Performance of Laying Hens Fed Diets Containing DAS-59122-7 Maize Grain Compared with Diets Containing Nontransgenic Maize Grain

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ABSTRACT An experiment using 216 Hy-Line W-36 pullets was conducted to evaluate transgenic maize grain containing the *cry*34Ab1 and *cry*35Ab1 genes from a *Bacillus thuringiensis* (*Bt*) strain and the phosphinothricin acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes*. Expression of the *cry*34Ab1 and *cry*35Ab1 genes confers resistance to corn rootworms, and the *pat* gene confers tolerance to herbicides containing glufosinate-ammonium. Pullets (20 wk of age) were placed in cage lots (3 hens/cage, 2 cages/lot) and were randomly assigned to 1 of 3 corn-soybean meal dietary treatments (12 lots/treatment) formulated with the following maize grains: near-isogenic control (control), conventional maize, and transgenic test corn line 59122 containing event DAS-59122-7. Differences between 59122 and control group means were evaluated with statistical significance at $P < 0.05$. Body weight and gain, egg production, egg mass, and feed efficiency for hens fed the 59122 corn were not significantly different from the respective values for hens fed diets formulated with control maize grain. Egg component weights, Haugh unit measures, and egg weight class distribution were similar regardless of the corn source. This research indicates that performance of hens fed diets containing 59122 maize grain, as measured by egg production and egg quality, was similar to that of hens fed diets formulated with near-isogenic corn grain.

Key words: Cry34Ab1, Cry35Ab1, corn rootworm, egg production, egg quality

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INTRODUCTION

Western corn rootworms are responsible for a large amount of crop damage in the United States each year (Metcalf, 1986). Thus, the development of a corn rootworm-resistant maize strain has great economic and biological benefits. The transgenic maize line 59122 has been modified to contain event DAS-59122-7, consisting of the *cry*34Ab1 and *cry*35Ab1 genes from *Bacillus thuringiensis* (*Bt*) Berliner strain PS149B1 and the phosphinothricin acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes*. Coexpression of the Cry34Ab1 and Cry35Ab1 proteins confers resistance to corn rootworms, and expression of the *pat* protein confers tolerance to herbicides containing glufosinate-ammonium as the active ingredient (i.e., Liberty, Bayer AG, Leverkusen, Germany).

Some previous studies have evaluated the nutritional value of transgenic maize grains for broiler chickens by feeding diets containing transgenic maize grain and also diets composed of nontransgenic maize with a comparable genetic background (Brake and Vlachos, 1998; Sidhu et al., 2000; Brake et al., 2003, 2005; Taylor et al., 2003a,b,c, 2005). Few such studies have been conducted with laying hens (Aulrich et al., 1998; Aeschbacher et al., 2005; Halle et al., 2006). The objective of the current study was to compare the performance of laying hens fed diets containing 59122 transgenic corn with the performance of those fed diets containing nontransgenic control corn.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee (IACUC) and the Biosafety committees of the University of Illinois approved all animal care, housing, and handling procedures. Two hundred sixteen Hy-Line W-36 pullets were obtained from Hy-Line Hatchery (Warren, IN) and fed the same diet until 20 wk of age. This common diet met or exceeded all nutritional requirements of developing pullets (NRC, 1994). The pullets were then moved into a caged layer house of commercial design with water and feed provided ad libitum. The birds were randomly placed into "cage lots" (2 adjacent raised wire cages, 30 × 46 cm each, containing 3 hens per cage) for a 4-wk acclimation period. Cage lots were randomly assigned to 3 dietary treatments consisting of standard corn-soybean

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Table 1. Nutrient composition of test corns and soybean meal used to prepare test diets (as-is basis)

Ingredient	Control corn	59122 transgenic corn	3394 commercial corn	Soybean meal
DM (%)	88.50	88.33	88.71	88.42
Protein (%)	7.71	8.49	7.18	48.67
Fat (%)	3.18	3.07	3.07	1.35
Gross energy (kcal/kg)	3,923	3,906	3,912	4,175
Fiber (%)	1.9	1.8	1.8	3.0
Ash (%)	1.33	1.25	1.26	6.67
Calcium (%)	0.01	0.00	0.00	0.30
Phosphorus (%)	0.24	0.27	0.23	0.67
Arg (%)	0.31	0.37	0.31	3.66
Cys (%)	0.16	0.17	0.15	0.66
His (%)	0.21	0.24	0.20	1.25
Ile (%)	0.26	0.30	0.23	2.17
Leu (%)	0.93	1.06	0.81	3.71
Lys (%)	0.23	0.25	0.24	3.03
Met (%)	0.16	0.16	0.16	0.63
Phe (%)	0.35	0.42	0.33	2.40
Thr (%)	0.26	0.28	0.25	1.85
Trp (%)	0.07	0.07	0.07	0.66
Tyr (%)	0.14	0.22	0.18	1.57
Val (%)	0.37	0.41	0.34	2.35
Ala (%)	0.58	0.65	0.52	2.08
Asp (%)	0.54	0.60	0.52	5.71
Glu (%)	1.45	1.65	1.32	9.27
Gly (%)	0.28	0.31	0.27	2.04
Pro (%)	0.68	0.81	0.67	2.86
Ser (%)	0.34	0.39	0.32	2.40

meal mash diets formulated with a nontransgenic near-isogenic control corn (corn with a genetic background similar to the transgenic corn; control), a nontransgenic commercial corn (Pioneer hybrid 3394), or transgenic corn grain containing event DAS-59122-7 (59122). Each dietary treatment was assigned to 12 cage lots (replicates) for a total of 72 hens per treatment.

Pioneer Hi-Bred, grew all corn sources. The 59122 grain was sourced from corn plants that received 2 sequential applications of glufosinate-ammonium herbicide (Liberty). The control and commercial hybrid grains were produced in isolation (201 m) from the 59122 maize production plot to avoid cross-pollination. Eurofins Laboratories (Des Moines, IA) analyzed samples of each grain source and soybean meal for moisture (930.15), CP (990.03), crude fat (920.39), crude fiber (962.09), ash (942.05), calcium, phosphorus (985.01 with modification), tryptophan (988.15 with modification), sulfur-containing amino acids Met and Cys (994.12 with modification), and all other amino acids (982.30 with modification), all according to Association of Official Analytical Chemists International methods (2007). Pioneer Hi-Bred (Urbandale, IA) performed the gross energy analysis (1271 bomb calorimeter, Parr Instruments, Moline IL). The analyzed nutrient compositions of the test corns and soybean meal (Table 1) were used in diet formulations. The analyzed protein content of the control, 59122 transgenic, and commercial corn was 7.7, 8.5, and 7.2%, respectively, and the analytical values for other proximate components, minerals, and amino acids were similar among the 3 corns.

The corns were ground to a mean particle size (650 to 700 μm) and all diets were fed in mash form. The composition of the test diets is provided in Table 2. All diets contained equal amounts of corn grain (64.75%) and were balanced based on analyzed nutrient composition data to contain equal amounts of nitrogen, gross energy, sulfur amino acids, Lys, Thr, Trp, and Arg. Altering the level of fat inclusion changed dietary energy concentration. The diet containing transgenic corn 59122 was mixed last to reduce the potential for cross-contamination. Diet samples from each batch preparation were submitted to Dairyland Laboratories Inc. (Arcadia, WI) for analysis of moisture (930.15), CP (990.03), crude fat (920.39), ash (942.05), calcium, and phosphorus (985.01), all according to Association of Official Analytical Chemists International methods (2007); samples were submitted to Eurofins Labratories for amino acid analysis by methods described previously. Gross energy was determined by Pioneer as described previously.

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). The experiment was started in September and completed in December 2005. Lighting was provided in 15-h photoperiods at the beginning of phase 1, and then increased by 0.5 h weekly to 17 h by the end of phase 1; the 17-h photoperiod was maintained throughout the remaining phases. Live body weights were taken at the start of the study and at the end of each phase, and weight gains were calculated for each phase. Feed intakes (g/hen per d) were measured weekly.

Eggs were collected daily, and egg production and egg mass (grams of egg produced per day) were determined weekly for each phase. Egg weight, number of cracked eggs, and egg grade measures were determined on eggs collected on 2 d of egg production during the last week

Table 2. Ingredient composition of test diets (%)

Item	Pretrial diet[1]	Control corn	59122 transgenic corn	3394 commercial corn
Ingredient				
Ground corn	68.85	64.75	64.75	64.75
Soybean meal	18.40	22.50	21.55	23.20
Meat and bone meal	2.50	0.00	0.00	0.00
Soybean oil	0.00	1.14	1.41	0.95
Solka-Floc (cellulose)[2]	0.00	0.48	1.15	0.00
Limestone	8.50	8.93	8.93	8.91
Dicalcium phosphorus	1.00	1.35	1.36	1.34
NaCl	0.30	0.30	0.30	0.30
Vitamin premix[3]	0.20	0.20	0.20	0.20
Trace mineral premix[4]	0.15	0.15	0.15	0.15
dl-Met	0.10	0.15	0.15	0.15
Choline chloride, 60%	0.00	0.05	0.05	0.05
Analysis[5]				
Protein (%)	16.0	15.6	15.9	15.8
ME (kcal/kg)	2,809	2,906	2,907	2,906
Calcium (%)	3.83	4.21	4.15	4.22
Total P (%)	0.63	0.57	0.61	0.57
Nonphytate P (%)	0.41	0.35	0.35	0.35
Lys (%)	0.80	0.83	0.84	0.83
Met + Cys (%)	0.62	0.64	0.65	0.64

[1]Fed from 20 to 24 wk.

[2]Fiber Sales and Development, Urbana, OH.

[3]Provided per kilogram of diet: retinyl A acetate, 4,400 IU; cholecalciferol, 25 μg; DL-α-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[4]Provided as milligrams per kilogram of diet: manganese, 75 from manganese oxide; iron, 75 from iron sulfate; zinc, 75 from zinc oxide; copper, 5 from copper sulfate; iodine, 0.35 from ethylene diamine dihydroiodide; selenium, 0.2 from sodium selenite.

[5]Values for the pretrial diet are calculated values. Values for ME (ME_n) and nonphytate P for the other diets are calculated; all other values are analyzed.

of each phase. Egg component weights (albumen, yolk, and wet shell) and Haugh units were determined on 4 eggs/cage-lot during the last week of each phase.

Statistical Analysis

Performance and egg quality data were summarized for each phase and for the entire experiment. For all data, the cage lot (2 adjacent cages) was considered to be the experimental unit. Data were analyzed by using PROC MIXED of SAS (SAS Institute, 1990); the model included treatment, phase, and the treatment × phase interaction as fixed effects, and pen (treatment) was designated as a random effect. The true comparison of interest in this trial was that of the 59122 transgenic group vs. the control group. Therefore, estimate statements were used to generate the P-values for comparisons of individual measures; similarity to the control treatment was established when the difference between the 59122 transgenic corn and control treatment groups was not statistically significant (P

Table 3. Body weight gain, feed intake, egg production, and production efficiency of hens fed diets containing control or 59122 transgenic corn[1]

Response[2]	Control	59122 transgenic corn	3394[3] commercial corn	SEM
BW gain (g)	53.6	58.9	62.6	3.3
Feed intake (g/hen per d)	97.6	98.2	99.6	0.9
Egg production (hen-day %)	94.0	95.0	94.9	1.1
Egg weight (g)	55.6	55.7	56.0	0.3
Egg mass[4] (g of egg produced/d)	52.3	52.9	53.1	0.7
Feed efficiency[5] (g of egg/g of feed)	0.536	0.539	0.533	0.007

[1]Values are means for 12 replicate groups of 6 hens each.

[2]Means for the control corn and 59122 corns were not different ($P > 0.05$) for any of the phases or the overall period. Phase 1 = 24 to 28 wk; phase 2 = 28 to 32 wk; phase 3 = 32 to 36 wk.

[3]Least squares means included for reference purposes only. The true comparison of interest is 59122 vs. the control treatment.

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

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

Table 4. Component weights and Haugh unit measures of eggs produced from hens fed diets containing control or 59122 corn[1]

Quality trait[2]	Control	59122	3394[3]	SEM
Albumen weight (g)	33.0	32.9	32.8	0.3
Yolk weight (g)	15.1	15.1	15.3	0.1
Wet shell weight (g)	7.5	7.5	7.5	0.1
Haugh units	95.0	95.0	94.6	0.6

[1]Values are means for 12 replicate groups of 6 hens each.

[2]Means for the control corn and 59122 corns were not different ($P > 0.05$) for any of the phases or the overall period.

[3]Least squares means included for reference purposes only. The true comparison of interest is 59122 vs. the control treatment.

> 0.05). False discovery rate, as described by Benjamini and Hochberg (1995), was applied across all measurements to control for multiplicity. Data from the commercial corn hybrid treatment (3394) were used in the estimation of experimental variability; least squares mean values were generated for 3394, but comparisons between 59122 and 3394 were generated only in the event that there were significant differences between the 59122 transgenic and control treatment groups after the false discovery rate was applied. Corn and diet nutrient concentrations for the entire feeding period were summarized by using PROC MEANS of SAS (SAS Institute, 1990).

RESULTS AND DISCUSSION

Body weight gain and feed intake were not affected ($P > 0.05$) by dietary treatment (Table 3). Egg production was similar between hens fed the 59122 transgenic corn diet and those fed the near-isogenic control diet (Table 3). Egg weight, egg mass, and feed efficiency were also not different ($P > 0.05$) between hens fed diets containing the control or 59122 transgenic corn. Phase changes ($P < 0.05$) were not unexpected and reflect typical hen performance and production (data not shown).

Egg component (albumen, yolk, and wet shell) weights were not different ($P > 0.05$) between hens fed the control or 59122 diet (Table 4). Haugh unit values did not differ ($P > 0.05$) between hens fed the control or 59122 transgenic corn diet, and values fell within the Grade AA category. Weight class distributions (extra large, large, medium, small, and peewee) were not different ($P > 0.05$) between hens fed the control or 59122 transgenic corn diet (data not shown).

The results found in this study strongly suggest that there were no performance differences between laying hens fed diets made with transgenic corn when compared with laying hens fed diets made with nontransgenic corn. The *Bt* corn was genetically modified for protection from feeding damage by devastating insect pests and to provide tolerance to an herbicide. No modifications were made to the nutritional composition of the corn (Herman et al., 2007); thus, the nutritional value of DAS-59122-7 corn should be equal to the original line. Several previous studies conducted with broiler chickens (Brake and Vlachos, 1998; Sidhu et al., 2000; Brake et al., 2003, 2005; Taylor et al., 2003a,b,c, 2005) and with laying hens (Aulrich et al., 1998; Aeschbacher et al., 2005; Halle et al., 2006) have shown that growth and laying hen performance has not been influenced by crops genetically modified to confer pesticide resistance or herbicide tolerance. The results of this study are in agreement with these previous studies. The benefits of insect-protected corn, such as increased yield, better plant health, and decreased reliance on chemical pesticides, are all of great importance to the corn grower, and all contribute to economic and environmental benefits.

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