METABOLISM AND NUTRITION

Comparison of broiler performance and carcass yields when fed transgenic maize grain containing event DP-Ø9814Ø-6 and processed fractions from transgenic soybeans containing event DP-356Ø43-5

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ABSTRACT The performance of broilers fed diets containing maize grain from event DP-Ø9814Ø-6 (98140; *gat*4621 and *zm-hra* genes) and processed fractions (meal, hulls, and oil) from soybeans containing event DP-356Ø43-5 (356043; *gat*4601 and *gm-hra* genes) was evaluated in a 42-d feeding study. Diets were produced with nontransgenic maize grain and soybean fractions from controls with comparable genetic backgrounds to 98140 and 356043 (control), 98140 maize and 356043 soybean (98140 + 356043), or 3 commercially available nontransgenic maize and soybean combinations. Ross 708 broilers (n = 120/group; 50% male, 50% female) were fed diets in 3 phases: starter (d 0 to 21), grower (d 22 to 35), and finisher (d 36 to 42). Starter diets contained (on average) 63% maize and 28% soybean meal, grower diets 66% maize and 26% soybean meal, and finisher diets 72% maize and 21% soybean meal; soybean hulls and oils were held constant at 1.0 and 0.5%, respectively, across all diets in all phases. Weight gain, feed intake, and mortality-adjusted feed efficiency were calculated for d 0 to 42. Standard organ and carcass yield data were collected on d 42. Data were analyzed using a mixed model ANOVA with differences between control and 98140 + 356043 group means considered significant at $P < 0.05$. Reference group data were used only to estimate experimental variability and to generate tolerance intervals. No significant differences were observed in weight gain, mortality, mortality-adjusted feed efficiency, organ yields, or carcass yields between broilers consuming diets produced with 98140 + 356043 and those consuming diets produced with control maize and soybean fractions. All values of response variables evaluated in the control and 98140 + 356043 groups fell within calculated tolerance intervals. Based on these results, it was concluded that the combination of genetically modified 98140 maize and 356043 soybean fractions was nutritionally equivalent to nontransgenic maize and soybean controls with comparable genetic backgrounds.

Key words: genetically modified maize, genetically modified soybean, broiler performance, carcass yield

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INTRODUCTION

The glyphosate acetyltransferase (*gat*) gene isolated from *Bacillus licheniformis* was functionally improved by a gene shuffling process to optimize the kinetics of the glyphosate acetyltransferase enzyme to acetylate glyphosate (Castle et al., 2004; Siehl et al., 2005). Maize (*Zea mays* L.) and soybean (*Glycine max*) plants were modified by integration of the *gat* and acetolactate synthase genes (*zm-hra* and *gm-hra* in maize and soybean, respectively) to produce events DP-Ø9814Ø-6 (**98140**) and DP-356Ø43-5 (**356043**), respectively. The *gat* gene is identified as *gat*4621 in maize plants and as *gat*4601 in soybean plants. The expressed proteins from *gat*4621 and *gat*4601 genes, GAT4621 and GAT4601, respectively, confer tolerance in planta to the herbicidal active ingredient glyphosate. Expression of the ZM-HRA and GM-HRA proteins, respectively encoded by the *zm-hra* and *gm-hra* genes, confers tolerance in planta to acetolactate synthase-inhibiting herbicides such as sulfonylurea and imidazolinone herbicides (Lee et al., 1988).

Broiler nutritional equivalency studies have focused on feeding a single genetically modified (**GM**) feedstuff (e.g., maize grain or processed soybean fractions) and evaluating growth performance and carcass yields against that of birds fed the corresponding non-GM feedstuff (Flachowsky et al., 2005). Although Deaville and Maddison (2005) fed a combination of GM maize (event MON810) and GM soybean meal (event GTS 40-3-2) to broilers to determine the fate of transgenic proteins, we are not aware of any published broiler

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nutritional equivalency studies to date that have used a combination of at least 2 sources of GM feedstuffs in a study. The objective of this study was to assess the nutritional performance of a combination of 98140 maize grain and processed fractions (meal, hulls, and oil) from 356043 soybean with that of a nontransgenic maize grain plus soybean fraction control by comparing the growth performance, organ yield, and carcass parts yields of growing broiler chickens fed diets containing either combination of maize grain and soybean fractions.

MATERIALS AND METHODS

Maize Grain Production and Characterization

Nontransgenic control maize with comparable genetic background to 98140 maize (control maize), commercially available nontransgenic Pioneer brand hybrids 33J56, 33P66, and 33R77, and 98140 maize were produced by Pioneer Hi-Bred (Johnston, IA) in a 2007 field production trial conducted near York, Nebraska. All maize grains from the production trial were used in this broiler trial and a concurrent laying hen study (McNaughton et al., 2010). Maize grain characterization and nutrient analysis methods were as described in McNaughton et al. (2010).

Soybean Production, Processing, and Characterization

Commercial nontransgenic Pioneer variety 93M14 soybeans and soybeans containing event 356043 were produced by Pioneer Hi-Bred in a field production trial conducted near York, Nebraska in 2007. Nontransgenic soybeans with a comparable genetic background to 356043 soybeans (control soybean) and commercially available nontransgenic Pioneer varieties 92M72 and 93B15 were produced in a concurrent field trial at the same location; these 3 soybean sources were also used with the above maize grains in a concurrent laying hen study (McNaughton et al., 2010). All soybean sources were processed into their respective meal, hull, and oil fractions under similar conditions at Texas A&M University (College Station, TX). Identity preservation procedures were followed throughout the processing and inventory systems to maintain the identity of the resulting processed fractions from each soybean source. Event-specific qualitative PCR analysis was performed on all meal and hull fractions to confirm the presence of event DP-356Ø43-5 in only 356043 fractions (DuPont Agricultural Biotechnology Regulatory Group, Wilmington, DE) and confirm that identity preservation was maintained through all harvest, shipping, inventory, and processing procedures. All meal and hull sources were evaluated for nutrient proximate composition, calcium, phosphorus, and mycotoxin content at Cumberland Valley Analytical Services (Hagerstown, MD). Dry matter (930.15), protein (990.03), fiber (978.10), ash (942.05), calcium and phosphorus (985.01), and mycotoxin (994.08, 995.15, and 986.17) analyses were performed according to AOAC (2000) methods; fat analysis was performed according to AOAC (1990) method 920.39. Amino acid analysis of meal and hull sources was conducted by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) in accordance with AOAC (2000) methods 988.15, 982.30, and 994.12. All fractions were analyzed for gross energy content with a bomb calorimeter (Parr Instruments Model 1271, Parr Instruments, Moline, IL) at Pioneer Hi-Bred. Carbohydrate values were calculated.

Birds and Housing

Pioneer Hi-Bred's Internal Animal Care and Use Committee approved all animal care, housing, and handling procedures used in this study. Animal care and use practices conformed to the guidelines of Federation of Animal Science Societies (1999). Commercial broilers (Ross 708) were obtained at hatch (trial d 0) from a commercial Maryland hatchery; birds were feather-sexed at the hatchery and then transported to AHPharma Inc. (farm 1; Tyaskin, MD) in June 2008. Sufficient numbers of broilers were obtained to ensure availability of 840 healthy chicks (50% male, 50% female) for the conduct of the study. Broilers were evaluated upon receipt for signs of disease or other complications that may have affected the outcome of the study; bird health observations and actual number of birds received were documented. Broilers were weighed following examination, identified with a wingband, and placed randomly in 0.914 m × 1.219 m (3 ft × 4 ft) floor pens at a density of approximately 0.305 m^2 (1.0 ft^2) of available floor space per broiler; litter was provided as new pine shavings with a minimal amount of saw dust. The potential for cross-contamination was minimized by separating pens with a wire partition and not allowing pens to contact on any side. Broilers were housed in a room containing forced-air heaters and individual pen heat lamps with a cross-house ventilation system. A continuous 24-h lighting program was followed. AHPharma farm personnel observed birds 3 times daily for overall health, behavior and evidence of toxicity, and environmental conditions. No type of medication was administered during the entire feeding period. Mortalities were recorded and complete necropsy examinations were performed on all broilers found dead or moribund. Carcasses of necropsied broilers were disposed of according to local regulations via composting. Drinking water was provided for ad libitum consumption.

Experimental Design

This study was designed as a randomized complete block with 5 dietary treatments: control maize grain + control soybean fractions (control), 98140 maize grain

+ 356043 soybean fractions, and reference maize grain + soybean fraction combinations 33J56 + 92M72 (reference 1), 33P66 + 93B15 (reference 2), and 33R77 + 93M14 (reference 3). Two additional treatment groups were included in this study (referred to below as diet A and diet B), but individual data for these 2 groups are not reported here. There were 10 broilers/pen (5 males, 5 females) and 12 pens (replicates)/treatment for a total of 120 broilers/treatment. All dietary treatments were fed to the broilers from the day of hatch (trial d 0) to 42 d of age.

Diets

A 3-phase feeding system was used in this study (starter, d 0 to 21; grower, d 22 to 35; and finisher, d 36 to 42), with all diets offered as a mash feed for ad libitum consumption. Each phase diet was formulated to meet the nutrient requirements of a typical commercial broiler diet using NRC (1994) as a guideline. Requirements for protein, lysine, methionine, cystine, calcium, and phosphorus were met by adjusting the concentrations of ingredients other than maize grain and soybean fractions. Diets were prepared at the Pioneer Livestock Nutrition Center Feed Mill (Polk City, IA). Maize grain sources were individually cleaned and milled (375 kg) to an average particle diameter of 650 to 750 μm using a Bliss Experimental hammer mill (Bliss Manufacturing, Ponca City, OK). Grain sources were milled in the order of control maize, 33J56, 33P66, 33R77, and 98140 to minimize the potential for cross-contamination. Individual maize and soybean meal sources were added to the indicated diets in as similar amounts across treatments within each source type and phase as the formulation program would allow; maize and soybean meal quantities were within approximately 2 and 3 percentage units, respectively, within each source type across treatments within each phase. Reference maize grain and soybean meal pairings were based upon the nutrient profiles of each source. Soybean hulls and oils were held constant at 1.0 and 0.5%, respectively, across all treatments in all phases. All diets were formulated within each phase to the same ME level: starter, 3,135 kcal of ME/kg; grower, 3,164 kcal of ME/kg; and finisher, 3,186 kcal of ME/kg. Phase diets for each maize grain + soybean fraction source were mixed in the following order to minimize the potential for cross-contamination: control, references, diet A, diet B, and 98140 + 356043. Mixing equipment was flushed with nontransgenic soybean hulls before diet preparation. All diets were prepared using a ribbon mixer (Sudenga M750, Sudenga Industries Inc., George, IA), and the mixer was cleaned between each phase diet using compressed air and vacuum. Mixing equipment was flushed with nontransgenic soybean hulls between each maize grain + soybean fraction combination. Whole maize grain kernels were observed in all prepared diets in quantities that were determined to potentially affect feed intake. Each diet was passed through a screen sized to retain only the whole grain kernels; the whole kernels were weighed and an equal amount of ground maize grain from the respective maize grain source was added back to each diet. Diets were remixed with equipment and clean-out procedures as described previously. Diets were subsampled following remixing and samples were composited for all nutrient analyses as described previously. All diets were evaluated by ELISA for expression of GAT4621 and ZM-HRA proteins using samples collected at the time of each diet preparation (beginning, middle, and end of diet production) to determine whether the diets were blended homogeneously and to confirm that the proteins were absent from control and reference diets. GAT4621 and ZM-HRA protein stability over the duration of each feeding phase was evaluated by ELISA on samples collected from 98140 + 356043 diets at the start and end of each diet phase. The presence of event DP-356Ø43-5 in 98140 + 365043 diets and its absence from all other diets was determined in the composite diet samples using event-specific qualitative PCR.

Measurements

Body weights and feed weights (including amount of feed added and amount remaining) were determined every 7 d. Body weight gain, feed intake, and mortality-corrected feed:gain ratio (feed efficiency) were calculated for d 0 through 42. All surviving birds were killed on study d 42 by cervical dislocation and subjected to a gross necropsy. Carcass and carcass parts yield data were collected from 4 male and 4 female broilers/pen (n = 96 broilers/treatment) and included carcass yield (postchilled), thighs, breasts, wings, legs, abdominal fat (including fat around gizzard), kidneys, and whole liver. Combined total mass was recorded for all parts considered as pairs (e.g., legs, thighs, both sides of the breast). Kidney and liver weights were expressed as percentages of whole live bird weight. Carcass yield was expressed as the percentage of whole live bird weight, and parts yields were expressed as the percentage of postchilled dressed carcass weight. Bird carcasses and remaining diets were disposed of by composting, conforming to local and state regulations.

Statistical Analysis

The mean value of data from control and 98140 + 356043 groups was calculated for each variable to test the hypothesis that growth performance, organ yield, and carcass yield would be different between broiler chickens fed diets containing 98140 maize grain + 356043 soybean fractions and those fed diets containing the nontransgenic control maize grain + control soybean fractions. Data were analyzed using a mixed model ANOVA (PROC MIXED of SAS, version 9.1, SAS Institute Inc., Cary, NC). Statistical analysis of live performance data was determined on a per pen basis and did not consider gender whereas analysis of carcass data was determined on a per animal basis and did consider gender. The model used for live performance data analysis was $Y_{ij} = U + T_i + B_j + e_{ij}$, where Y_{ij} = observed pen response, U = overall mean, T_i = treatment effect, B_j = random block effect, and e_{ij} = residual error. The model used for carcass data analysis was $Y_{ijkl} = U + T_i + B_j + G_k + (TG)_{ik} + e_{ijkl}$, where Y_{ijkl} = observed bird response, U = overall mean, T_i = treatment effect, B_j = random block effect, G_k = gender effect, $(TG)_{ik}$ = treatment by gender interaction, and e_{ijkl} = residual error. Estimate statements were used to generate comparisons for each measure with differences between means considered statistically significant at $P < 0.05$. False discovery rate as described by Benjamini and Hochberg (1995) was applied across all response variables analyzed to control the false positive rate. The false discovery rate-adjusted *P*-value was reviewed if statistically significant differences generated from the estimate comparison statement were observed for a measure.

Data generated from broilers fed reference diets were used in the estimation of experimental variability; least squares means were generated for each reference group, but comparisons between individual reference groups and control or 98140 + 356043 groups were not generated. Reference group data were used to construct a 95% tolerance interval containing 99% of the observed performance and carcass trait values from birds fed nontransgenic commercially available maize + soybean fraction diets, as described by Graybill (1976). These tolerance intervals were a supplement to the statistical comparisons and their purpose was to estimate the expected response range of broilers obtained from the same supplier and exposed to the same conditions as broilers fed control or 98140 + 350643 diets. Data from control and 98140 + 356043 groups were evaluated to determine whether the observed values were contained within the tolerance interval; observed response values within the tolerance interval were considered to be similar to the response of broilers fed nontransgenic commercially available maize grain + soybean fraction diets. Because of expected yield differences between male and female broilers, tolerance intervals for organ and carcass variables were created by gender.

RESULTS AND DISCUSSION

Maize Grain Characterization and Nutrient Composition

Event-specific real-time PCR testing confirmed the presence of the insert from event DP-Ø9814Ø-6 in 98140 maize grain and its absence from all other maize grains (data not shown). An ELISA analysis confirmed the expression of GAT4621 and ZM-HRA proteins (7.0 and 0.27 ng/mg of grain, respectively) from the event in 98140 maize grain and the absence of these proteins from all other maize grains [lower limit of quantitation (**LLOQ**) = 0.11 and 0.27 ng/mg of grain for GAT4621 and ZM-HRA, respectively]. Nutrient profiles were comparable between control, 98140, and reference maize grains (Table 1). The presence of mycotoxins in the maize grains was limited to fumonisins, with fumonisin B_1 detected (0.1 to 4.8 mg/kg) in all maize grains and fumonisins B_2 and B_3 detected (<2 and <1 mg/kg, respectively) in all maize grains except reference maize grain 33P66 (data not shown).

Soybean Fraction Characterization and Nutrient Composition

Real-time qualitative PCR analysis confirmed the presence of event DP-356Ø43-5 in 356043 fractions (soybean meal and hulls) and its absence from control and reference fractions. Slight variations in concentrations of protein, fat, and energy were noted between control, 356043, and reference soybean meals (Table 2); however, these nutrient levels were similar in the prepared diets fed to the broilers. Concentrations of most macronutrients were similar between control and 356043 soybean hull sources (approximately 25 and 26% protein, 12 and 13% fat, and 4,836 and 4,892 kcal/kg of gross energy, respectively); a wider range of values was observed for reference soybean hulls (approximately 18 to 29% protein, 9 to 13% fat, and 4,569 to 4,745 kcal/kg of gross energy).

Macronutrient concentrations in all soybean hull sources were higher than typically observed in commercial soybean hulls, indicating that the hulls contained a higher amount of bean meat as a result of poor separation between the hull and bean. None of the soybean meals or hulls contained measurable concentrations of mycotoxins (data not shown). Soybean oil energy values (Table 2) were similar between 356043 and reference sources; control soybean oil energy values were slightly lower than those of the other oils.

Diet Characterization and Nutrient Composition

An ELISA analysis of diet samples collected for homogeneity evaluation confirmed that both GAT4621 and ZM-HRA proteins were absent from diets produced with control and reference maize grains (GAT4621 LLOQ = 0.054 ng/mg of diet; ZM-HRA LLOQ = 0.068 ng/mg of diet). An ELISA analysis of diets produced with 98140 maize grain confirmed the presence of GAT4621 protein (6.1, 6.0, and 6.2 ng/mg of diet in starter, grower, and finisher diets, respectively) and ZM-HRA protein (0.089, 0.093, and 0.098 ng/mg of diet in starter, grower, and finisher diets, respectively). Diets produced with 98140 maize grain were blended homogeneously and the protein concentrations were stable for the duration of the respective feeding phases (data not shown). Event-specific real-time PCR testing confirmed the presence of the insert from event DP-356Ø43-5 in 98140 + 356043 diets and its absence from

Table 1. Analyzed nutrient composition[1] (as-fed basis) of maize grain sources[2]

Analyzed composition	Control maize	98140	33J56	33P66	33R77
Proximate (% unless noted)					
Moisture	12.2	12.9	11.9	12.6	12.6
Protein	7.1	7.9	7.6	7.4	6.7
Fat	3.3	3.3	3.1	3.6	3.6
Fiber	1.6	1.6	1.6	1.6	1.6
Ash	1.6	1.4	1.5	1.8	1.7
Carbohydrate[3]	75.8	74.5	75.9	74.6	75.4
Calcium	0.02	0.01	0.01	0.01	0.01
Phosphorus	0.22	0.22	0.22	0.26	0.24
Gross energy (kcal/kg)	3,949	3,923	3,948	3,926	3,929
ME[4] (kcal/kg)	3,475	3,452	3,474	3,455	3,458
Essential amino acid (%)					
Arginine	0.39	0.42	0.39	0.39	0.36
Lysine	0.26	0.27	0.27	0.27	0.25
Histidine	0.21	0.23	0.23	0.23	0.21
Isoleucine	0.27	0.30	0.28	0.28	0.25
Leucine	0.89	1.03	0.93	0.93	0.82
Methionine	0.17	0.16	0.17	0.17	0.15
Phenylalanine	0.37	0.41	0.38	0.37	0.33
Threonine	0.26	0.29	0.27	0.27	0.25
Tryptophan	0.06	0.06	0.06	0.06	0.05
Valine	0.37	0.40	0.38	0.38	0.35

[1]Each value represents the mean of 2 samples.

[2]98140: maize grain from event DP-Ø9814Ø-6 (*gat*4621 and *zm-hra* genes). Nontransgenic control maize with comparable genetic background to 98140 maize (control maize), commercially available nontransgenic Pioneer brand hybrids 33J56, 33P66, and 33R77, and 98140 maize were produced by Pioneer Hi-Bred (Johnston, IA) in a 2007 field production trial conducted near York, Nebraska.

[3]Carbohydrate values calculated as 100% − (% protein + % fat + % moisture + % ash).

[4]ME values calculated for maize grain using conversion factors based upon internal Pioneer Hi-Bred data.

Table 2. Analyzed nutrient composition[1] (as-fed basis) of soybean meal and oil sources[2]

Analyzed composition	Control soybean	356043	92M72	93B15	93M14
Meal					
Proximate (% unless noted)					
Moisture	7.2	2.9	5.4	4.9	3.9
Protein	48.7	51.6	49.8	49.3	51.4
Fat	1.0	1.0	1.8	1.5	2.0
Fiber	4.7	3.9	3.2	4.3	2.8
Ash	7.1	5.6	7.4	7.1	6.8
Carbohydrate[3]	36.0	38.9	35.6	37.2	35.9
Calcium	0.36	0.36	0.31	0.36	0.41
Phosphorus	0.75	0.68	0.79	0.70	0.72
Gross energy (kcal/kg)	4,372	4,592	4,504	4,475	4,538
ME[4] (kcal/kg)	2,549	2,677	2,626	2,609	2,645
Essential amino acid (%)					
Arginine	3.32	3.68	3.56	3.47	3.75
Lysine	2.83	3.07	2.97	3.00	3.12
Histidine	1.28	1.42	1.32	1.27	1.36
Isoleucine	2.27	2.48	2.41	2.36	2.42
Leucine	3.79	4.18	3.93	3.81	4.11
Methionine	0.69	0.71	0.72	0.72	0.72
Phenylalanine	2.41	2.64	2.49	2.42	2.60
Threonine	1.83	2.00	1.86	1.79	1.99
Tryptophan	0.74	0.73	0.80	0.73	0.75
Valine	2.32	2.66	2.51	2.38	2.56
Oil[5] (kcal/kg)					
Gross energy	9,374	9,420	9,419	9,417	9,424
ME[4]	6,749	6,782	6,782	6,780	6,785

[1]Each value represents the mean of 2 samples.

[2]356043: soybeans containing event DP-356Ø43-5 (*gat*4601 and *gm-hra* genes). Commercial nontransgenic Pioneer variety 93M14 soybeans and soybeans containing event 356043 were produced by Pioneer Hi-Bred (Johnston, IA) in a field production trial conducted near York, Nebraska, in 2007. Nontransgenic soybeans with a comparable genetic background to 356043 soybeans (control soybean) and commercially available nontransgenic Pioneer varieties 92M72 and 93B15 were produced in a concurrent field trial at the same location.

[3]Carbohydrate values calculated as 100% − (% protein + % fat + % moisture + % ash).

[4]ME values calculated for using conversion factors based upon internal Pioneer Hi-Bred data.

[5]Each cell represents the mean of multiple determinations (2 minimum) performed on a single sample.

Table 3. Ingredient[1] and analyzed nutrient[2] compositions (as-fed basis) of starter (d 0 to 21) diets[3]

Item	Control	98140 + 356043	Reference 1	Reference 2	Reference 3
Ingredient (%)					
Maize	62.460	64.131[4]	62.836	62.960	62.680
Soybean meal	27.580	27.395	28.318	27.547	28.131
Soybean hulls	1.000	1.000	1.000	1.000	1.000
Sysco soybean oil	0.500	0.500	0.500	0.500	0.500
Protein blend[5]	4.473	2.802	3.287	4.024	3.747
DL-Methionine	0.268	0.310	0.250	0.256	0.273
L-Lysine-HCl	0.133	0.109	0.094	0.081	0.058
Limestone	0.801	0.872	0.914	0.845	0.811
Dicalcium phosphate	1.725	1.815	1.737	1.725	1.735
Sodium chloride	0.435	0.442	0.440	0.437	0.438
Vitamin-mineral premix[6]	0.625	0.625	0.625	0.625	0.625
Analyzed nutrient composition					
Proximate (% unless noted)					
Moisture	8.5	8.3	8.6	8.8	7.7
Protein	23.1	21.4	22.8	21.8	22.0
Fat	2.9	3.4	3.1	3.4	3.6
Fiber	1.8	2.5	2.0	2.5	2.9
Ash	5.0	4.9	5.4	4.8	5.5
Calcium	0.80	0.83	0.77	0.83	0.85
Phosphorus	0.67	0.67	0.70	0.76	0.75
Gross energy (kcal/kg)	4,036	4,026	4,025	4,034	4,065
Essential amino acid (%)					
Arginine	1.40	1.39	1.40	1.36	1.44
Histidine	0.61	0.57	0.55	0.58	0.59
Isoleucine	0.97	0.97	0.96	0.91	0.92
Leucine	2.01	2.10	2.01	1.93	1.92
Lysine	1.26	1.15	1.21	1.18	1.18
Methionine	0.58	0.59	0.53	0.54	0.54
Phenylalanine	1.09	1.10	1.06	1.04	1.06
Threonine	0.83	0.84	0.82	0.81	0.86
Tryptophan	0.24	0.24	0.24	0.25	0.27
Valine	1.19	1.17	1.15	1.08	1.09

[1]Diets were formulated to contain the following: ME, 3,135 kcal/kg; protein, 22.00%; lysine, 1.20%; and methionine + cystine, 1.02%.

[2]Each value represents 1 determination.

[3]Control: control maize grain + control soybean fractions; 98140 + 356043: 98140 maize grain + 356043 soybean fraction; reference 1: 33J56 maize grain + 92M72 soybean fraction; reference 2: 33P66 maize grain + 93B15 soybean fraction; reference 3: 33R77 maize grain + 93M14 soybean fraction. See Tables 1 and 2 for further details.

[4]The actual percentage of corn was 63.898% as a result of a calculation error in adding back ground grain during the remixing process.

[5]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%.

[6]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.

control and reference diets (data not shown). Individual phase diets were formulated based on the analyzed nutrient concentrations of the maize grain and soybean meal sources. The diets produced from control, test, and reference maize grain + soybean fractions were all similar in proximate, energy, mineral, and amino acid composition in each diet phase (Tables 3, 4, and 5).

Performance Response Variables

No statistically significant differences were found in growth performance, mortality, or mortality-adjusted feed efficiency between control and 98140 + 356043 groups (Table 6). Furthermore, all growth performance measures for broilers fed control or 98140 + 356043 diets fell within the tolerance intervals calculated for this study using data obtained from broilers consuming diets produced with commercially available nontransgenic maize grain and soybean fraction sources.

Organ and Carcass Yields

Kidney yields and liver yields (Table 6) were not significantly different between control and 98140 + 356043 groups. No statistically significant differences were observed for carcass or individual parts yields between the control and 98140 + 356043 diet groups (Table 6). Additionally, all observed organ yield and carcass and individual parts yield values fell within tolerance intervals calculated for this study, indicating that organ and carcass and parts yields of broilers fed control or 98140 + 356043 diets were similar to those of broilers fed diets prepared with commercially available nontransgenic maize grain and soybean fraction sources.

Genetically modified crops have been widely adopted in the United States since their commercial introduction in 1996. The development of herbicide-tolerant crops has allowed growers more flexibility and ease in weed management practices and reduced cultivation costs

Table 4. Ingredient[1] and analyzed nutrient[2] compositions (as-fed basis) of grower (d 22 to 35) diets[3]

Item	Control	98140 + 356043	Reference 1	Reference 2	Reference 3
Ingredient (%)					
Maize	64.959	66.662[4]	65.372	65.510	65.145
Soybean meal	25.608	25.245	26.240	25.455	26.053
Soybean hulls	1.000	1.000	1.000	1.000	1.000
Sysco soybean oil	0.500	0.500	0.500	0.500	0.500
Protein blend[5]	4.296	2.775	3.177	3.915	3.713
DL-Methionine	0.191	0.232	0.176	0.180	0.196
L-Lysine-HCl	0.101	0.084	0.066	0.055	0.032
Limestone	0.754	0.821	0.861	0.797	0.762
Dicalcium phosphate	1.581	1.665	1.593	1.576	1.587
Sodium chloride	0.385	0.392	0.390	0.387	0.388
Vitamin-mineral premix[6]	0.625	0.625	0.625	0.625	0.625
Analyzed nutrient composition					
Proximate (% unless noted)					
Moisture	8.6	8.1	8.6	9.0	7.9
Protein	20.9	21.1	21.3	20.9	21.8
Fat	3.0	3.5	3.1	3.5	3.2
Fiber	1.9	2.6	1.9	2.1	2.6
Ash	4.7	4.6	4.9	4.8	4.8
Calcium	0.71	0.81	0.80	0.86	0.80
Phosphorus	0.65	0.67	0.70	0.74	0.68
Gross energy (kcal/kg)	4,041	4,031	4,020	4,027	4,060
Essential amino acid (%)					
Arginine	1.44	1.40	1.41	1.34	1.47
Histidine	0.62	0.57	0.56	0.53	0.60
Isoleucine	0.98	0.96	0.97	0.90	0.94
Leucine	2.03	2.01	2.05	1.94	1.94
Lysine	1.24	1.16	1.18	1.13	1.18
Methionine	0.50	0.54	0.49	0.47	0.51
Phenylalanine	1.11	1.07	1.07	1.02	1.08
Threonine	0.84	0.81	0.83	0.78	0.86
Tryptophan	0.23	0.24	0.23	0.24	0.25
Valine	1.21	1.15	1.16	1.09	1.12

[1]Diets were formulated to contain the following: ME, 3,164 kcal/kg; protein, 21.00%; lysine, 1.12%; and methionine + cystine, 0.92%.

[2]Each value represents 1 determination.

[3]Control: control maize grain + control soybean fractions; 98140 + 356043: 98140 maize grain + 356043 soybean fraction; reference 1: 33J56 maize grain + 92M72 soybean fraction; reference 2: 33P66 maize grain + 93B15 soybean fraction; reference 3: 33R77 maize grain + 93M14 soybean fraction. See Tables 1 and 2 for further details.

[4]The actual percentage of corn was 66.582% as a result of a calculation error in adding back ground grain during the remixing process.

[5]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%.

[6]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.

(Johnson et al., 2008). Herbicide-tolerant GM maize and soybeans accounted for 22 and 91%, respectively, of all corn and soybean acres planted in the United States in the 2009 growing season (USDA Economic Research Service, 2010).

The current feeding study was conducted to compare the nutritional performance of herbicide-tolerant 98140 maize grain fed in combination with herbicide-tolerant 356043 soybean fractions with those of non-transgenic maize and soybean fraction combinations with a comparable genetic background. Information about feedstuffs used to formulate broiler chicken diets is limited primarily to nutritional proximates, amino acids, calcium, phosphorus, and gross energy values. Nutrient values of all maize grains used in this trial fell within the ranges of conventionally bred precommercial and commercial maize grains produced from controlled field trials in the United States (International Life Sciences Institute, 2010). Fumonisin presence in the maize sources was not a concern for diet production because the concentration of total fumonisins ($B_1 + B_2 + B_3$) in the prepared diets was calculated to be well below the recommended total ration maximum of 50 mg/kg (US Food and Drug Administration, 2005), even when included at the highest rate (72% average inclusion in finisher phase diets). Soybean meal nutrient values of control, 356043, and commercial varieties used in this study were similar to those of solvent-extracted dehulled 48% and 50% soybean meals (van Eys et al., 2004). All maize grains and soybean meal sources were considered suitable for commercial-type broiler diet production because no large differences in key nutrients (e.g., protein, energy, essential amino acids) were observed between the sources within each feedstuff (grain, soybean meal) that would have limited their inclusion amount. The quantity of soybean hulls added to the diets was limited to 1.0% because of their high protein, fat, and energy concentrations that resulted from poor

Table 5. Ingredient[1] and analyzed nutrient[2] compositions (as-fed basis) of finisher (d 36 to 42) diets[3]

Item	Control	98140 + 356043	Reference 1	Reference 2	Reference 3
Ingredient (%)					
Maize	71.159	72.946	71.664	71.835	71.264
Soybean meal	20.988	20.197	21.368	20.545	21.179
Soybean hulls	1.000	1.000	1.000	1.000	1.000
Sysco soybean oil	0.500	0.500	0.500	0.500	0.500
Protein blend[4]	2.605	1.437	1.641	2.385	2.359
DL-Methionine	0.230	0.268	0.219	0.219	0.234
L-Lysine-HCl	0.261	0.260	0.237	0.229	0.207
Limestone	0.787	0.845	0.881	0.830	0.790
Dicalcium phosphate	1.504	1.575	1.519	1.489	1.499
Sodium chloride	0.342	0.347	0.345	0.343	0.343
Vitamin-mineral premix[5]	0.625	0.625	0.625	0.625	0.625
Analyzed nutrient composition					
Proximate (% unless noted)					
Moisture	8.9	8.4	8.8	9.1	8.0
Protein	19.1	17.8	18.3	18.8	18.0
Fat	3.3	3.3	3.2	3.7	3.7
Fiber	1.6	2.0	2.0	2.0	2.1
Ash	4.4	4.5	4.3	4.6	4.5
Calcium	0.72	0.76	0.73	0.80	0.77
Phosphorus	0.62	0.60	0.63	0.64	0.63
Gross energy (kcal/kg)	4,008	4,008	3,987	3,992	4,038
Essential amino acid (%)					
Arginine	1.17	1.10	1.22	1.18	1.12
Histidine	0.48	0.47	0.49	0.47	0.46
Isoleucine	0.80	0.78	0.82	0.77	0.77
Leucine	1.79	1.76	1.78	1.76	1.67
Lysine	1.16	1.05	1.16	1.12	1.02
Methionine	0.49	0.51	0.53	0.55	0.45
Phenylalanine	0.91	0.89	0.92	0.90	0.87
Threonine	0.71	0.66	0.72	0.71	0.65
Tryptophan	0.20	0.20	0.20	0.20	0.20
Valine	0.97	0.94	0.98	0.93	0.94

[1]Diets were formulated to contain the following: ME, 3,186 kcal/kg; protein, 18.00%; lysine, 1.08%; and methionine + cystine, 0.85%.

[2]Each value represents 1 determination.

[3]Control: control maize grain + control soybean fractions; 98140 + 356043: 98140 maize grain + 356043 soybean fraction; reference 1: 33J56 maize grain + 92M72 soybean fraction; reference 2: 33P66 maize grain + 93B15 soybean fraction; reference 3: 33R77 maize grain + 93M14 soybean fraction. See Tables 1 and 2 for further details.

[4]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%.

[5]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.

separation between the hull and bean during processing. The ME values calculated for ration formulation from the analyzed gross energy values of the oils indicated that soy oil values (6,749 to 6,785 kcal of ME/kg) were lower compared with typical crude or refined soy oil values (8,370 to 10,212 kcal of ME/kg; NRC, 1994). Because of the lower nutritional quality relative to typical crude or refined soy oil, quantities of the soy oils were limited to 0.5% across all diets.

Nutritional equivalency studies of GM feedstuffs are conducted using broiler chickens because they are a rapidly growing species of commercial importance and are sensitive to nutritional deficiencies (International Life Sciences Institute, 2003). The performance of the chickens fed different diets in this study was compared using standard performance variables along with organ and carcass yields. No significant differences in BW, weight gain, organ yield, or carcass yields were observed between broiler chickens consuming diets prepared with grain from 98140 maize and fractions from 356043 soybean or nontransgenic control maize + soybean fractions with comparable genetic background. Previous feeding trials conducted with the individual events showed no difference in performance or yields between broilers fed processed fractions from 356043 soybean or its nontransgenic control fractions (McNaughton et al., 2007a) or between broilers fed 98140 maize grain or its nontransgenic control (McNaughton et al., 2008a). These results are consistent with previous broiler feeding trials conducted with grain or soybean meal from herbicide-tolerant GM plants that demonstrated similar weight gains and feed conversion between groups of broilers fed diets with transgenic grain or soybean meal or nontransgenic control grain or soybean meal with comparable genetic backgrounds (Hammond et al., 1996; Sidhu et al., 2000; Taylor et al., 2003, 2007). Nutritional performance trials of transgenic grains in other species, such as rodents, routinely include measures of organ weights (Hammond et al., 2004; MacKenzie et al., 2007; Appenzeller et al., 2008, 2009). Organ yields,

Table 6. Growth performance,[1] prechill organ yields,[2] and postchill carcass and parts yields[3] of broilers fed diets containing nontransgenic control maize grain and soybean fractions or diets containing 98140 maize grain and 356043 soybean fractions[4]

Item	Control	98140 + 356043	SEM	Control vs. 98140 + 356043: FDR *P*-value[7]	Control vs. 98140 + 356043: Raw *P*-value[8]	Tolerance interval[5]	Reference group[6]: 1	Reference group[6]: 2	Reference group[6]: 3
Growth performance									
Initial weight, d 0 (g)	47.4	47.1	0.2	1.00	0.32	45.0–49.4	47.3	47.0	47.3
Final weight, d 42 (g)	1,731.4	1,731.3	19.2	1.00	0.83	1,522.5–1,969.8	1,752.3	1,744.2	1,741.9
Mortality[9] (%)	0.83	0.83	0.82	1.00	1.00	0.00–10.50	0.83	0.00	1.67
Feed:gain, 0–42 d[10] (g:g)	1.870	1.860	0.015	1.00	0.80	1.693–2.045	1.872	1.870	1.864
Prechill organ yield (%)									
Kidney									
Overall	2.14	2.12	0.05	1.00	0.76		2.02	2.05	2.08
Males	2.19	2.11	0.07	1.00	0.35	0.80–3.33	2.02	2.06	2.11
Females	2.08	2.13	0.07	1.00	0.62	0.68–3.38	2.01	2.03	2.04
Liver									
Overall	3.53	3.51	0.05	1.00	0.77		3.55	3.51	3.54
Males	3.54	3.60	0.08	1.00	0.58	1.93–5.14	3.58	3.48	3.56
Females	3.53	3.42	0.08	1.00	0.33	2.01–5.05	3.53	3.53	3.52
Postchill carcass and parts yield (%)									
Carcass									
Overall	71.03	70.77	0.37	1.00	0.60		70.57	70.85	70.72
Males	71.09	70.76	0.49	1.00	0.62	62.07–79.47	70.28	71.14	70.90
Females	70.97	70.78	0.49	1.00	0.77	60.74–80.57	70.87	70.56	70.54
Breast									
Overall	26.65	26.64	0.23	1.00	0.97		26.84	26.69	26.71
Males	26.52	26.54	0.31	1.00	0.98	21.06–32.70	27.22	26.68	26.72
Females	26.77	26.74	0.31	1.00	0.94	20.46–32.77	26.46	26.69	26.69
Thigh									
Overall	15.83	15.93	0.14	1.00	0.60		15.96	15.84	15.98
Males	15.64	15.65	0.20	1.00	0.97	12.00–19.88	15.98	16.02	15.82
Females	16.02	16.22	0.20	1.00	0.48	11.77–20.07	15.95	15.67	16.13
Leg									
Overall	14.43	14.40	0.13	1.00	0.87		14.28	14.37	14.38
Males	14.51	14.37	0.18	1.00	0.56	10.60–18.19	14.58	14.32	14.28
Females	14.35	14.43	0.18	1.00	0.73	10.79–17.80	13.98	14.43	14.48
Wing									
Overall	10.66	10.62	0.08	1.00	0.69		10.58	10.49	10.63
Males	10.61	10.61	0.11	1.00	1.00	8.34–12.85	10.62	10.51	10.66
Females	10.71	10.62	0.11	1.00	0.57	8.11–12.94	10.53	10.46	10.59
Abdominal fat									
Overall	1.46	1.51	0.04	1.00	0.35		1.41	1.42	1.50
Males	1.38	1.46	0.05	1.00	0.30	0.30–2.52	1.42	1.34	1.46
Females	1.53	1.55	0.05	1.00	0.78	0.42–2.53	1.40	1.50	1.53

[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 weight. Individual treatment least squares means represent 12 pens/treatment group with 8 birds/pen.

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

[4]98140: maize grain from event DP-Ø9814Ø-6 (*gat*4621 and *zm-hra* genes); 356043: soybeans containing event DP-356Ø43-5 (*gat*4601 and *gm-hra* genes).

[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 reference 1, reference 2, and reference 3 diets.

[6]Commercial group least squares means included for reference purposes only. The comparison of interest was control vs. 98140 + 356043. Reference 1: 33J56 maize grain + 92M72 soybean fraction; reference 2: 33P66 maize grain + 93B15 soybean fraction; reference 3: 33R77 maize grain + 93M14 soybean fraction. See Tables 1 and 2 for further details.

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

[8]Nonadjusted *P*-value.

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

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

such as those of liver and kidney, may indicate effects on broiler health resulting from dietary inadequacies or the presence of antinutritional factors (Whitehead et al., 1978; Keagy et al., 1987; Bailey et al., 2000; Farran et al., 2005). The organ yield results in this study are consistent with those of previous studies in which no significant differences in organ yields were observed between broilers fed diets prepared with transgenic grain or feed fractions and those fed diets with grain or feed fractions from nontransgenic controls with comparable genetic background (McNaughton et al., 2007a,b, 2008a,b).

Statistical analysis of all data in this study resulted in rejection of the hypothesis of expected growth performance, organ yield, and carcass yield differences between birds fed nontransgenic control maize and control soybean fractions and those fed 98140 maize + 356043 soybean fractions. The results from this study demonstrated that grain obtained from maize plants containing event DP-Ø9814Ø-6 and processed fractions from soybeans containing event DP-356Ø43-5 fed in combination were nutritionally equivalent to a combination of grain and soybean fractions obtained from nontransgenic maize and soybean plants with comparable genetic backgrounds.

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