Evaluation of broiler performance and carcass yields when fed diets containing corn grain from transgenic stacked-trait product DAS-Ø15Ø7-1xDAS-59122-7xMON-ØØ81Ø-6xMON-ØØ6Ø3-6

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Primary Audience: Researchers, Nutritionists

SUMMARY

The nutritional equivalency of grain produced from transgenic stacked-trait corn DAS-Ø15Ø7-1xDAS-59122-7xMON-ØØ81Ø-6xMON-ØØ6Ø3-6 (1507x59122xMON810xNK603) with combined insect resistance and herbicide tolerance was evaluated in a 42-d feeding trial with broiler chickens. Broilers consuming diets produced with grain from unsprayed or sprayed 1507x59122xMON810xNK603 corn plants performed as well as broilers consuming diets produced with nontransgenic near-isogenic control grain, and the broilers produced organ, carcass, and parts yields similar to those of broilers fed diets produced with the control grain. Additionally, all performance, organ, and carcass measures from the control, 1507x59122x-MON810xNK603, and 1507x59122xMON810xNK603(S) groups were within the range of values from broilers fed diets containing nontransgenic commercially available hybrids. It was concluded, based on these results, that grain from 1507x59122xMON810xNK603 corn (unsprayed or sprayed with an herbicide mixture) was nutritionally equivalent to grain from nontransgenic near-isogenic corn.

Key words: broiler performance, carcass yield, herbicide tolerance, insect resistance, transgenic corn

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DESCRIPTION OF PROBLEM

The development of biotechnology-derived crops has provided growers with numerous benefits, including improved yields, decreased pest management costs and pesticide usage, and higher returns [1]. Genetically modified (**GM**) corn hybrids have been widely adopted in the United States since their commercial introduction in 1996. These GM hybrids with insect resistance, herbicide tolerance, or a combination of both traits, accounted for 85% of all US corn planted in 2009 [2] and 26% of global corn hectares [3]. The first stacked-trait corn products were commercialized in 2006 and have been adopted rapidly, with 75% of the GM corn hybrids planted in 2009 having double- or triple-stacked traits [3].

Transgenic stacked-trait corn (*Zea mays* L.) line DAS-Ø15Ø7-1xDAS-59122-7xMON-

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ØØ81Ø-6xMON-ØØ6Ø3-6 (1507x59122x-MON810xNK603) was produced by conventional breeding methods to provide a combination of in planta insect resistance and herbicide tolerance. The expressed Cry1F protein from event DAS-Ø15Ø7-1 confers resistance to such pests as the fall armyworm (*Spodoptera frugiperda*), corn earworm (*Helicoverpa zea*), Southwestern corn borer (*Diatraea grandiosella* [Dyar]), European corn borer (*Ostrinia nubilalis*), and Western bean cutworm (*Striacosta albicosta* [Smith]) [4–6]. The Cry34Ab1 and Cry35Ab1 proteins expressed from event DAS-59122-7 constitute an active insecticidal crystal protein that confers resistance to such coleopteran pests [7, 8] as Western corn rootworm (*Diabrotica virgifera virgifera*). Event MON-ØØ81Ø-6 expresses the Cry1Ab protein, which confers resistance to certain lepidopteran pests [9], including European corn borer (*O. nubilalis*). Herbicide tolerance is provided through the expressed phosphinothricin acetyltransferase (**PAT**) protein from events DAS-Ø15Ø7-1 and DAS-59122-7, which confers tolerance to the herbicidal active ingredient glufosinate-ammonium, and through the 5-enolpyruvylshikimate-3-phosphate synthase (**CP4 EPSPS**) protein expressed from event MON-ØØ6Ø3-6, which confers tolerance to herbicides containing glyphosate as the active ingredient.

In previous work with broilers fed diets formulated with corn grain from individual events DAS-Ø15Ø7-1 [10], MON-ØØ6Ø3-6 [11], MON-ØØ81Ø-6 [12], and DAS-59122-7 [13], it was demonstrated that there were no differences in nutritional performance and carcass variables between broilers consuming diets formulated with grains from those events and broilers fed diets containing nontransgenic grains. Compositional evaluation of stacked-trait products is recommended to determine whether there are any unintended effects on nutrient composition and nutrient digestibility caused by combining the individual events, and a poultry feeding trial may be used to determine nutritional equivalence [14]. The objective of this study was to evaluate the nutritional performance of broilers fed diets formulated with corn grain from stacked-trait product 1507x59122x-MON810xNK603 by comparing growth performance (as measured by BW and FE), organ yields, and carcass parts yields with those of broilers fed diets containing nontransgenic near-isogenic control corn grain.

MATERIALS AND METHODS

Corn Grain Characterization and Composition Analysis

All corn sources were grown by Pioneer Hi-Bred International Inc. [15]. Two plots of 1507x59122xMON810xNK603 test corn grain were produced, with 1 plot [1507x59122xMON810xNK603(S)] sprayed with 2 applications of glyphosate [16] plus glufosinate [17] and the other plot left unsprayed. Control corn grain was obtained from nontransgenic plants with a genetic background similar to 1507x59122xMON810xNK603 corn plants. Commercially available nontransgenic Pioneer brand hybrids 33H25, 33M15, and 33D11 were included as reference corn grain sources. The control and reference corn plants were produced in plots located 201 m from the 1507x59122xMON810xNK603 plots to avoid cross-pollination. Neither the control nor reference corn plants were sprayed with glyphosate or glufosinate herbicides. The nontransgenic near-isogenic control was included to evaluate the effect of the gene additions, whereas the reference corn grains were included to estimate the expected response range of broilers obtained from the same supplier and exposed to the same conditions as broilers fed the control, 1507x59122xMON810xNK603, or 1507x59122xMON810xNK603(S) diet.

Duplicate samples of each corn source were submitted for proximate, mineral (calcium and phosphorous), and mycotoxin analysis [18]; amino acid analysis [19]; and gross energy analysis [15]. The presence of individual events DAS-Ø15Ø7-1, DAS-59122-7, MON-ØØ81Ø-6, and MON-ØØ6Ø3-6 in the test corn grains and their absence from the control and reference corn grains was confirmed by using event-specific qualitative polymerase chain reaction analysis [20], and all corn grains were evaluated by enzyme-linked immunosorbent assay (**ELISA**) for transgenic protein expression levels [15].

Birds and Housing

All bird care, housing, and handling procedures were approved by the Internal Animal Care and Use Committee of Pioneer Hi-Bred, and conformed to bird care and use practices referenced in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* [21]. Healthy Ross 708 broiler chicks obtained on the day of hatch from a commercial Maryland hatchery and transported to the facility were assigned to 6 treatments in a randomized complete block design: control, 33H25, 33M15, 33D11, 1507x59122xMON810xNK603, and 1507x59122xMON810xNK603(S). There were 10 broilers per pen (5 males and 5 females) and 12 pens per treatment. Broilers were fed their respective dietary treatments from the day of hatching (trial d 0) to 42 d of age. Floor pens (0.914 × 1.219 m) with fresh shavings were separated by a wire partition and did not touch other pens from any side to minimize the potential for cross-contamination. Broilers were housed and managed under conditions similar to those of a commercial facility. Daily observations, housing environment management, and general broiler care were in accordance with standard facility procedures. Drinking water was provided for ad libitum consumption.

Diet Preparation

Mash-type diets were offered for ad libitum consumption in the starter (d 0 to 21), grower (d 22 to 35), and finisher (d 36 to 42) phases. Each phase diet was formulated to meet the nutrient requirements of a typical commercial broiler diet, using the NRC requirements [22] as a guideline within each phase. Corn grains were added to the indicated diets in equal amounts within each phase. Essential (protein, lysine, methionine, cystine, calcium, and phosphorus) nutrient requirements were met and equalized within each phase by adjusting the concentrations of noncorn ingredients. All diets were formulated to the same ME level (starter diets, 3,135 kcal of ME/kg; grower diets, 3,164 kcal of ME/kg; and finisher diets, 3,186 kcal of ME/kg); noncorn energy sources were allowed to float to equalize the ME level within each phase. No type of medication was administered during the entire feeding period. Diet samples were analyzed for proximates, minerals, amino acids, and gross energy [15, 18, 19]. All diets

Table 1. Analyzed nutrient profiles (as-is basis) of corn sources fed to broilers

Nutrient	Control	1507x59122x MON810xNK603	1507x59122x MON810xNK603(S)[1]	33H25	33M15	33D11
Proximate or mineral, %						
Moisture	14.3	15.3	14.2	14.7	15.2	13.9
Protein	6.9	6.6	6.7	6.1	7.6	7.3
Fat	3.4	3.4	3.4	3.0	3.0	3.1
Fiber	1.5	1.7	1.6	1.5	1.6	1.6
Ash	1.5	1.5	1.7	1.4	1.3	1.4
Calcium	0.01	0.01	0.01	0.01	0.01	0.01
Phosphorus	0.25	0.27	0.29	0.27	0.24	0.25
Gross energy, kcal/kg	3,848	3,783	3,835	3,787	3,785	3,838
ME,[2] kcal/kg	3,386	3,329	3,375	3,332	3,331	3,377
Essential amino acid, %						
Arginine	0.36	0.33	0.34	0.32	0.38	0.37
Lysine	0.25	0.24	0.24	0.24	0.27	0.25
Histidine	0.22	0.20	0.20	0.19	0.23	0.22
Isoleucine	0.26	0.25	0.24	0.22	0.30	0.26
Leucine	0.92	0.81	0.83	0.72	1.01	0.84
Methionine	0.17	0.16	0.17	0.16	0.16	0.17
Methionine + cystine	0.33	0.32	0.32	0.32	0.32	0.35
Phenylalanine	0.37	0.33	0.34	0.30	0.40	0.35
Threonine	0.26	0.23	0.24	0.22	0.27	0.24
Tryptophan	0.06	0.05	0.05	0.05	0.05	0.06
Valine	0.36	0.34	0.34	0.31	0.40	0.35

[1](S) indicates sprayed with 2 applications of glyphosate [16] plus glufosinate [17].

[2]ME values calculated for corn grain using conversion factors based on internal Pioneer Hi-Bred (Johnston, IA) data.

were also evaluated for transgenic protein expression and homogeneity by ELISA [15], using samples collected at the beginning, middle, and end of each diet production. The stability of the expressed transgenic proteins over the duration of each respective feeding phase was evaluated by using ELISA [15] on samples collected from the 1507x59122xMON810xNK603 and 1507x59122xMON810xNK603(S) diets at the beginning and end of each diet phase.

Performance and Carcass Measures

Body weights and feed weights (including amount of feed added and amount remaining) were determined every 7 d to calculate BW gain, feed intake, and mortality-corrected feed:gain ratio (FE) for d 0 through 42. All surviving birds were euthanized on study d 42, and carcass, organ, and carcass parts yield data were collected from 4 males and 4 females per pen.

Table 2. Ingredient[1] and analyzed nutrient (as-is basis) compositions of starter diets fed to broilers

Item	Control	1507x59122x MON810xNK603	1507x59122x MON810xNK603(S)[2]	33H25	33M15	33D11
Ingredient, %						
Corn	63.000	63.000	63.000	63.000	63.000	63.000
Soybean meal	24.528	23.292	24.285	23.228	23.680	24.496
Soybean oil	0.812	1.139	0.803	0.814	1.668	1.111
Protein blend[3]	8.036	9.008	8.335	9.473	7.967	7.722
DL-Methionine	0.153	0.143	0.150	0.118	0.174	0.160
L-Lysine hydrochloride	0.046	0.066	0.052	0.050	0.066	0.059
Limestone	0.683	0.660	0.709	0.638	0.688	0.698
Dicalcium phosphate	1.666	1.617	1.590	1.604	1.678	1.675
Choline chloride	0.031	0.034	0.031	0.034	0.033	0.031
Sodium chloride	0.421	0.417	0.420	0.415	0.421	0.422
Vitamin-trace mineral premix[4]	0.625	0.625	0.625	0.625	0.625	0.625
Analyzed nutrient composition						
Proximate or mineral, %						
Moisture	13.2	13.5	12.3	13.2	13.8	13.2
Protein	23.1	22.1	21.8	22.9	22.4	22.0
Fat	3.7	3.7	3.7	3.4	4.0	3.6
Fiber	2.0	2.0	2.2	2.0	2.0	2.1
Ash	5.0	4.8	4.6	4.7	4.9	5.1
Calcium	0.87	0.90	0.95	0.89	0.86	0.90
Phosphorus	0.66	0.65	0.71	0.63	0.66	0.69
Gross energy, kcal/kg	3,946	3,933	3,971	3,939	3,960	3,936
Essential amino acid, %						
Arginine	1.44	1.44	1.42	1.58	1.40	1.42
Histidine	0.52	0.58	0.57	0.59	0.57	0.56
Isoleucine	0.98	0.96	0.96	1.07	0.95	0.92
Leucine	2.02	2.00	1.97	2.08	1.98	1.95
Lysine	1.10	1.16	1.13	1.20	1.14	1.13
Methionine	0.43	0.43	0.41	0.43	0.47	0.44
Methionine + cystine	0.97	0.93	0.91	1.04	0.97	0.98
Phenylalanine	1.11	1.10	1.09	1.18	1.08	1.08
Threonine	0.87	0.88	0.85	0.92	0.83	0.86
Tryptophan	0.26	0.26	0.25	0.25	0.24	0.24
Valine	1.27	1.28	1.27	1.42	1.24	1.19

[1]Diets were formulated to contain protein, 23.00%; lysine, 1.20%; and methionine + cystine, 1.02%.

[2](S) indicates sprayed with 2 applications of glyphosate [16] plus glufosinate [17].

[3]Protein blend manufactured by 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%. The ME value for this product was 4,471 kcal/kg.

[4]Vitamin-mineral premix supplied (minimum) the following 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 3. Ingredient[1] and analyzed nutrient (as-is basis) compositions of grower diets fed to broilers

Item	Control	1507x59122x MON810xNK603	1507x59122x MON810xNK603(S)[2]	33H25	33M15	33D11
Ingredient, %						
Corn	67.500	67.500	67.500	67.500	67.500	67.500
Soybean meal	21.123	19.804	20.864	19.736	20.218	21.088
Soybean oil	0.734	1.085	0.724	0.737	1.651	1.055
Protein blend[3]	7.210	8.248	7.530	8.747	7.134	6.874
DL-Methionine	0.118	0.107	0.115	0.081	0.140	0.125
L-Lysine hydrochloride	0.097	0.117	0.103	0.101	0.118	0.111
Limestone	0.673	0.648	0.701	0.625	0.678	0.689
Dicalcium phosphate	1.547	1.495	1.466	1.481	1.561	1.558
Sodium chloride	0.374	0.370	0.372	0.368	0.374	0.375
Vitamin-trace mineral premix[4]	0.625	0.625	0.625	0.625	0.625	0.625
Analyzed nutrient composition						
Proximate, %						
Moisture	13.3	13.7	12.3	13.4	13.9	13.1
Protein	21.1	20.9	21.2	20.7	20.4	20.8
Fat	3.6	3.9	3.8	3.4	4.0	3.5
Fiber	2.2	1.9	2.1	2.1	2.0	2.1
Ash	4.7	4.6	4.9	4.5	4.4	4.5
Calcium	0.88	0.94	0.82	0.84	0.82	0.78
Phosphorus	0.71	0.70	0.64	0.65	0.62	0.61
Gross energy, kcal/kg	3,925	3,933	3,975	3,912	3,928	3,933
Essential amino acid, %						
Arginine	1.34	1.34	1.44	1.38	1.34	1.46
Histidine	0.50	0.58	0.55	0.51	0.56	0.56
Isoleucine	0.92	0.88	0.97	0.93	0.93	0.91
Leucine	1.93	1.95	1.98	1.85	1.99	1.97
Lysine	1.05	1.15	1.19	1.06	1.12	1.18
Methionine	0.40	0.46	0.42	0.38	0.47	0.41
Methionine + cystine	0.90	0.95	0.97	0.94	0.96	0.89
Phenylalanine	1.04	1.06	1.10	1.04	1.07	1.08
Threonine	0.80	0.85	0.87	0.82	0.82	0.88
Tryptophan	0.20	0.22	0.22	0.23	0.26	0.22
Valine	1.20	1.19	1.29	1.25	1.22	1.20

[1]Diets were formulated to contain protein, 21.00%; lysine, 1.12%; and methionine + cystine, 0.92%.

[2](S) indicates sprayed with 2 applications of glyphosate [16] plus glufosinate [17].

[3]Protein blend manufactured by 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%. The ME value for this product was 4,471 kcal/kg.

[4]Vitamin-mineral premix supplied (minimum) the following 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.

Statistical Analysis

The 2 comparisons of interest in this study were the control vs. 1507x59122x-MON810xNK603 and the control vs. 1507x59122xMON810xNK603(S). Data were analyzed using a mixed-model ANOVA [23, 24]. The false discovery rate was applied across all response variables analyzed to control the false positive rate [25]. The false discovery rate-adjusted *P*-value was reviewed if a statistically significant difference ($P < 0.05$) was observed for a trait. Reference group data were used in the mixed model analysis to improve the estimation of experimental variability for each trait with means generated for each reference group, but comparisons between individual reference groups and the control, 1507x59122x-MON810xNK603, or 1507x59122xMON810 xNK603(S) group were not performed. Reference group data were primarily used to construct a tolerance interval containing 99% of the ob-

Table 4. Ingredient[1] and analyzed nutrient (as-is basis) compositions of finisher diets fed to broilers

Item	Control	1507x59122x MON810xNK603	1507x59122x MON810xNK603(S)[2]	33H25	33M15	33D11
Ingredient, %						
Corn	74.000	74.000	74.000	74.000	74.000	74.000
Soybean meal	16.213	14.766	15.928	14.692	15.220	16.174
Soybean oil	0.553	0.938	0.542	0.557	1.559	0.905
Protein blend[3]	5.684	6.822	6.035	7.369	5.601	5.316
DL-Methionine	0.156	0.144	0.153	0.115	0.181	0.164
L-Lysine hydrochloride	0.278	0.301	0.285	0.283	0.302	0.294
Limestone	0.702	0.675	0.733	0.650	0.707	0.719
Dicalcium phosphate	1.460	1.403	1.371	1.388	1.475	1.472
Sodium chloride	0.329	0.325	0.328	0.323	0.330	0.331
Vitamin-trace mineral premix[4]	0.625	0.625	0.625	0.625	0.625	0.625
Analyzed nutrient and amino acid composition						
Proximate, %						
Moisture	13.4	13.7	12.5	13.6	14.1	13.3
Protein	18.5	18.1	18.5	18.7	18.4	18.5
Fat	4.0	4.1	3.8	3.3	4.2	3.7
Fiber	1.9	2.0	2.5	2.0	1.9	2.2
Ash	4.2	4.4	4.7	4.4	4.4	4.4
Calcium	0.77	0.81	0.85	0.70	0.73	0.77
Phosphorus	0.60	0.62	0.71	0.57	0.57	0.57
Gross energy, kcal/kg	3,892	3,917	3,932	3,895	3,892	3,902
Essential amino acid, %						
Arginine	1.17	1.07	1.18	1.13	1.14	1.12
Histidine	0.45	0.46	0.48	0.46	0.46	0.45
Isoleucine	0.80	0.72	0.80	0.75	0.75	0.75
Leucine	1.76	1.59	1.69	1.62	1.74	1.65
Lysine	1.07	1.05	1.13	1.02	1.10	1.07
Methionine	0.41	0.43	0.43	0.35	0.48	0.41
Methionine + cystine	0.84	0.87	0.87	0.80	0.90	0.83
Phenylalanine	0.93	0.84	0.91	0.87	0.91	0.88
Threonine	0.72	0.66	0.74	0.69	0.71	0.68
Tryptophan	0.20	0.19	0.22	0.18	0.20	0.20
Valine	1.07	0.97	1.01	1.01	0.99	1.00

[1]Diets were formulated to contain protein, 18.00%; lysine, 1.08%; and methionine + cystine, 0.85%.

[2](S) indicates sprayed with 2 applications of glyphosate [16] plus glufosinate [17].

[3]Protein blend manufactured by 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%. The ME value for this product was 4,471 kcal/kg.

[4]Vitamin-mineral premix supplied (minimum) the following 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.

served performance (excluding mortality), organ, and carcass trait values at a 95% confidence level [26]. These commercial hybrid-based tolerance intervals were a supplement to the statistical comparisons, with their purpose being to estimate the expected response range of broilers obtained from the same supplier and exposed to the same conditions as broilers fed the near-isoline control or test corn diet. Data from the control, 1507x59122xMON810xNK603, and 1507x59122xMON810xNK603(S) groups were evaluated to determine whether the observed values were contained within the tolerance interval, and those observed values within the tolerance interval were considered similar to feeding nontransgenic corn grain. Organ and carcass

Table 5. Growth performance,[1] prechill organ yields,[2] and postchill carcass and parts yields[3] of broilers fed diets containing nontransgenic control corn grain or diets containing 1507x59122xMON810xNK603 or 1507x59122xMON810xNK603(S)[4] corn grain

					Control vs. 1507x59122x MON810xNK603		Control vs. 1507x59122x MON810xNK603(S)			Reference corn[6]		
Item	Control	1507x59122x MON810x NK603	1507x59122x MON810x NK603(S)	SEM	FDR *P*-value[7]	Raw *P*-value[8]	FDR *P*-value	Raw *P*-value	Tolerance interval[5]	33H25	33M15	33D11
Growth performance												
Initial BW (d 0), g	47.1	46.9	46.9	0.2	1.00	0.56	0.90	0.64	44.8 to 49.5	47.4	47.3	46.9
Final BW (d 42), g	1,729.9	1,721.1	1,744.4	19.4	1.00	0.75	0.90	0.60	1,510.6 to 1,979.3	1,748.4	1,763.9	1,722.6
Mortality, %	2.50	1.67	1.67	1.37	1.00	0.64	0.90	0.64		0.83	0.00	1.67
FE[9] (0 to 42 d), g/g	1.871	1.875	1.873	0.015	1.00	0.88	0.99	0.94	1.693 to 2.049	1.869	1.868	1.877
Prechill organ yield												
Kidney, %												
Overall	2.10	2.09	2.13	0.05	1.00	0.92	0.90	0.62		2.05	2.11	2.10
Males	2.12	2.12	2.16	0.07	1.00	0.98	0.90	0.68	0.73 to 3.44	2.05	2.13	2.07
Females	2.07	2.06	2.10	0.07	1.00	0.91	0.92	0.77	0.75 to 3.42	2.05	2.08	2.14
Liver, %												
Overall	3.59	3.58	3.56	0.05	1.00	0.97	0.91	0.72		3.54	3.49	3.49
Males	3.59	3.55	3.64	0.07	1.00	0.72	0.90	0.65	2.02 to 5.02	3.54	3.48	3.53
Females	3.59	3.62	3.49	0.07	1.00	0.77	0.90	0.35	1.92 to 5.07	3.54	3.50	3.44
Postchill carcass and parts yield												
Carcass, %												
Overall	70.78	71.36	71.13	0.39	1.00	0.30	0.90	0.52		71.07	71.07	71.39
Males	70.87	71.00	71.42	0.50	1.00	0.86	0.90	0.44	61.86 to 79.80	70.96	70.42	71.11
Females	70.69	71.72	70.84	0.50	1.00	0.15	0.93	0.83	61.75 to 81.30	71.18	71.72	71.67
Breast, %												
Overall	26.82	26.58	26.43	0.22	1.00	0.45	0.90	0.22		26.65	26.57	26.43
Males	26.65	26.81	26.54	0.30	1.00	0.71	0.92	0.80	20.75 to 32.50	26.73	26.66	26.49
Females	26.99	26.36	26.32	0.30	1.00	0.14	0.90	0.11	19.99 to 32.97	26.57	26.48	26.38
Thigh, %												
Overall	15.89	15.99	15.77	0.15	1.00	0.66	0.90	0.55		15.87	15.99	15.68
Males	15.82	15.99	15.69	0.21	1.00	0.54	0.90	0.66	11.72 to 19.93	15.72	16.04	15.71
Females	15.97	15.98	15.85	0.21	1.00	0.97	0.90	0.67	11.63 to 20.11	16.02	15.95	15.64
Leg, %												
Overall	14.47	14.34	14.25	0.12	1.00	0.45	0.90	0.22		14.49	14.25	14.06
Males	14.57	14.57	14.37	0.18	1.00	1.00	0.90	0.42	10.69 to 18.05	14.70	14.31	14.10
Females	14.37	14.10	14.14	0.18	1.00	0.29	0.90	0.35	10.50 to 17.82	14.28	14.18	14.02

Continued

Table 5 (Continued). Growth performance,[1] prechill organ yields,[2] and postchill carcass and parts yields[3] of broilers fed diets containing nontransgenic control corn grain or diets containing 1507x59122xMON810xNK603 or 1507x59122xMON810xNK603(S)[4] corn grain

Item	Control	1507x59122x MON810x NK603	1507x59122x MON810x NK603(S)	SEM	Control vs. 1507x59122x MON810xNK603 FDR *P*-value[7]	Control vs. 1507x59122x MON810xNK603 Raw *P*-value[8]	Control vs. 1507x59122x MON810xNK603(S) FDR *P*-value	Control vs. 1507x59122x MON810xNK603(S) Raw *P*-value	Tolerance interval[5]	Reference corn[6] 33H25	Reference corn[6] 33M15	Reference corn[6] 33D11
Wing, %												
Overall	10.54	10.56	10.54	0.10	1.00	0.88	0.99	0.99		10.57	10.47	10.42
Males	10.55	10.68	10.47	0.12	1.00	0.45	0.90	0.61	8.48 to 12.68	10.59	10.70	10.45
Females	10.53	10.44	10.62	0.12	1.00	0.61	0.90	0.61	7.90 to 12.89	10.55	10.25	10.39
Abdominal fat, %												
Overall	1.51	1.48	1.53	0.03	1.00	0.44	0.90	0.67		1.49	1.45	1.50
Males	1.49	1.48	1.53	0.05	1.00	0.88	0.90	0.62	0.55 to 2.45	1.51	1.45	1.54
Females	1.53	1.47	1.54	0.05	1.00	0.40	0.99	0.96	0.43 to 2.49	1.47	1.44	1.46

[1]Individual treatment growth performance means represent 12 pens/treatment group, with 10 birds/pen.

[2]Prechill organ yields calculated as percentage of live bird BW. Individual treatment least squares means represent 12 pens/treatment group, with 8 birds/pen.

[3]Carcass yield calculated as percentage of live bird BW; parts yield calculated as percentage of postchill carcass weight. Individual treatment least squares means represent 12 pens/treatment group, with 8 birds/pen.

[4](S) indicates sprayed with 2 applications of glyphosate [16] plus glufosinate [17].

[5]Lower and upper limits of a 95% tolerance interval on 99% of the observed performance, organ yield, and postchill carcass and parts yield trait values from birds fed 33H25, 33M15, and 33D11 reference corn diets.

[6]Commercial corn least squares means included for reference purposes only. The comparisons of interest were 1) control vs. 1507x59122xMON810xNK603 and 2) control vs. 1507x59122xMON810xNK603(S).

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

[8]Nonadjusted *P*-value.

[9]FE was calculated as grams of feed intake per gram of BW gain and was adjusted for mortality.

tolerance intervals were created by sex because of the expected yield differences between male and female broilers.

RESULTS AND DISCUSSION

Corn Grain Characterization and Composition

The presence of the individual DAS-Ø15Ø7-1, DAS-59122-7, MON-ØØ81Ø-6, and MON-ØØ6Ø3-6 events in the 1507x59122xMON810xNK603 and 1507x-59122xMON810xNK603(S) corn grains, and their absence from the control and reference corn grains, was confirmed with event-specific real-time polymerase chain reaction testing. Expression of Cry1F (3.9 and 3.0 ng/mg of grain, respectively), Cry34Ab1 (29 and 23 ng/mg of grain, respectively), Cry35Ab1 (0.62 and 0.84 ng/mg grain, respectively), PAT (0.10 and 0.09 ng/mg grain, respectively), Cry1Ab (0.23 and 0.29 ng/mg grain, respectively), and CP4 EPSPS (5.0 and 12 ng/mg grain, respectively) proteins in the 1507x59122xMON810xNK603 and 1507x59122xMON810xNK603(S) corn grains, and their absence from all other corn sources, was confirmed by ELISA analysis. Analyzed nutrient values of the control, 1507x59122xMON810xNK603, and 1507x59122xMON810xNK603(S) corn grains were similar in composition to each other and to the reference corn grains (Table 1). Additionally, nutrient values of all corn grains used in this trial fell within the ranges of conventionally bred precommercial and commercial corn produced from controlled field trials in the United States [27]. All grain sources were considered suitable for the production of commercial-type broiler diets because no differences in key nutrients that would have affected inclusion rates were observed between the corn grains. The presence of mycotoxins in the corn grains was limited to fumonisin B_1 (control, 1507x59122x-MON810xNK603, 33H25, and 33M15 grains) and deoxynivalenol (control and reference grains), which were found at very low (<1 mg/kg) concentrations. The limited presence of fumonisins and deoxynivalenol in the corn sources was not a concern because diet production resulted in further dilution of the concentrations to well below guidelines [28] for dietary total fumonisins (fumonisin B_1 + fumonisin B_2 + fumonisin B_3) or deoxynivalenol (50 and 5 mg/kg, respectively) when included up to the maximum corn inclusion level (finisher phase, 74%).

Diet Characterization and Composition

The absence of Cry1F, Cry34Ab1, Cry-35Ab1, PAT, Cry1Ab, and CP4 EPSPS proteins from the diets produced with control or reference grains and the presence of all expressed transgenic proteins in the diets produced with 1507x59122xMON810xNK603 and 1507x59122xMON810xNK603(S) grains were confirmed with ELISA analysis of diet samples collected for homogeneity evaluation (data not shown). The diets produced with each corn grain were blended homogeneously and the expressed proteins were stable for the duration of the respective feeding phases (data not shown). Starter, grower, and finisher diets produced using grain from individual corn sources were all similar in analyzed proximate, gross energy, and amino acid concentrations in each feeding phase (Tables 2, 3, and 4).

Performance and Carcass Response Variables

Nutritional equivalency studies have long been conducted using broiler chickens because they are a rapidly growing species whose diet typically contains a high concentration of corn grain, and because BW gain and mortality are sensitive indicators of changes in the nutritional quality of their diet [29]. Body weight, BW gain, mortality, and mortality-adjusted FE (Table 5) did not differ between broilers fed the control diet and broilers fed the 1507x59122xMON810x NK603 or 1507x59122xMON810xNK603(S) diets. Further, all growth performance values for broilers fed the control, 1507x59122xMON810x NK603, or 1507x59122xMON810xNK603(S) diet fell within the tolerance intervals calculated from broilers consuming the commercial reference diets (33H25, 33M15, and 33D11). No statistically significant differences were observed for organ, carcass, or individual parts yields between the control and 1507x59122xMON810xNK603 or 1507x59122xMON810xNK603(S) groups (Table 5). Additionally, all observed organ, carcass, and parts yield values fell within the calculated tolerance intervals. Therefore, organ, carcass, and parts yields of broilers fed the control, 1507x59122xMON810xNK603, or 1507x59122xMON810xNK603(S) diet were similar to those of broilers fed the nontransgenic diets prepared with commercial reference corn grains.

These results are consistent with previous broiler feeding trials conducted with grain from transgenic stacked-trait corn plants [11, 12, 30–33], which demonstrated similar growth performance and carcass yields between groups of broilers fed diets with transgenic grain or nontransgenic near-isogenic control grain. The results of the sprayed corn group 1507x59122xMON810xNK603(S) are also consistent with those of previous studies [34, 35], in which no difference was found in performance measures or carcass yields between broiler fed diets containing nontransgenic near-isogenic corn grain or transgenic corn grain from plants sprayed with glufosinate-ammonium herbicides. Dietary nutrient inadequacies [36–38] or the presence of antinutritional factors [39–41] may affect the liver and kidney yields of broilers. The organ yield results in this study are consistent with those of previous studies, in which no biologically significant differences in organ yields were observed between broilers fed diets prepared with transgenic corn grain [13, 35] or feed fractions [42, 43] and those fed diets with corn grain or feed fractions from nontransgenic controls.

CONCLUSIONS AND APPLICATIONS

1. Growth performance measures, and organ, carcass, and individual parts yields of broilers fed diets containing transgenic corn grain 1507x59122x-MON810xNK60 (from unsprayed or sprayed plants) were similar to the respective values for broilers fed diets formulated with nontransgenic corn grains.
2. The analyzed nutrient composition of transgenic corn grain 1507x59122x-MON810x NK603 (from unsprayed or sprayed plants) was similar to that of its nontransgenic near-isogenic control and to nontransgenic commercially available hybrids.
3. In this study, it was demonstrated that corn grain obtained from plants containing the stacked-trait product 1507x59122xMON810xNK603 with combined insect resistance and herbicide tolerance is nutritionally equivalent to grain obtained from nontransgenic plants.

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