (356043 + Gly/SU), untreated 356043 soybeans (356043), and control soybeans were compared in the current

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Table 9. Postchill carcass, breast, and thigh yields[1] of broilers fed diets containing Optimum GAT meal; hulls and oil from untreated (356043) and glyphosate/sulfonylurea-treated (356043 + Gly/SU) soybean plants; non-transgenic near-isoline control soybean meal, hulls, and oil; and meal, hulls, and oil from commercial reference soybeans (93B86, 93B15, and 93M40)

Item	Control	356043	356043 + Gly/SU	Tolerance interval[2]
Carcass (%)—overall				
Mean[3]	70.54	70.39	70.98	
SEM	0.35	0.35	0.35	
FDR[4]		0.89	0.89	
P-value[5]		0.75	0.36	
Carcass (%)—males				
Mean	69.81	70.29	70.47	61.96 to 79.74
SEM	0.47	0.47	0.47	
FDR		0.89	0.89	
P-value		0.46	0.31	
Carcass (%)—females				
Mean	71.27	70.48	71.49	60.86 to 80.41
SEM	0.47	0.47	0.47	
FDR		0.89	0.89	
P-value		0.22	0.74	
Breast (%)—overall				
Mean	26.84	26.83	26.55	
SEM	0.22	0.22	0.22	
FDR		0.98	0.89	
P-value		0.98	0.36	
Breast (%)—males				
Mean	27.14	26.72	26.87	20.73 to 32.90
SEM	0.31	0.31	0.31	
FDR		0.89	0.89	
P-value		0.34	0.54	
Breast (%)—females				
Mean	26.55	26.94	26.24	20.82 to 32.92
SEM	0.31	0.31	0.31	
FDR		0.89	0.89	
P-value		0.36	0.47	
Thigh (%)—overall				
Mean	15.98	16.09	16.06	
SEM	0.16	0.16	0.16	
FDR		0.89	0.89	
P-value		0.63	0.73	
Thigh (%)—males				
Mean	16.15	15.96	16.13	11.77 to 19.75
SEM	0.21	0.21	0.21	
FDR		0.89	0.98	
P-value		0.53	0.96	
Thigh (%)—females				
Mean	15.82	16.21	15.98	11.70 to 20.29
SEM	0.21	0.21	0.21	
FDR		0.89	0.89	
P-value		0.19	0.58	

[1]Carcass yield calculated as percentage of live bird weight; parts yield calculated as percentage of postchill carcass weight.

[2]Lower and upper limits of a 95% tolerance interval on 99% of the observed postchill carcass and parts yield trait values from birds fed 93B86, 93B15, and 93M40 reference soybean diets.

[3]Control, 356043, and 356043 + Gly/SU treatment means (n = 12 pens/treatment group with 10 birds/pen).

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

[5]Nonadjusted *P*-value.

study. Processed fractions of 3 additional non-GM commercial reference soybeans (93B86, 93B15, and 93M40) obtained from the same field trial were also included. Information about the nutrient composition of soybean fractions necessary for formulating broiler chicken diets is limited primarily to nutritional proximates, amino acids, Ca, P, and gross energy values. In this study, no nutrient compositional differences were identified between the soybean meal and hull fractions from these different soybean sources. These results indicated that the processed fractions from these different soybeans were suitable for the production of broiler chicken diets.

Processed fractions (meal, hulls, and crude oil) from these different soybeans were used to produce starter, grower, and finisher broiler chicken diets in a manner consistent with that used by commercial poultry farmers

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Table 10. Postchill leg, wing, and abdominal fat yields[1] of broilers fed diets containing Optimum GAT meal; hulls and oil from untreated (356043) and glyphosate/sulfonylurea-treated (356043 + Gly/SU) soybean plants; nontransgenic near-isoline control soybean meal, hulls, and oil; and meal, hulls, and oil from commercial reference soybeans (93B86, 93B15, and 93M40)

Item	Control	356043	356043 + Gly/SU	Tolerance interval[2]
Leg (%)—overall				
Mean[3]	14.49	14.31	14.21	
SEM	0.13	0.13	0.13	
FDR[4]		0.89	0.89	
P-value[5]		0.36	0.15	
Leg (%)—males				
Mean	14.68	14.44	14.32	10.71 to 17.80
SEM	0.18	0.18	0.18	
FDR		0.89	0.89	
P-value		0.35	0.16	
Leg (%)—females				
Mean	14.29	14.18	14.10	10.66 to 18.19
SEM	0.18	0.18	0.18	
FDR		0.89	0.89	
P-value		0.67	0.46	
Wing (%)—overall				
Mean	10.60	10.69	10.54	
SEM	0.09	0.09	0.09	
FDR		0.89	0.89	
P-value		0.42	0.60	
Wing (%)—males				
Mean	10.69	10.71	10.57	8.25 to 12.80
SEM	0.11	0.11	0.11	
FDR		0.98	0.89	
P-value		0.92	0.42	
Wing (%)—females				
Mean	10.50	10.66	10.51	8.25 to 12.88
SEM	0.11	0.11	0.11	
FDR		0.89	0.98	
P-value		0.30	0.94	
Abdominal fat (%)—overall				
Mean	1.46	1.53	1.51	
SEM	0.04	0.04	0.04	
FDR		0.89	0.89	
P-value		0.25	0.37	
Abdominal fat (%)—males				
Mean	1.50	1.52	1.53	0.52 to 2.52
SEM	0.05	0.05	0.05	
FDR		0.89	0.89	
P-value		0.75	0.65	
Abdominal fat (%)—females				
Mean	1.42	1.53	1.49	0.39 to 2.52
SEM	0.05	0.05	0.05	
FDR		0.89	0.89	
P-value		0.16	0.37	

[1]Parts yield calculated as percentage of postchill carcass weight.

[2]Lower and upper limits of a 95% tolerance interval on 99% of the observed postchill carcass and parts yield trait values from birds fed 93B86, 93B15, and 93M40 reference soybean diets.

[3]Control, 356043, and 356043 + Gly/SU treatment means (n = 12 pens/treatment group with 10 birds/pen).

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

[5]Nonadjusted *P*-value.

(McNaughton et al., 2007). No compositional differences were identified in the diets from each respective phase regardless of the source of soybeans.

The performance of these different diets was compared using standard nutritional performance variables and organ and carcass yields. No significant differences in BW, weight gain, or carcass yields were observed among broiler chickens consuming diets prepared with processed soybean fractions from 356043, 356043 + Gly/SU, near-isoline control, or reference soybeans. Organ yields from broiler chickens have not previously been reported in nutritional comparison trials of grains from transgenic crops. However, in addition to nutritional performance and carcass traits, they are indicators of overall broiler health. Liver weights in chickens are sensitive to changes from nutritional deficiencies in diets (Velu et al., 1971; Carew et al., 2005). The kidney weights of chickens are also sensitive to change from dietary differences, as best documented from studies with mycotoxins (Edrington et al., 1997; Morris et al., 1999; Farran

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et al., 2005). Further, liver and kidney weights are among the organs that have been assessed in nutritional performance trials of grains from transgenic crops in other species such as rodents (Hammond et al., 2004, 2006a,b; MacKenzie et al., 2007; Malley et al., 2007). In the current study, there were no biologically significant differences in organ yield between broiler chickens consuming diets formulated with processed fractions obtained from Optimum GAT soybeans and those consuming diets formulated with feed fractions from near-isoline nontransgenic control soybeans.

The results from this study demonstrated that the processed fractions obtained from Optimum GAT soybeans are nutritionally equivalent to the fractions obtained from a near-isoline nontransgenic control and commercially available non-GM reference soybeans.

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