Evaluation of Nutritional Equivalency of Corn Grain from DAS-Ø15Ø7-1 (Herculex* I) in the Diets of Laying Hens[1]

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Primary Audience: Researchers, Nutritionists

SUMMARY

Grain from transgenic corn line TC1507 (Herculex* I) from Pioneer Hi-Bred International Inc. and Dow AgroSciences LLC, which expresses the Cry1F protein from *Bacillus thuringiensis* to provide protection from lepidopteran pests of corn, was compared with its isoline equivalent and 2 conventional corn strains in a 16-wk laying hen feeding trial. Egg production and production efficiency of hens fed the diet formulated with transgenic grain TC1507 were similar to those of hens fed diets formulated with isoline or nontransgenic conventional corns. Hens fed TC1507 had similar egg qualities as those fed nontransgenic grain diets. Diet × phase interactions were noted for Haugh unit and Roche color fan score. Hens fed conventional corn 2 had a poorer Haugh unit score compared with hens fed the other 3 diets. Conventional corn 1 had greater levels of xanthophylls compared with the other corn treatments, resulting in increased Roche color fan score for eggs produced by hens fed this diet. Overall, hens fed the transgenic Herculex* I corn grain containing the Cry1F protein performed as well as hens fed the isoline equivalent of Herculex* I and hens fed the conventional corn grains.

Key words: Herculex* I transgenic corn, laying hen, Cry1F protein, corn

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DESCRIPTION OF PROBLEM

A transgenic corn line containing the *cry*1F gene (event DAS-Ø15Ø7–1, TC1507) from *Bacillus thuringiensis* var. *aizawai* and the phosphinothricin acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes* has been developed through collaboration between Pioneer Hi-Bred International Inc [1]. and Dow AgroSciences LLC [2]. The *cry*1F gene encodes the Cry1F protein, which has demonstrated insecticidal activity toward the European corn borer, southwestern corn borer, fall armyworm, black cutworm, and western bean cutworm [3–5]. The commercial product trait name is Herculex* I [1].

There is a limited number of papers published testing the nutritional value and equivalency of new corn varieties versus their near isogenic and

[1]Herculex* I Insect Protection by Dow AgroSciences and Pioneer Hi-Bred. Herculex is a registered trademark of Dow AgroSciences LLC.

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conventional corn counterparts in laying hens [6, 7]. Most of the studies reported have been conducted with broiler chickens [8–12]. McNaughton and Zeph [8] were the first to report on the nutritional evaluation of TC1507 corn expressing the Cry1F protein in broiler diets. Their study compared the Cry1F corn to 4 commercial corn lines from the eastern United States in broiler chickens from 1 to 42 d of age. They reported mean body weight and feed conversion to be statistically similar among treatment groups and concluded that Cry1F corn was nutritionally equivalent to commercial corn grains when fed to broiler chickens. Several research reports [9–12] have been written regarding the comparison of commercial corn with YieldGard MON 810 corn containing the Cry1A(b) protein, which is similar to the Cry1F protein in Herculex* I corn. These studies, in which the MON 810 event was fed alone or in stacked combinations with herbicide-tolerant and insect-resistant events (GA21, NK603, MON 863, and MON 88017), concluded that broilers performed equally on the test corn compared with the commercial varieties for growth, feed conversion, and carcass yields and characteristics. The authors stated that the genetically modified corn containing the insect-protected and herbicide-tolerant traits were as wholesome in broiler diets as their respective nontransgenic control corn grains.

Because research conducted to date on corn containing *cry*1F or *cry*1A genes has been conducted mostly in broiler chickens, it is appropriate for this trial to test the nutritional equivalency of corn containing event TC1507 and expressing the Cry1F protein in laying hen diets.

MATERIALS AND METHODS

Trial Design, Diet Preparation, and Housing Conditions

All procedures used in this experiment were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Healthy pullets (Bovans White [13]) were obtained from a pullet supplier at 16 wk of age and fed a common diet to 21 wk of age. This diet was formulated to meet the nutritional needs of developing pullets [14]. Birds were randomly placed in cages (7 hens/cage at 68 in.^{2}/hen, in accordance with University of Nebraska's poultry nutrition best management practices) at 21 wk of age. A randomized complete block design was used. The number of pens per treatment was selected to adequately detect, at $P < 0.05$, a 5% difference from the mean using a type I error rate of 0.05 and a type II error rate of 0.20. Cages (12 cages per treatment, 336 hens total) were randomly assigned to 4 dietary treatments. Mash diets were formulated according to NRC guidelines [14] with the energy requirement (2,880 kcal/kg of ME) being met by the inclusion of corn and limited amounts of added tallow; diets were formulated to be isonitrogenous and isocaloric. The 4 dietary treatments were as follows: a near-isoline control (same genetic background excluding the TC1507 event), Herculex* I—a transgenic corn containing event TC1507 and expressing the Cry1F protein (TC1507), conventional corn 1, and conventional corn 2. The isoline control treatment was included to evaluate the effects of the gene addition, whereas inclusion of the conventional corn sources allowed an additional comparison between hens fed the transgenic TC1507 corn grain and those fed commercially available nontransgenic corn sources. Cry1F (TC1507) corn was approved by the Food and Drug Administration for food and feed use in the United States (May 18, 2001) before the start of the laying hen trial on June 4, 2002. All corn sources were grown and harvested by Pioneer Hi-Bred International Inc. [1]. The control corn plot was grown under isolation distances (201-m border) from the TC1507 corn plot to reduce the possibility of cross-pollination. Nutrient analysis for corn varieties and soybean meal protein, amino acids, fat, fiber, and gross energy were conducted on samples submitted by Pioneer Hi-Bred International to Eurofins Laboratories [15]; gross energy analysis was performed by Pioneer Hi-Bred International [1]. Results of the analysis were available before feed ration formulation and are given in Table 1. Table 2 shows the calculated and analyzed feed nutrient values for each experimental treatment. Nutrient analysis for dietary protein, calcium, and amino acids was performed on each diet also at Eurofins Laboratories [15]. Diets were fed for a 16-wk period divided into four 4-wk periods called phases.

Big Dutchman [16] cages were 20 × 24 in. in a stacked tier cage system with manure belts.

Table 1. Analyzed nutrient compositions of test corns and soybean meal used in diets[1]

Nutrient	Corn 1 (isoline)	Corn 2 (TC1507)	Corn 3 (conventional corn 1)	Corn 4 (conventional corn 2)	Soybean meal
Dry matter, %	83.92	86.89	86.04	86.47	88.68
Protein, %	7.80	8.39	8.26	7.98	47.27
Fat, %	3.1	3.1	2.8	2.8	0.52
Fiber, %	1.4	1.4	1.4	2.1	3.0
Gross energy, cal/g	3,868	3,894	3,852	3,889	4,180
Ash, %	1.06	1.02	1.15	1.28	5.35
Calcium, %	0.005	0.008	0.007	0.003	0.35
Phosphorus, %	0.22	0.22	0.23	0.26	0.65
Alanine, %	0.52	0.55	0.52	0.52	1.88
Arginine, %	0.25	0.28	0.30	0.34	3.12
Aspartic acid, %	0.28	0.34	0.31	0.57	5.34
Cystine, %	0.16	0.17	0.18	0.15	0.68
Glutamic acid, %	1.15	1.25	1.21	1.34	8.35
Glycine, %	0.25	0.26	0.26	0.30	1.89
Histidine, %	0.19	0.20	0.20	0.22	3.18
Isoleucine, %	0.22	0.23	0.23	0.24	1.96
Leucine, %	0.81	0.87	0.83	0.84	3.27
Lysine, %	0.22	0.23	0.24	0.24	2.68
Methionine, %	0.17	0.15	0.16	0.14	0.64
Phenylalanine, %	0.31	0.33	0.33	0.34	2.18
Proline, %	0.57	0.60	0.60	0.61	2.22
Serine, %	0.26	0.29	0.27	0.36	2.32
Threonine, %	0.18	0.20	0.18	0.28	1.81
Tryptophan, %	0.05	0.06	0.06	0.07	0.66
Tyrosine, %	0.08	0.12	0.12	0.11	1.46
Valine, %	0.31	0.33	0.33	0.33	2.04
Xanthophylls, µg/kg	10.8	11.0	15.2	9.5	0

[1]All values on an as-fed basis.

Housing conditions simulated those of typical commercial conditions with 4 cfm/hen ventilation but no supplemental cooling. Lighting was provided in 16-h photoperiods (16L:8D) with artificial light at a minimum of 0.5 foot candles. Cages were blocked by tier levels with dietary treatments randomized equally within blocks.

Performance and Egg Quality Measures

Live hen weights were taken at the start of the study and every 4 wk during the trial. Hens were fed ad libitum, and weekly feed intakes (g/hen per day) were calculated as follows: each week on the same day, excess feed in the feeders was weighed back to measure unconsumed feed, and that amount was subtracted from the amount added to the feeders.

Egg production (number and percentage per hen) was measured daily. Egg weight was measured on 2-d total egg production every 4 wk. Albumen, yolk and shell weights, Haugh units, and Roche color scores were recorded on 4 eggs/pen every 4 wk according to procedures as reported by Novak [17]. In the event of hen mortality, an evaluation was performed at the University of Nebraska Veterinary Diagnostic Laboratory to determine if mortality was treatment-related.

Statistical Analysis

Performance (body weight and weight gain, feed intake, egg production, and feed efficiency) and egg quality (egg weights, Haugh units, Roche color fan score, albumen, yolk, yolk solids, and wet shell) data were summarized and averaged for the entire experiment. Data were analyzed using the MIXED procedure of SAS [18]. Planned comparisons between fixed effects were by Fisher's protected LSD ($P < 0.05$). The model included diet, phase, and diet × phase as fixed effects; phase reflected laying hen age effects in the data. Block and time were used in the random statement when weekly measures were

Table 2. Ingredient and calculated and analyzed nutrient compositions of test diets

Diet	Corn 1 (isoline)	Corn 2 (TC1507)	Corn 3 (conventional corn 1)	Corn 4 (conventional corn 2)
Ground corn	59.8	60.4	60.5	60.0
Soybean meal	24.4	23.8	23.7	24.1
Limestone	9.2	9.3	9.3	9.3
Dicalcium phosphorus	1.93	1.94	1.94	1.93
Tallow	3.8	3.74	3.7	3.8
Salt	0.40	0.40	0.40	0.40
Methionine	0.23	0.24	0.23	0.25
Lysine	0.08	0.09	0.09	0.08
Trace mineral premix	0.075	0.075	0.075	0.075
Vitamin premix	0.075	0.075	0.075	0.075
Calculated nutrient content				
ME, kcal/kg	2,880	2,880	2,880	2,880
Protein, %	16.5	16.5	16.5	16.5
Lysine, %	0.85	0.85	0.85	0.85
Total sulfur amino acids, %	0.74	0.74	0.74	0.74
Calcium, %	4.00	4.00	4.00	4.00
Available phosphorus, %	0.45	0.45	0.45	0.45
Analyzed nutrient content[1]				
Dry matter, %	86.95	87.10	86.97	87.03
Protein, %	17.03	16.66	16.55	16.28
Calcium, %	4.38	4.27	4.21	4.24
Arginine, %	0.96	0.93	0.94	0.95
Cystine, %	0.25	0.27	0.25	0.25
Lysine, %	0.88	0.86	0.87	0.86
Methionine, %	0.44	0.44	0.44	0.44
TSAA, %	0.69	0.71	0.69	0.69
Threonine, %	0.67	0.64	0.65	0.65
Tryptophan, %	0.22	0.20	0.22	0.21
Tyrosine, %	0.45	0.44	0.44	0.43

[1]Values are the average of 3 samples.

taken; when measures were taken during the last week of each phase, only block was used in the random statement. For all data, the pen was considered to be the experimental unit.

RESULTS AND DISCUSSION

Hen Performance

Three mortalities occurred during the experimental period. Yolks or soft shells were present in the abdominal cavities of 2 of the 3 hens, contributing to the primary cause of death (egg yolk peritonitis). Deaths were not diet-related. Dietary corn treatment significantly affected ($P < 0.05$) feed intake, egg production, and production efficiency during this trial (Table 3). Waste and spillage were minimal, because hens were fed daily. Overall, feed intake was low but was acceptable for a summertime trial during which ambient temperatures often rose above 32°C. Hens fed the conventional corn 1 diet ate more than hens fed diets containing transgenic corn TC1507 or conventional corn 2. Feed intake was not different between hens fed the transgenic corn TC1507 diet and those fed the diet formulated with its near-isoline control grain. Egg production averaged near 90% for the 16-wk trial, which is close to industry standards for this strain (Bovan White [13]) of Single Comb White Leghorn hens. Production was greater for hens fed diets formulated with transgenic corn TC1507 or conventional corn 2 as compared with isoline corn. The lower feed intake observed with TC1507 and conventional corn 2 dietary treatments, coupled with their greater egg production, resulted in greater production efficiencies for those 2 groups. Weight gain was also positive during this study. Hens gained similarly among all 4 treatment groups, with weight gain increasing ($P < 0.0001$) with each succes-

Table 3. Dietary corn treatment effects on performance variables: Feed intake, egg production, and hen weight gain

Item	Corn type	Feed intake (g/hen/d)	Egg production (hen day,%)	Production efficiency (g of egg/g of feed)	Hen weight gain[1] (g)
Diet					
1	Isoline	96.2[ab]	89.75[b]	0.512[b]	41.3
2	TC1507	95.2[bc]	91.64[a]	0.528[a]	37.2
3	Conventional corn 1	96.6[a]	90.50[ab]	0.514[b]	43.6
4	Conventional corn 2	94.6[c]	91.25[a]	0.528[a]	38.3
SEM		2.1	1.41	0.014	4.4
P-values					
Diet		0.0082	0.0237	0.0003	NS
Phase		NS	NS	NS	<0.0001
Diet × phase		NS	NS	NS	NS

[a–c]Data means with different superscripts are significantly different.

[1]Average weight gain per phase.

sive phase (8.8, 20.6, 52.6, and 78.4 g, respectively), as was expected. Overall, hens fed the diet formulated with transgenic grain TC1507 performed as well as hens fed diets formulated with isoline or nontransgenic conventional corns. Consistency in poultry performance and production parameters between transgenic grains and conventional control and commercial grains is in agreement with much of the previously published laying hen [7] and broiler data [8–12].

Egg Quality Measures

Egg weight was similar across all 4 dietary corn treatment groups (Table 4); weights were lowest in phase 1 (52.8 g) and peaked in phase 3 (56.6 g). The majority (>63%) of eggs produced in this trial fell into the grade A category, and the distribution in this category was similar across treatments (data not shown). Overall diet and phase effects for both Haugh units and Roche color fan score were confounded by significant ($P < 0.0001$) diet × phase interactions; however, when evaluating the gene effect, the diet × phase interactions between the isoline and TC1507 dietary treatments were not significant (Haugh units, $P = 0.97$; Roche color fan score, $P = 0.14$). Haugh units indicate albumen quality by correlating albumen height, egg weight, and internal egg temperature. Generally, egg Haugh units were lower for hens fed the conventional corn 2 dietary treatment relative to the other 3 dietary corn treatments. Haugh units also decreased over time (from 80.5 to 60.9), as would be expected given the decrease in albumen quality that is observed with increased hen age [19]. Roche color fan score (1 = pale yellow to 15 = reddish-orange) was generally greater for hens fed the diet formulated with conventional corn 1. The scores for the other 3 dietary treatments

Table 4. Dietary corn treatment effects on egg variables: Egg weight, egg Haugh units, and Roche color fan score

Item	Corn type	Egg weight (g)	Egg Haugh units	Roche color fan score
Diet				
1	Isoline	54.2	75.1	8.39
2	TC1507	54.2	74.8	7.92
3	Conventional corn 1	54.3	75.3	10.20
4	Conventional corn 2	54.3	69.8	8.26
SEM		0.3	0.9	0.12
P-values				
Diet		NS	<0.0001	<0.0001
Phase		<0.0001	<0.0001	0.0538
Diet × phase		NS	0.0024	<0.0001

Table 5. Dietary corn treatment effects on albumen, yolk, and shell measurements

Item	Corn type	Albumen	Yolk	Yolk solids	Wet shell
Diet		%			
1	Isoline	56.74[b]	27.51	53.66	12.41
2	TC1507	57.75[ab]	27.76	53.52	12.45
3	Conventional corn 1	58.81[a]	28.23	54.17	12.60
4	Conventional corn 2	57.20[b]	27.66	54.55	12.56
SEM		0.51	0.25	0.36	0.11
P-values					
Diet		0.0101	NS	NS	NS
Phase		NS	<0.0001	<0.0001	0.0066
Diet × phase		NS	NS	NS	NS

[a,b]Data means with different superscripts are significantly different.

were more typical of a corn-soybean meal basal laying hen ration (7 to 8 on the average). A score of 10 indicates an intensive yellow, yellow-orange yolk, which would be beneficial to egg producers seeking heightened egg yolk color. The greater Roche color score observed with conventional corn 1 diet was directly related to the xanthophyll content of the corn source for that diet. This corn had approximately 1.4 to 1.6 times more xanthophyll content compared with isoline, TC1507, and conventional corn 2 sources (Table 1). Roche color score decreased slightly over the 4 phases, from 8.83 to 8.46, remaining in the average (7 to 8) range. The diet × phase interaction for Roche color score appeared to be driven by the conventional corn sources, particularly the conventional corn 2 diet treatment in which Roche color score increased over the 4 phases (7.73 to 8.81), in contrast to the other treatments in which values decreased over time (isoline, 8.67 to 7.73; TC1507, 8.69 to 7.31; and conventional corn 1, 10.24 to 9.98). It is unclear why the color scores increased for the conventional corn 2 dietary treatment, especially when this corn source contained the least amount of xanthophyll.

Dietary corn significantly ($P < 0.05$) affected percentage of egg albumen (Table 5). Hens fed diets containing isoline and conventional corn 2 had less albumen in the egg compared with hens fed conventional corn 1. The TC1507 (Herculex* I) transgenic corn treatment percentage of albumen did not differ from the other dietary corn treatments. The lower percentage of albumen for the conventional corn 2 diet correlates with a lower egg Haugh unit for the same diet. There was no obvious explanation for less albumen and poor Haugh units for this dietary corn treatment. Dietary protein was only slightly lower for the conventional corn 2 dietary treatment (Table 2) than for the other diets, and amino acid analysis showed an adequate and equal level of TSAA and lysine in that diet as compared with the other diets. Percentage of yolk, yolk solids, and wet shell were not significantly affected by dietary corn treatment. Yolk percentage increased over the phases (26.82 to 29.44%), as was expected. Yolk solids decreased in phases 2 and 4 (53.02 and 53.09% vs. 55.13 and 54.65%) in response to the high environmental temperatures that occurred during those phases. Wet shell percentage was also negatively affected by the hot temperatures in phase 2 (12.19% versus 12.54 to 12.64%). Similarity between transgenic and near-isoline albumen, yolk, and shell measures is consistent with previously published work [7].

CONCLUSIONS AND APPLICATIONS

1. Egg production parameters for hens fed the transgenic grain TC1507 (Herculex* I, *cry*1F gene) corn were comparable to the respective values for hens fed diets formulated with nontransgenic corns.
2. Egg quality measures were equivalent for the transgenic grain (TC1507, *cry*1F gene) and near-isoline control grain but differed among the conventional grains tested for egg albumen Haugh units and egg yolk color scores.

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