Nutritional equivalency evaluation of transgenic maize grain from event DP-Ø9814Ø-6 and transgenic soybeans containing event DP-356Ø43-5: Laying hen performance and egg quality measures

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ABSTRACT The objective of this study was to compare the nutritional performance of laying hens fed maize grain from event DP-Ø9814Ø-6 (98140; *gat4621* and *zm-hra* genes) and processed soybean meal from soybeans containing event DP-356Ø43-5 (356043; *gat4601* and *gm-hra* genes), individually or in combination, with the performance of hens fed diets containing nontransgenic maize and soybean meal. Healthy pullets (n = 216) placed in cages (3 hens/cage) were randomly assigned to 9 dietary treatments (8 cages/treatment): nontransgenic controls 1, 2, and 3 (comparable genetic background controls for 98140, 356043, and 98140 + 356043, respectively); reference 1, reference 2, and reference 3 (commercially available nontransgenic maize-soybean meal sources); and 98140 (test 1), 356043 (test 2), and 98140 + 356043 (test 3). The experiment was divided into three 4-wk phases (24 to 28 wk, 28 to 32 wk, and 32 to 36 wk of age), during which time hens were fed mash diets. Performance (BW, feed intake, and egg production) and egg quality data were collected. Data were analyzed using a mixed model ANOVA; differences between the control and respective test group means were considered significant at $P < 0.05$. Data generated from the reference groups were used only in the estimation of experimental variability and in generating the tolerance interval. Body weight and BW gain, egg production, and production efficiency for hens fed the test diets were similar to the respective values for hens fed the corresponding control diets. Haugh unit measures and egg component weights were similar between the respective test and control groups, and no differences were observed in quality grades or crack measures. All observed values of the control and test groups were within the calculated tolerance intervals. This research indicates that the performance and egg quality of hens fed diets containing 98140 maize grain, 356043 soybean meal, or a combination of the 2 was comparable with that of hens fed diets formulated with nontransgenic maize grain or soybean meal control diets with comparable genetic backgrounds.

Key words: GAT4621, GAT4601, laying hen performance, egg production, egg quality

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INTRODUCTION

The adoption of biotechnology has increased crop yields, which in turn increases food availability and supply. Additionally, biotechnology adoption reduces production costs, which will ultimately help reduce food prices. Hectares of biotech crops produced in the United States in 2008 increased to 62.5 million from their initial adoption in 1996, with 85% of the 35.3 million maize hectares planted to biotech traits. Economic gains from the use of biotech crops in the United States from 1996 to 2007 have totaled $44 billion dollars; 44% of the economic gains have been due to substantial yield gains, and 56% have been due to a reduction in production costs, which includes a reduction in pesticides of 359,000 t of active ingredient (James, 2008).

Pioneer Hi-Bred has developed a glyphosate acetyltransferase (*gat*) gene isolated from *Bacillus licheniformis*. Maize plants (*Zea mays* L.) were modified by the insertion of the *gat4621* and corn acetolactate synthase (*zm-hra*) genes to produce event DP-Ø9814Ø-6 (**98140**); transgenic soybean (*Glycine max*) line DP-356Ø43-5 (**356043**) was produced by the insertion of the *gat4601* and soybean acetolactate synthase (*gm-hra*) genes. The expressed transgenic proteins confer in planta tolerance to both the herbicidal active ingredient glyphosate and to acetolactate synthase-inhibiting herbicides.

Published papers evaluating the nutritional equivalence of transgenic feedstuffs in laying hens are lim-

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ited. Previous studies have evaluated the nutritional value of transgenic maize (Rasmussen et al., 2007; Jacobs et al., 2008; Scheideler et al., 2008a,b), with few published studies on feeding transgenic soybean meal to laying hens (Mejia et al., 2010). The objective of this study was to evaluate the potential nutritional impact of 98140 maize grain and 356043 soybean meal in laying hen diets, separately or in combination, as compared with diets containing nontransgenic control maize grain and soybean meal with comparable genetic backgrounds or commercially available nontransgenic maize grain and soybean meal.

MATERIALS AND METHODS

Maize Grain and Soybean Meal Sources

All maize grain sources, with the exception of an additional commercially available nontransgenic source (Pioneer brand hybrid 33T56), were grown by Pioneer Hi-Bred in a 2007 field production trial conducted near York, Nebraska. Control maize (**091 Maize**), with a genetic background comparable with 98140 maize, and commercially available nontransgenic Pioneer hybrids 33J56, 33P66, and 33R77 were produced 201 m from the 98140 maize production plot to avoid cross-pollination. The 98140 maize plants were treated with an 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). Neither control nor reference maize plants were treated with the herbicides described previously. Soybean meal, with the exception of a purchased nontransgenic soybean meal source (**SBM A**), was produced from soybeans grown by Pioneer in a 2007 field production trial conducted near York, Nebraska. Nontransgenic control soybeans (**091 Soy**), with a genetic background comparable with 356043 soybeans; commercially available nontransgenic Pioneer varieties 92M72 and 93B15; and 356043 soybeans were processed into meal at Texas A&M University (College Station, TX), with identity preservation procedures followed throughout the processing and inventory systems to maintain the identity of each soybean source and the resulting processed meal fractions.

Maize Grain and Soybean Meal Characterization

All maize grain sources were evaluated by ELISA for expression of GAT4621 and ZM-HRA proteins expressed by *gat4621* and corn acetolactate synthase (*zm-hra*) genes, respectively; Cry1F protein expressed by the *cry*1F gene from *Bacillus thuringiensis* var. *aizawai*; Cry34Ab1 and Cry35Ab1 proteins expressed from *cry*34Ab1 and *cry*35Ab1 genes, respectively, from *Bacillus thuringiensis* (Bt) Berliner strain PS149B1; PAT protein expressed by the phosphinothricin acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes*; and the 5-enolpyruvylshikimate-3-phosphate synthase protein (CP4 EPSPS) expressed from *Agrobacterium* sp. strain CP4. Analysis of the additional proteins (Cry1F through CP4 EPSPS) was primarily to confirm the absence of transgenic proteins in the Pioneer hybrid 33T56 maize source. The ELISA testing confirmed the presence of GAT4621 and ZM-HRA proteins (7.0 and 0.27 ng/mg of grain, respectively) in only the 98140 maize source, and their absence from all other maize sources (GAT4621 lower limit of quantitation = 0.11 ng/mg of grain; ZM-HRA lower limit of quantitation = 0.27 ng/mg of grain). Samples of 091 Maize, 98140, 33J56, 33P66, and 33R77 maize grain sources were also submitted for real-time qualitative PCR analysis for the presence of event DP-Ø9814Ø-6; testing confirmed the presence of the event in 98140 maize grain and its absence from all other maize grains (data not shown). Event-specific real-time PCR testing confirmed the presence of event DP-35Ø643-5 in the 356043 soybean meal and its absence from the 091 Soy, 92M72, and 93B15 soybean meals (data not shown). Duplicate samples of maize grain and soybean meal sources were evaluated for nutrient proximate composition, calcium, phosphorous, and mycotoxin content (maize sources only) at Cumberland Valley Analytical Services (Hagerstown, MD). Analytical determinations were conducted according to standard methods for DM (AOAC, 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), calcium and phosphorus (AOAC, 2000; method 985.01), and mycotoxins (AOAC, 2000; methods 994.08, 995.15, and 986.17). Amino acid content of the maize grain and soybean meal sources was determined at the University of Missouri (Columbia, MO) in accordance with AOAC (2000) methods 988.15, 982.30, and 994.12. All maize grain and soybean meal 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). Analyzed nutrient compositions of the maize grain (Table 1) and soybean meal (Table 2) sources were used in diet formulations. Each maize grain source was milled to a consistent geometric mean particle size (within 650 to 750 μm) at the Pioneer Livestock Nutrition Center Feed Mill (Polk City, IA) using a Bliss Experimental hammer mill (Bliss Manufacturing, Ponca City, OK). Grains were ground in the following order to minimize the potential for cross-contamination of nontransgenic maize grain with transgenic maize grain: 091 Maize, 33T56, 33J56, 33P66, 33R77, and 98140. Samples from each ground maize source were analyzed on site for particle size according to the method of Pfost (1976).

Birds and Housing

Bird care and use practices during the trial were in accordance with the Guide for the Care and Use of Ag-

Table 1. Analyzed particle size, nutrient composition, and mycotoxin profile[1] (as-fed basis) of maize grains used to formulate diets

Item	33T56	091 Maize	98140	33J56	33P66	33R77
Particle size (μm)	651	684	702	724	669	688
Proximate						
Moisture (%)	15.05	12.15	12.90	11.85	12.55	12.55
Protein (%)	6.7	7.1	7.9	7.6	7.4	6.7
Fat (%)	3.4	3.3	3.3	3.1	3.6	3.6
Fiber (%)	2.0	1.6	1.6	1.6	1.6	1.6
Ash (%)	1.5	1.6	1.4	1.5	1.8	1.7
Calcium (%)	0.01	0.02	0.01	0.01	0.01	0.01
Phosphorus (%)	0.25	0.22	0.22	0.22	0.26	0.24
Gross energy (kcal/kg)	3,844	3,949	3,923	3,948	3,926	3,929
Essential amino acid (%)						
Arginine	0.34	0.39	0.42	0.39	0.39	0.36
Cysteine	0.15	0.16	0.16	0.17	0.17	0.15
Histidine	0.25	0.27	0.30	0.28	0.28	0.25
Isoleucine	0.81	0.89	1.03	0.93	0.93	0.82
Leucine	0.26	0.26	0.27	0.27	0.27	0.25
Lysine	0.14	0.17	0.16	0.17	0.17	0.15
Methionine	0.34	0.39	0.42	0.39	0.39	0.36
Phenylalanine	0.32	0.37	0.41	0.38	0.37	0.33
Threonine	0.24	0.26	0.29	0.27	0.27	0.25
Tryptophan	0.06	0.06	0.06	0.06	0.06	0.05
Valine	0.32	0.37	0.40	0.38	0.38	0.35
Mycotoxin[2] (mg/kg)						
Fumonisin B_1	0.2	2.1	4.0	4.8	0.1	3.3
Fumonisin B_2	Negative	0.7	1.2	1.6	Negative	1.0
Fumonisin B_3	Negative	0.2	0.5	0.6	Negative	0.5

[1]Each value represents the mean of 2 samples. 33T56 = commercially available nontransgenic Pioneer (Johnston, IA) brand hybrid; 091 Maize = control maize with a genetic background comparable with 98140 maize; 98140 = maize grain from event DP-Ø9814Ø-6; 33J56, 33P66, and 33R77 = commercially available nontransgenic Pioneer hybrids.

[2]Detection limits for fumonisins were 0.1 mg/kg.

ricultural Animals in Agricultural Research and Teaching (FASS, 1999). The study plan was reviewed and approved by the Pioneer Hi-Bred Animal Care and Use Committee. Healthy pullets (Babcock B300 White Leghorn) were raised to 17 wk of age in cages at Slonaker Farms (Glengary, WV) under conditions common to commercial pullet rearing. Standard corn- and soybean meal-based diets sufficient in nutrient content to meet the needs of growing pullets and to achieve a targeted BW of at least 1,000 g at 17 wk were fed during this time. Pullets were transferred to AHPharma farm 1 (Tyaskin, MD) at 17 wk of age; this transfer coincided with the time point under commercial conditions at which they would be moved from pullet to layer

Table 2. Analyzed nutrient composition[1] (as-fed basis) of soybean meals used to formulate diets

Analyzed composition	SBM A	091 Soy	356043	92M72	93B15
Proximate					
Moisture (%)	13.24	8.09	6.84	5.74	5.65
Protein (%)	49.6	48.7	49.9	49.8	49.3
Fat (%)	0.6	1.0	1.6	1.8	1.5
Fiber (%)	2.9	4.7	3.6	3.2	4.3
Ash (%)	6.0	7.1	7.4	7.4	7.1
Calcium (%)	0.32	0.36	0.28	0.31	0.36
Phosphorus (%)	0.68	0.75	0.77	0.79	0.70
Gross energy (kcal/kg)	4,141	4,372	4,434	4,504	4,475
Essential amino acid (%)					
Arginine	3.60	3.32	3.60	3.56	3.47
Cysteine	0.63	0.62	0.66	0.76	0.67
Histidine	1.28	1.28	1.37	1.32	1.27
Isoleucine	2.23	2.27	2.47	2.41	2.36
Leucine	3.72	3.79	4.07	3.93	3.81
Lysine	3.03	2.83	3.09	2.97	3.00
Methionine	0.65	0.69	0.72	0.72	0.72
Phenylalanine	2.49	2.41	2.59	2.49	2.42
Threonine	2.10	1.83	1.89	1.86	1.79
Tryptophan	0.64	0.74	0.81	0.80	0.73
Valine	2.32	2.32	2.57	2.51	2.38

[1]Each value represents the mean of 2 samples. SBM A = purchased nontransgenic soybean meal; 091 Soy = soybean meal from nontransgenic control soybeans with a genetic background comparable with 356043; 356043 = soybean meal from soybeans containing event DP-356Ø43-5; 92M72 and 93B15 = soybean meal from commercially available nontransgenic Pioneer (Johnston, IA) varieties.

houses. Hens were placed into cages for an acclimation and preconditioning period. Two corn- and soybean meal-based preconditioning diets were fed over a 7-wk period, with the first diet (2976 kcal/kg of ME, 14.5% protein, 2.25% calcium, and 0.35% available phosphorus) fed from 17 to 20 wk of age, and the second diet (2966 kcal/kg of ME, 16.0% protein, 2.50% calcium, and 0.40% available phosphorus) fed from 20 to 24 wk of age (start of study). Two preconditioning diets were deemed necessary to prevent kidney stones in young pullets, stimulate egg production, and address the different nutritional requirements commonly used for egg production.

A randomized block design was used to assign cages to 9 dietary treatments: 091 Maize + SBM A (control 1; control for 98140); 091 Soy + 33T56 (control 2; control for 356043), 091 Maize + 091 Soy (control 3; control for 98140+356043); nontransgenic commercial maize and soybean meal groups 33J56 + 92M72, 33P66 + 93B15, and 33R77 + SBM A (reference 1, reference 2, and reference 3, respectively); and test groups 98140 (test 1), 356043 (test 2), and 98140 + 356043 (test 3). The individual reference group maize and soybean meal combinations were made based on the analyzed nutrient content of each source. Each treatment was fed to 8 cages of hens (replicates), for a total of 24 hens/treatment (216 total hens). This number of replicates was determined to be adequate to detect a 5% difference from the mean using an α level of 0.05 and a β level of 0.20 (International Life Sciences Institute, 2003).

Hens were housed in a room containing forced-air heaters with a cross-house ventilation system. Pullets were housed (3 hens/cage) in free-standing single-tier cages at a density of 697 cm^2 (108 $in.^2$) of available floor space per laying hen. Cages were separated by a wire partition to minimize the potential for cross-contamination. Incandescent lights were used in the lighting program, which incorporated 16 h of light (during daylight hours and anticipating the longest day of the year) and 8 h of darkness.

Diets

The experiment was divided into three 4-wk phases (phase 1, 24 to 28 wk; phase 2, 28 to 32 wk; and phase 3, 32 to 36 wk). Diets within each phase were formulated to meet nutrient requirements of a commercial laying hen using the NRC Nutrient Requirements for Poultry (NRC, 1994) as a guideline. Corn- and soybean meal-based diets were supplemented with phosphorus, calcium, salt, trace minerals and vitamins; diets were formulated to be isocaloric within each phase (Tables 3, 4, and 5). Maize grain sources were added to the indicated diets in amounts as similar as the formulation program would allow; maize grain quantities were within approximately 2 to 3 percentage units across treatments within each phase. A similar formulation strategy was followed with soybean meals, with quantities being within approximately 2 to 4 percentage units across treatments within each phase. Diets were prepared at the Pioneer Livestock Nutrition Center Feed Mill (Polk City, IA) with diets for each maize-soybean meal source mixed in the order of control diets, reference diets, and test diets to minimize the potential for cross-contamination of nontransgenic sources with transgenic sources. Diet samples were collected and submitted for proximate (including calcium and phosphorus), amino acid, and gross energy analyses; subsamples were also submitted for ELISA and PCR analyses to confirm the presence of the expressed transgenic proteins and events in the 3 test diets and their absence from all control and reference diets.

Measurements

Body weights were taken at the beginning and end of each phase; feed consumption was measured for each phase. Egg production (number laid) was determined once every 2 wk and averaged for each phase; egg mass and feed efficiency measures were calculated from egg production, egg weights, and feed intake. Egg weight, crack, and grade measures were collected for 2 d of egg production (minimum of 4 eggs) during the last week of each phase. Egg component weights (albumen, yolk, and shell) and Haugh units were determined on 4 eggs/cage once every 2 wk during each phase.

Statistical Analysis

Performance (as measured by BW, BW gain, feed intake, and egg production), and egg quality data were summarized for each phase and for the entire experiment. The cage was the experimental unit for all data. Three hypotheses tested in this study were that performance and egg quality would be different between laying hens fed an individual control diet and those fed their respective test diet. Hen performance, Haugh unit, and egg component weight data were analyzed using the MIXED procedure of SAS (SAS Institute, 1990). The model included treatment, phase, and the treatment × phase interaction as fixed effects; cage(treatment) was designated as a random effect for performance data analysis and cage(treatment) and series time(phase) were designated as random effects for egg quality data analysis. Estimate statements were used to generate comparisons between controls and the respective test groups for each measure; observed P-values generated from the estimate comparison statement determined whether the mean of the control group was statistically different from the mean of the respective test group, with differences considered significant at $P \leq 0.05$. False discovery rate (**FDR**), as described by Benjamini and Hochberg (1995), was applied across all measures analyzed to control the false positive rate; the FDR-adjusted P-value was reviewed if statistically significant differences generated from the estimate comparison statement were observed for a measure.

Table 3. Ingredient[1] and analyzed nutrient compositions (as-fed basis) of phase 1 diets fed to laying hens

Item	Control 1	Test 1	Control 2	Test 2	Control 3	Test 3	Reference 1	Reference 2	Reference 3
Ingredient (% of diet)									
Ground maize	69.629	71.077	70.229	70.309	68.782	70.391	69.301	69.363	69.483
Soybean meal	15.961	14.296	13.632	13.834	17.447	15.857	16.516	16.993	15.506
Protein blend[2]	2.777	2.902	4.614	4.329	2.109	2.000	2.000	2.000	3.411
Soybean oil	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Limestone[3]	7.703	7.720	7.658	7.702	7.8081	7.789	7.815	7.764	7.705
Dicalcium phosphate	2.213	2.224	2.128	2.128	2.2181	2.209	2.596	2.176	2.177
Sodium chloride	0.510	0.509	0.503	0.504	0.5221	0.513	0.563	0.513	0.507
Vitamin-mineral premix[4]	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625
DL-Methionine	0.276	0.295	0.255	0.254	0.283	0.299	0.263	0.271	0.278
L-Lysine hydrochloride	0.256	0.301	0.308	0.265	0.272	0.266	0.269	0.245	0.258
Analyzed nutrient composition									
Proximate									
Moisture (%)	10.8	11.8	12.3	12.1	10.3	11.0	10.2	10.1	10.8
Protein (%)	15.7	15.7	15.2	16.4	15.5	15.0	15.4	15.6	16.6
Fat (%)	2.6	2.5	2.9	3.3	2.8	2.7	2.4	3.0	2.7
Fiber (%)	2.9	1.5	2.8	2.2	2.6	2.3	2.1	2.1	2.1
Ash (%)	10.0	10.5	9.9	10.2	10.9	11.3	11.1	11.1	9.4
Calcium (%)	3.25	3.43	3.25	3.16	3.33	3.62	3.48	3.44	3.12
Phosphorus (%)	0.68	0.73	0.68	0.66	0.69	0.71	0.73	0.74	0.49
Gross energy (kcal/kg)	3,611	3,541	3,561	3,604	3,637	3,580	3,589	3,628	3,653
Essential amino acid (%)									
Arginine	1.01	1.00	0.95	0.93	0.98	1.01	0.98	0.98	1.01
Cysteine	0.32	0.34	0.36	0.33	0.29	0.30	0.31	0.30	0.35
Histidine	0.46	0.46	0.43	0.44	0.46	0.47	0.45	0.45	0.45
Isoleucine	0.65	0.66	0.65	0.65	0.65	0.66	0.65	0.65	0.67
Leucine	1.44	1.50	1.43	1.43	1.42	1.49	1.43	1.44	1.44
Lysine	1.02	1.04	1.00	0.99	1.03	1.04	1.01	1.00	1.02
Methionine	0.48	0.56	0.45	0.50	0.51	0.50	0.49	0.50	0.45
Phenylalanine	0.78	0.79	0.76	0.76	0.77	0.79	0.76	0.77	0.78
Threonine	0.63	0.63	0.63	0.72	0.63	0.63	0.63	0.61	0.62
Tryptophan	0.19	0.17	0.17	0.18	0.19	0.19	0.20	0.20	0.20
Valine	0.81	0.84	0.85	0.85	0.81	0.83	0.80	0.79	0.85

[1]Diets were formulated to contain 2,932 kcal/kg of ME, 15.5% protein, 3.55% calcium, and 0.50% available phosphorus. Controls 1, 2, and 3 = nontransgenic controls with a genetic background comparable to 98140, 356043, and 98140 + 356043; test 1 = maize grain from event DP-Ø9814Ø-6 (98140); test 2 = soybean meal from soybeans containing event DP-356Ø43-5 (356043); test 3 = 98140 + 356043; reference 1, reference 2, and reference 3 = commercially available nontransgenic maize-soybean meal sources.

[2]Protein blend manufactured by the Papillion Agricultural Company (Easton, MD). Analyzed composition (as-fed basis): moisture, 8.25%; protein, 80.61%; gross energy, 5,082 kcal/kg; arginine, 5.03%; lysine, 3.03%; methionine, 0.71%; methionine + cystine, 4.05%; threonine, 3.75%; and tryptophan, 0.57%.

[3]Contained a minimum of 95% calcium.

[4]Vitamin-mineral premix supplied (minimum) per kilogram of diet: selenium, 0.3 mg; vitamin A, 1,703 IU; vitamin D_3, 568 ICU; vitamin E, 3.7 IU; menadione, 0.2 mg; vitamin B_{12}, 0.002 mg; biotin, 0.01 mg; choline, 92 mg; folic acid, 0.3 mg; niacin, 8.5 mg; pantothenic acid, 2.3 mg; pyridoxine, 0.2 mg; riboflavin, 1.1 mg; and thiamine, 0.3 mg.

Table 4. Ingredient[1] and analyzed nutrient compositions (as-fed basis) of phase 2 diets fed to laying hens

Item	Control 1	Test 1	Control 2	Test 2	Control 3	Test 3	Reference 1	Reference 2	Reference 3
Ingredient (% of diet)									
Ground maize	67.257	68.659	67.647	67.746	66.235	67.787	66.653	66.770	67.119
Soybean meal	18.989	17.378	17.079	17.332	19.467	17.693	18.341	18.817	18.547
Protein blend[2]	2.000	2.119	3.616	3.260	2.000	2.000	2.000	2.000	2.611
Soybean oil	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Limestone[3]	0.500	0.500	0.500	0.500	1.052	1.194	1.730	1.192	0.500
Dicalcium phosphate	7.850	7.867	7.812	7.868	7.841	7.905	7.889	7.874	7.852
Sodium chloride	2.041	2.052	1.955	1.956	2.014	2.027	2.022	1.998	2.006
Vitamin-mineral premix[4]	0.412	0.412	0.406	0.407	0.413	0.413	0.413	0.413	0.410
DL-Methionine	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625
L-Lysine hydrochloride	0.175	0.193	0.156	0.155	0.168	0.181	0.143	0.153	0.176
Analyzed nutrient composition									
Proximate									
Moisture (%)	88.9	88.0	88.4	88.2	90.0	89.5	90.5	90.3	89.1
Protein (%)	16.1	16.0	16.6	16.5	16.0	16.2	15.5	15.9	16.6
Fat (%)	2.7	2.5	2.5	2.9	2.4	2.7	2.5	2.7	2.5
Fiber (%)	2.8	1.8	2.9	3.5	2.8	3.2	3.2	2.8	2.2
Ash (%)	9.9	10.2	11.1	10.8	11.0	11.4	10.9	10.1	11.6
Calcium (%)	3.24	3.01	3.79	3.37	3.28	4.08	3.49	3.33	4.05
Phosphorus (%)	0.69	0.63	0.70	0.67	0.63	0.73	0.66	0.69	0.77
Gross energy (kcal/kg)	3,619	3,555	3,564	3,591	3,631	3,581	3,671	3,677	3,589
Essential amino acid (%)									
Arginine	1.07	1.04	0.98	1.03	0.99	1.04	1.02	1.02	1.04
Cysteine	0.31	0.31	0.33	0.33	0.29	0.31	0.32	0.30	0.32
Histidine	0.48	0.47	0.46	0.47	0.46	0.48	0.47	0.47	0.47
Isoleucine	0.68	0.69	0.69	0.68	0.67	0.67	0.66	0.67	0.69
Leucine	1.45	1.56	1.47	1.45	1.45	1.55	1.45	1.49	1.42
Lysine	0.98	0.94	0.93	0.99	0.91	0.97	0.95	0.93	0.98
Methionine	0.43	0.43	0.41	0.48	0.39	0.43	0.41	0.39	0.42
Phenylalanine	0.80	0.82	0.80	0.80	0.79	0.83	0.79	0.79	0.79
Threonine	0.66	0.64	0.64	0.76	0.63	0.66	0.68	0.64	0.64
Tryptophan	0.21	0.19	0.20	0.20	0.22	0.19	0.20	0.20	0.20
Valine	0.86	0.84	0.86	0.85	0.83	0.82	0.81	0.81	0.85

[1]Diets were formulated to contain 2,888 kcal/kg of ME, 16.8% protein, 3.56% calcium, and 0.47% available phosphorus. Controls 1, 2, and 3 = nontransgenic controls with a genetic background comparable to 98140, 356043, and 98140 + 356043; test 1 = maize grain from event DP-Ø9814Ø-6 (98140); test 2 = soybean meal from soybeans containing event DP-356Ø43-5 (356043); test 3 = 98140 + 356043; reference 1, reference 2, and reference 3 = commercially available nontransgenic maize-soybean meal sources.

[2]Protein blend manufactured by the Papillion Agricultural Company (Easton, MD). Analyzed composition (as-fed basis): moisture, 8.25%; protein, 80.61%; gross energy, 5,082 kcal/kg; arginine, 5.03%, lysine, 3.03%; methionine, 0.71%; methionine + cystine, 4.05%; threonine, 3.75%; and tryptophan, 0.57%.

[3]Contained a minimum of 95% calcium.

[4]Vitamin-mineral premix supplied (minimum) per kilogram diet: selenium, 0.3 mg; vitamin A, 1,703 IU; vitamin D_3, 568 ICU; vitamin E, 3.7 IU; menadione, 0.2 mg; vitamin B_{12}, 0.002 mg; biotin, 0.01 mg; choline, 92 mg; folic acid, 0.3 mg; niacin, 8.5 mg; pantothenic acid, 2.3 mg; pyridoxine, 0.2 mg; riboflavin, 1.1 mg; and thiamine, 0.3 mg.

Table 5. Ingredient[1] and analyzed nutrient compositions (as-fed basis) of phase 3 diets fed to laying hens

Item	Control 1	Test 1	Control 2	Test 2	Control 3	Test 3	Reference 1	Reference 2	Reference 3
Ingredient (% of diet)									
Ground maize	66.309	67.706	66.634	66.747	65.238	66.769	65.646	65.760	66.263
Soybean meal	19.167	17.774	19.231	19.515	19.615	17.858	18.498	18.972	19.716
Protein blend[2]	2.000	2.000	2.416	2.014	2.000	2.000	2.000	2.000	2.000
Soybean oil	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Limestone[3]	1.273	1.174	0.500	0.500	1.901	2.048	2.581	2.049	0.769
Dicalcium phosphate	7.848	7.870	7.862	7.924	7.839	7.903	7.887	7.871	7.877
Sodium chloride	2.043	2.054	1.966	1.968	2.016	2.028	2.024	2.001	2.014
Vitamin-mineral premix[4]	0.413	0.413	0.411	0.412	0.413	0.413	0.413	0.413	0.412
DL-Methionine	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625
L-Lysine hydrochloride	0.176	0.196	0.180	0.178	0.169	0.182	0.144	0.154	0.189
Analyzed nutrient composition									
Proximate									
Moisture (%)	89.1	88.2	88.3	88.4	90.0	89.7	90.8	90.4	89.0
Protein (%)	15.8	16.9	15.9	15.8	15.9	16.6	15.6	15.9	17.1
Fat (%)	2.6	2.6	2.7	2.9	2.8	2.6	2.6	2.8	2.3
Fiber (%)	3.0	2.6	2.9	3.2	3.5	3.9	3.6	3.9	3.2
Ash (%)	11.5	10.9	11.1	10.9	10.7	11.8	10.8	10.7	12.0
Calcium (%)	3.83	3.23	3.64	3.42	3.66	4.13	3.33	3.69	3.66
Phosphorus (%)	0.74	0.66	0.71	0.70	0.70	0.81	0.68	0.73	0.72
Gross energy (kcal/kg)	3,543	3,579	3,550	3,595	3,623	3,581	3,648	3,645	3,563
Essential amino acid (%)									
Arginine	1.04	1.02	1.01	1.06	1.02	1.07	1.06	1.01	1.03
Cysteine	0.29	0.30	0.30	0.33	0.29	0.30	0.33	0.29	0.30
Histidine	0.47	0.46	0.46	0.48	0.48	0.48	0.48	0.46	0.46
Isoleucine	0.67	0.67	0.68	0.70	0.68	0.66	0.69	0.67	0.68
Leucine	1.46	1.49	1.47	1.48	1.45	1.48	1.48	1.45	1.41
Lysine	0.95	0.97	0.95	0.95	0.95	0.97	1.01	0.94	0.96
Methionine	0.45	0.49	0.41	0.43	0.42	0.45	0.43	0.37	0.42
Phenylalanine	0.80	0.80	0.80	0.82	0.80	0.80	0.81	0.78	0.78
Threonine	0.65	0.70	0.66	0.67	0.65	0.65	0.69	0.61	0.63
Tryptophan	0.21	0.20	0.21	0.21	0.21	0.20	0.20	0.20	0.20
Valine	0.82	0.83	0.83	0.86	0.82	0.82	0.85	0.81	0.83

[1]Diets were formulated to contain 2,855 kcal/kg of ME, 16.8% protein, 3.56% calcium, and 0.47% available phosphorus. Controls 1, 2, and 3 = nontransgenic controls with a genetic background comparable to 98140, 356043, and 98140 + 356043; test 1 = maize grain from event DP-Ø9814Ø-6 (98140); test 2 = soybean meal from soybeans containing event DP-356Ø43-5 (356043); test 3 = 98140 + 356043; reference 1, reference 2, and reference 3 = commercially available nontransgenic maize-soybean meal sources.

[2]Protein blend manufactured by the Papillion Agricultural Company (Easton, MD). Analyzed composition (as-fed basis): moisture, 8.25%; protein, 80.61%; gross energy, 5,082 kcal/kg; arginine, 5.03%, lysine, 3.03%; methionine, 0.71%; methionine + cystine, 4.05%; threonine, 3.75%; and tryptophan, 0.57%.

[3]Contained a minimum of 95% calcium.

[4]Vitamin-mineral premix supplied (minimum) per kilogram of diet: selenium, 0.3 mg; vitamin A, 1,703 IU; vitamin D_3, 568 ICU; vitamin E, 3.7 IU; menadione, 0.2 mg; vitamin B_{12}, 0.002 mg; biotin, 0.01 mg; choline, 92 mg; folic acid, 0.3 mg; niacin, 8.5 mg; pantothenic acid, 2.3 mg; pyridoxine, 0.2 mg; riboflavin, 1.1 mg; and thiamine, 0.3 mg.

For each hypothesis tested, data generated from the 3 reference groups and the nonrelevant control groups were used in the estimation of experimental variability; least squares means were generated for each reference treatment, but comparisons between individual reference groups and control or test treatment groups were not reported. Instead, for each measure these data were used to construct a 95% tolerance interval containing 99% of the observed values from birds fed nontransgenic commercially available maize grain and soybean meal diets, as described by Graybill (1976). These tolerance intervals were a supplemental comparison in the event of differences still being statistically significant after FDR adjustments were made. If an individual data point from the control or corresponding test group was contained within the tolerance interval, that value was considered to be similar to feeding commercially available nontransgenic maize grain or soybean meal.

Quality grade and crack category distributions were analyzed by the Cochran-Mantel-Haenszel test using the FREQ procedure of SAS (SAS Institute, 1990), with differences between the control and respective test groups considered significant at $P \leq 0.05$. Grade and crack categories were ordered from highest or most desirable category (Grade AA, no cracks) to the lowest or least desirable category (Grade B, broken eggs) before analysis.

RESULTS AND DISCUSSION

Maize Grain, Soybean Meal, and Diet Compositions

Nutrient profiles were comparable within the maize grain (Table 1) and soybean meal sources (Table 2). Mycotoxin analysis showed the presence of fumonisin B_1 in all maize grains at levels of 0.1 to 4.8 mg/kg; low levels (<2 mg/kg) of fumonisins B_2 and B_3 were detected in all grains except Pioneer hybrids 33T56 and 33P66 maize grains. Analyzed nutrient compositions of the diets fed are presented by phase in Tables 3, 4, and 5. Concentrations of individual nutrients were similar between the diets produced with the maize grain and soybean meal sources. The ELISA analysis confirmed the presence of the GAT4621 and ZM-HRA proteins in all phases of the test 1 and test 3 diets, and their absence from all phases of all other diets (data not shown). Qualitative real-time PCR analysis using a primer and probe set specific for event DP-356Ø43-5 confirmed the presence of the event in all phases of the test 2 and test 3 diets, and its absence from all phases of all other diets (data not shown).

No treatment × phase interaction was observed for any performance or egg quality measure, and phase effects ($P < 0.05$) were as would be expected and reflected the typical production cycle of a laying hen. Therefore, only the overall treatment data are presented, with data tables presented and discussed in order of control 1 vs. test 1, control 2 vs. test 2, and control 3 vs. test 3.

Control 1 vs. Test 1

Body weight, BW gain, and feed intake were not different ($P > 0.05$) between hens fed the control 1 diets and those fed the test 1 diets (Table 6). No mortalities occurred during this trial. Egg production (number laid, hen-day percentage, egg mass) and feed efficiency were similar between hens fed the test 1 diet and those fed the control 1 diet. Haugh unit values did not differ ($P > 0.05$) between hens fed the control 1 or test 1 diet. Egg component (albumen, yolk, and shell) weights were similar between the control 1 and test 1 treatment groups. The range of values observed with the control 1 and test 1 treatment groups was within the tolerance interval calculated for each measure evaluated, indicating that the observed values were considered to be similar to those of hens fed commercially available nontransgenic maize grain diets.

Control 2 vs. Test 2

Body weight, BW gain, and feed intake were not different ($P > 0.05$) between hens fed the control 2 diets and those fed the test 2 diets (Table 7). Egg production (number laid, hen-day percentage, egg mass) and feed efficiency were also similar between hens fed the control 2 diet and those fed the test 2 diet. Haugh unit values did not differ ($P > 0.05$) between hens fed the control 2 or test 2 diet. Egg component (albumen, yolk, and shell) weights were also similar between the control 2 and test 2 treatment groups. The range of values observed with the control 2 and test 2 treatment groups was within the tolerance interval calculated for each measure evaluated, indicating that the observed values were considered to be similar to those of hens fed commercially available nontransgenic soybean meal diets.

Control 3 vs. Test 3

Body weight, BW gain, and feed intake were not different ($P > 0.05$) between hens fed the control 3 diets and those fed the test 3 diets (Table 8). Egg production (number laid, hen-day percentage, and egg mass) and feed efficiency was also similar between the 2 treatment groups. Haugh unit values did not differ ($P > 0.05$) between hens fed the control 3 diet and those fed the test 3 diet. Egg component (albumen, yolk, and shell) weights were also similar between the control 3 and test 3 treatment groups. The range of values observed with the control 3 and test 3 treatment groups was within the tolerance interval calculated for each measure evaluated, indicating that the observed values were considered to be similar to those of hens fed commercially available nontransgenic maize grain and soybean meal diets.

Table 6. Control 1 vs. test 1[1]: hen performance, egg production, and egg measures of laying hens fed the control 1 or test 1 diet

	Control 1 vs. test 1						Reference group[2]				
Item	Control 1	Test 1	SEM	FDR *P*-value[3]	Raw *P*-value[4]	Tolerance interval[5]	Control 2	Control 3	Reference 1	Reference 2	Reference 3
Hen performance											
Initial BW, wk 24 (g)	1,461.3	1,418.6	30.6	0.37	0.33	844.1 to 2,012.0	1,443.9	1,394.6	1,436.8	1,415.8	1,449.1
Final BW, wk 36 (g)	1,536.0	1,488.0	32.1	0.37	0.29	1,128.6 to 1,864.9	1,513.1	1,462.5	1,504.9	1,481.8	1,521.4
BW gain (g)	74.7	69.4	3.6	0.37	0.30	−168.4 to 305.8	69.1	67.9	68.0	66.0	72.4
Feed intake (g/hen per d)	116.3	117.4	1.6	0.61	0.61	95.3 to 134.2	115.2	113.4	114.2	116.7	114.1
Egg production											
Eggs laid (no.)	73.0	71.9	0.5	0.37	0.13	48.2 to 96.4	72.1	72.3	72.3	72.2	72.7
Hen-day (%)	86.95	85.57	0.64	0.37	0.13	57.45 to 114.73	85.81	86.01	86.11	85.96	86.56
Egg weight (g)	51.5	51.1	0.3	0.37	0.23	32.3 to 70.8	51.7	51.4	51.8	51.6	51.3
Egg mass[6] (g of egg/d)	44.9	43.8	0.4	0.37	0.06	15.0 to 73.9	44.4	44.3	44.7	44.4	44.4
Feed efficiency[7] (g/g)	0.387	0.374	0.007	0.37	0.18	0.115 to 0.662	0.387	0.392	0.393	0.381	0.390
Egg measure											
Haugh units	71.62	71.19	1.05	0.95	0.68	47.53 to 92.78	69.73	70.91	70.20	69.90	70.03
Egg weight (g)	50.7	50.7	0.5	0.95	0.76	33.7 to 67.9	50.9	50.7	51.0	50.7	50.8
Albumen weight (g)	33.5	33.4	0.3	0.95	0.53	22.2 to 44.9	33.6	33.5	33.7	33.4	33.4
Yolk weight (g)	12.1	12.1	0.1	0.98	0.98	7.4 to 16.9	12.1	12.2	12.2	12.2	12.2
Shell weight (g)	5.1	5.1	0.1	0.95	0.52	3.2 to 7.0	5.2	5.0	5.1	5.1	5.1

[1]The control 1, test 1, control 2, control 3, reference 1, reference 2, and reference 3 treatment means represent 8 cages/treatment group with 3 hens/cage. Controls 1, 2, and 3 = nontransgenic controls with a genetic background comparable to 98140, 356043, and 98140 + 356043; test 1 = maize grain from event DP-Ø9814Ø-6 (98140); reference 1, reference 2, and reference 3 = commercially available nontransgenic maize-soybean meal sources.

[2]Control and reference group least squares means are included for reference purposes only. The comparison of interest was control 1 vs. test 1.

[3]*P*-value adjusted using the false discovery rate.

[4]Nonadjusted *P*-value.

[5]Lower and upper limits of a 95% tolerance interval on 99% of the observed values from hens fed the control 2, control 3, reference 1, reference 2, or reference 3 diet.

[6]Egg mass calculated as egg weight (g) × (egg production/100).

[7]Feed efficiency calculated as [egg weight (g) × (egg production/100)]/feed intake (g) per hen per day.

Table 7. Control 2 vs. test 2[1]: hen performance, egg production, and egg measures of laying hens fed the control 2 or test 2 diet

Item	Control 2 vs. test 2						Reference group[2]				
	Control 2	Test 2	SEM	FDR P-value[3]	Raw P-value[4]	Tolerance interval[5]	Control 1	Control 3	Reference 1	Reference 2	Reference 3
Hen performance											
Initial BW (g), wk 24	1,443.9	1,420.4	30.6	0.76	0.59	814.2 to 2,048.8	1,461.3	1,394.6	1,436.8	1,415.8	1,449.1
Final BW (g), wk 36	1,513.1	1,484.5	32.1	0.76	0.53	1,130.8 to 1,871.8	1,536.0	1,462.5	1,504.9	1,481.8	1,521.4
BW gain (g)	69.1	64.1	3.6	0.76	0.33	−170.7 to 310.3	74.7	67.9	68.0	66.0	72.4
Feed intake (g/hen per day)	115.2	116.6	1.6	0.76	0.51	95.4 to 134.4	116.3	113.4	114.2	116.7	114.1
Egg production											
Eggs laid (no.)	72.1	72.0	0.5	0.87	0.87	49.2 to 95.8	73.0	72.3	72.3	72.2	72.7
Hen-day (%)	85.81	85.66	0.64	0.87	0.87	58.54 to 114.10	86.95	86.01	86.11	85.96	86.56
Egg weight (g)	51.7	51.3	0.3	0.76	0.28	32.7 to 70.3	51.5	51.4	51.8	51.6	51.3
Egg mass[6] (g of egg/d)	44.4	44.0	0.4	0.76	0.48	15.9 to 73.2	44.9	44.3	44.7	44.4	44.4
Feed efficiency[7] (g/g)	0.387	0.379	0.007	0.76	0.41	0.118 to 0.659	0.387	0.392	0.393	0.381	0.390
Egg measure											
Haugh units	69.73	71.62	1.05	0.36	0.07	46.93 to 94.13	71.62	70.91	70.20	69.90	70.03
Egg weight (g)	50.9	50.8	0.5	0.87	0.61	33.8 to 67.7	50.7	50.7	51.0	50.7	50.8
Albumen weight (g)	33.6	33.6	0.3	0.87	0.87	22.3 to 44.7	33.5	33.5	33.7	33.4	33.4
Yolk weight (g)	12.1	12.1	0.1	0.87	0.76	7.3 to 17.1	12.1	12.2	12.2	12.2	12.2
Shell weight (g)	5.2	5.1	0.1	0.67	0.27	3.2 to 7.0	5.1	5.0	5.1	5.1	5.1

[1]Control 2, test 2, contro1 1, control 3, reference 1, reference 2, and reference 3 treatment means represent 8 cages/treatment group with 3 hens/cage. Controls 1, 2, and 3 = nontransgenic controls with a genetic background comparable to 98140, 356043, and 98140 + 356043; test 2 = soybean meal from soybeans containing event DP-356Ø43-5 (356043); reference 1, reference 2, and reference 3 = commercially available nontransgenic maize-soybean meal sources.

[2]Control and reference group least squares means are included for reference purposes only. The comparison of interest was control 2 vs. test 2.

[3]P-value adjusted using the false discovery rate.

[4]Nonadjusted P-value.

[5]Lower and upper limits of a 95% tolerance interval on 99% of the observed values from hens fed the control 1, control 3, reference 1, reference 2, or reference 3 diet.

[6]Egg mass calculated as egg weight (g) × (egg production/100).

[7]Feed efficiency calculated as [egg weight (g) × (egg production/100)]/feed intake (g) per hen per day.

Table 8. Control 3 vs. test 3[1]: hen performance, egg production, and egg measures of laying hens fed control 3 or test 3 diets

	Control 3 vs. test 3						Reference group[2]				
Item	Control 3	Test 3	SEM	FDR P-value[3]	Raw P-value[4]	Tolerance interval[5]	Control 1	Control 2	Reference 1	Reference 2	Reference 3
Hen performance											
Initial BW (g), wk 24	1,394.6	1,427.5	30.6	0.96	0.45	851.3 to 2,031.4	1,461.3	1,443.9	1,436.8	1,415.8	1,449.1
Final BW (g), wk 36	1,462.5	1,494.5	32.1	0.96	0.48	1,149.2 to 1,873.6	1,536.0	1,513.1	1,504.9	1,481.8	1,521.4
BW gain (g)	67.9	67.1	3.6	0.96	0.86	−174.2 to 314.3	74.7	69.1	68.0	66.0	72.4
Feed intake (g/hen per day)	113.4	115.3	1.6	0.96	0.39	95.8 to 134.8	116.3	115.2	114.2	116.7	114.1
Egg production											
Eggs laid (no.)	72.3	72.2	0.5	0.96	0.91	49.0 to 96.0	73.0	72.1	72.3	72.2	72.7
Hen-day (%)	86.01	85.91	0.64	0.96	0.91	58.33 to 114.23	86.95	85.81	86.11	85.96	86.56
Egg weight (g)	51.4	51.4	0.3	0.96	0.94	32.4 to 70.7	51.5	51.7	51.8	51.6	51.3
Egg mass[6] (g of egg/day)	44.3	44.3	0.4	0.96	0.96	15.7 to 73.5	44.9	44.4	44.7	44.4	44.4
Feed efficiency[7] (g/g)	0.392	0.386	0.007	0.96	0.56	0.114 to 0.662	0.387	0.387	0.393	0.381	0.390
Egg measure											
Haugh units	70.91	70.20	1.05	0.98	0.49	22.21 to 44.87	71.62	69.73	70.20	69.90	70.03
Egg weight (g)	50.7	50.7	0.5	0.98	0.98	33.8 to 67.8	50.7	50.9	51.0	50.7	50.8
Albumen weight (g)	33.5	33.5	0.3	0.98	0.93	46.4 to 94.2	33.5	33.6	33.7	33.4	33.4
Yolk weight (g)	12.2	12.2	0.1	0.98	0.81	7.5 to 16.9	12.1	12.1	12.2	12.2	12.2
Shell weight (g)	5.0	5.1	0.1	0.98	0.78	3.2 to 7.0	5.1	5.2	5.1	5.1	5.1

[1]Control 3, test 3, control 1, control 2, reference 1, reference 2, and reference 3 treatment means represent 8 cages/treatment group with 3 hens/cage. Controls 1, 2, and 3 = nontransgenic controls with a genetic background comparable to 98140, 356043, and 98140 + 356043; test 3 = maize grain from event DP-Ø9814Ø-6 (98140) + soybean meal from soybeans containing event DP-356Ø43-5 (356043); reference 1, reference 2, and reference 3 = commercially available nontransgenic maize-soybean meal sources.

[2]Control and reference group least squares means are included for reference purposes only. The comparison of interest was control 3 vs. test 3.

[3]P-value adjusted using the false discovery rate.

[4]Nonadjusted P-value.

[5]Lower and upper limits of a 95% tolerance interval on 99% of the observed values from hens fed the control 1, control 2, reference 1, reference 2, or reference 3 diet.

[6]Egg mass calculated as egg weight (g) × (egg production/100).

[7]Feed efficiency calculated as [egg weight (g) × (egg production/100)]/feed intake (g) per hen per day.

Table 9. Grade and crack data of eggs produced from hens fed dietary treatments[1]

Item	Control 1	Test 1	Control 2	Test 2	Control 3	Test 3	Reference range[2]
Eggs (no.)	124	119	129	129	128	127	127 to 131
Quality grade							
AA	32.26	30.25	28.68	29.46	27.34	27.56	28.24 to 30.77
A	53.23	57.98	59.69	60.47	60.94	62.20	52.76 to 62.60
B	14.52	11.76	11.63	10.08	11.72	10.24	9.16 to 16.54
P-value[3]		0.98		0.78		0.84	
Crack class							
No cracks	83.06	84.03	81.40	84.50	80.47	82.68	85.04 to 87.02
Hairline cracks	7.26	7.56	4.65	4.65	10.16	9.45	3.82 to 6.15
Open or web cracks	6.45	6.72	11.63	9.30	7.81	4.72	5.34 to 9.45
Broken egg	3.23	1.68	2.33	1.55	1.56	3.15	0.79 to 3.82
P-value		0.80		0.49		0.66	

[1]Value in treatment column is the percentage of eggs in that treatment that received the specified quality grade or crack class designation. Controls 1, 2, and 3 = nontransgenic controls with a genetic background comparable to 98140, 356043, and 98140 + 356043; test 1 = maize grain from event DP-Ø9814Ø-6 (98140); test 2 = soybean meal from soybeans containing event DP-356Ø43-5 (356043); test 3 = 98140 + 356043; references = reference 1, reference 2, and reference 3 (commercially available nontransgenic maize-soybean meal sources).

[2]Minimum and maximum values observed across all 3 reference groups. Values included for reference purposes only; the comparisons of interest were control 1 vs. test 1, control 2 vs. test 2, and control 3 vs. test 3.

[3]Cochran-Mantel-Haenszel *P*-value for comparison between each individual control group and its respective test group.

Egg Grade and Crack Data

Individual comparisons between the control groups and respective test groups showed no treatment differences ($P > 0.05$) for quality grade assignments or crack categories (Table 9). Most eggs (53 to 62%) from the control and test groups in this study were graded as Quality Grade A and the majority (80 to 85%) did not have any cracks.

The current study evaluated the potential nutritional impact of 98140 maize grain and 356043 soybean meal, separately or in combination, by comparing the performance of laying hens fed diets containing 98140, 356043, or 98140 + 356043 with those fed diets containing nontransgenic control maize grain and soybean meal with a comparable genetic background, or diets containing commercially available nontransgenic maize grain and soybean meal. The *gat4621* and *gat4601* genes inserted into maize and soybean plants, respectively, were functionally improved through gene shuffling to optimize the kinetics of the GAT enzyme to acetylate glyphosate (Castle et al., 2004; Siehl et al., 2005). The expressed proteins from the *gat4621* and *gat4601* genes, GAT4621 and GA4601, respectively, confer tolerance in planta to the herbicidal active ingredient glyphosate, and expression of the ZM-HRA and GM-HRA proteins encoded by the *zm-hra* and *gm-hra* genes, respectively, confers tolerance in planta to acetolactate synthase-inhibiting herbicides, such as sulfonylurea and imidazolinone herbicides. To date, no nutritional equivalency studies have been published in which the same transgenic trait in both maize and soybeans has been fed in combination.

The maize grain and soybean meal sources used in this study were deemed suitable for layer diet production because no major differences in key nutrients (proximates, amino acids, calcium, phosphorus, and gross energy) that would have limited their inclusion amount were observed between maize grain sources or between soybean meal sources. The presence of fumonisins FB_1, FB_2, and FB_3 in the maize sources were not a concern because the concentrations were well below the US Food and Drug Administration (2001) guideline (100 mg/kg) for total fumonisins, and diet production resulted in further dilution of the concentrations to well below the recommended total ration maximum of 50 mg/kg (US Food and Drug Administration, 2001). Layer diets were produced in a manner consistent with that used by commercial poultry producers. No differences in proximates, energy, mineral, or amino acid composition were identified in the diets from each phase regardless of maize grain and soybean meal source.

These results demonstrated no differences in nutritional performance of laying hens fed the transgenic diets (maize or soybean meal individually or in combination) when compared with laying hens fed the nontransgenic control diets; statistical analysis of the data in this study resulted in rejection of all hypotheses of expected performance and egg quality differences. Previous studies with laying hens (Aulrich et al., 1998; Aeschbacher et al., 2005; Halle et al., 2006) have shown that laying hen performance is not affected by crops conferring pesticide resistance or herbicide tolerance traits. Both the maize and soybean traits reported in this study have been fed individually to broiler chickens, with no negative effect on performance (McNaughton et al., 2007, 2008). The incorporation of the *gat* gene into maize and soybean seed allows the grain grower more flexibility and simplicity for effective weed control, which in turn reduces herbicide application, increases yields, and improves environmental benefits.

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