Comparison of broiler performance and carcass yields when fed diets containing genetically modified canola meal from event DP-Ø73496-4, near-isogenic canola meal, or commercial canola meals

J. McNaughton,*[1] M. Roberts,* D. Rice,† B. Smith,† B. Hong,† B. Delaney,† and C. Iiams†

*AHPharma, 116 W. Chestnut St., Salisbury, MD 21801; and †DuPont Pioneer, 7250 NW 62nd Ave, Johnston, IA 50131

ABSTRACT Genetically modified (GM) canola (*Brassica napus* L.) line containing event DP-Ø73496–4 (hereafter referred to as 73496 canola) was produced by the insertion of the glyphosate acetyltransferase (*gat*4621) gene derived from *Bacillus licheniformis*. Expression of the GAT4621 protein present in 73496 canola plants confers *in planta* tolerance to the herbicidal active ingredient glyphosate. The objective of this study was to compare the nutritional performance of broiler chickens fed canola meal from 73496 canola seed with that of broiler chickens fed non-GM canola meal in a 42-d feeding trial. Diets were prepared using meal processed from seed from unsprayed 73496 plants or from plants sprayed with an in-field application of glyphosate herbicide [73496(S)]. For comparison, additional diets were produced with canola meal obtained from the non-GM near-isogenic control or non-GM commercial reference DuPont Pioneer brand varieties 42H72, 42H73, 46A65, and 44A89. Diets were fed to Ross 708 broilers (n = 120/group, 50% male and 50% female) in 3 phases: starter and grower phases containing 10 or 20% canola meal, respectively, and a finisher phase with a common corn-soybean meal diet without any canola meal. No statistically significant differences were observed in growth performance measures or organ and carcass yields between broilers consuming diets produced with canola meal from unsprayed or sprayed 73496 seed and those consuming diets produced with canola meal from control seed. Additionally, all performance, organ, and carcass measures from control, 73496, and 73496(S) canola treatment groups were within tolerance intervals constructed using data from the reference canola groups. It was concluded from these results that meal processed from 73496 canola seed (unsprayed plants or plants sprayed with glyphosate) was nutritionally equivalent to meal processed from non-GM near-isogenic control canola seed.

Key words: broiler, canola, carcass yield, genetically modified, growth performance

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INTRODUCTION

A genetically modified (**GM**) canola (*Brassica napus* L.) line containing event DP-Ø73496–4 (hereafter referred to as 73496 canola) was produced by the insertion of the glyphosate acetyltransferase (*gat*4621) gene derived from *Bacillus licheniformis*. The *gat*4621 gene was functionally improved by a gene shuffling process to optimize the kinetics of glyphosate acetyltransferase activity for acetylating the herbicide glyphosate (Castle et al., 2004; Siehl et al., 2005). Expression of the GAT4621 protein encoded by the *gat*4621 gene confers tolerance *in planta* to the herbicidal active ingredient glyphosate.

Canola is the second-largest oil crop and feed meal (after soybean meal) and processed canola may be an economical protein source for animals with lower energy or lysine requirements (USDA-ERS, 2013). The development of GM crops such as herbicide-tolerant canola provides growers with such benefits as reduced input costs and better weed control (Gusta et al., 2011). Globally, 30% of the 31 million canola hectares planted in 2012 canola were a GM variety (James, 2012), and in North America nearly all (>90%) of the canola planted is GM. Compared with GM crops such as corn or soybeans, published nutritional equivalency feeding studies with GM canola are limited (Stanford et al., 2002; Brown et al., 2003; Taylor et al., 2004). The objective of this study was to evaluate the nutritional performance of broilers fed diets formulated with canola meal processed from 73496 canola seed by comparing growth performance (as measured by BW and feed efficiency), organ yields, and carcass parts yields with those

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[1]Corresponding author: mcnaughton@ahpharma.com

of broilers fed diets containing non-GM near-isogenic canola meal.

MATERIALS AND METHODS

Canola Seed Production and Meal Processing

All canola seed for this trial was produced in isolated plots in a US production trial. Two plots of 73496 test canola seed were produced. One plot [hereafter referred to as 73496(S)] was sprayed with 2 applications of glyphosate herbicide (Touchdown HiTech, Syngenta Crop Protection Inc., Greensboro, NC), whereas the other plot was not sprayed with glyphosate herbicide. Control canola seed was obtained from non-GM plants with a genetic background similar to 73496 canola plants. Reference canola seeds were commercially available non-GM DuPont Pioneer varieties 45H72, 45H73, 46A65, and 44A89. Neither control nor reference canola plants were sprayed with glyphosate herbicide. All canola sources were processed at GLP Technologies (Navasota, TX) under similar conditions to meet specifications of commercial solvent-extracted canola meal (with hulls). Identity preservation procedures were followed throughout the processing and inventory systems to maintain the identity of the resulting processed meal from each canola source.

Canola Meal Characterization and Composition Analysis

The presence of event DP-Ø73496–4 in the test canola meal and its absence from control and reference canola meals was confirmed using event-specific qualitative PCR analysis (DuPont Pioneer, Ankeny, IA), thus verifying that identity preservation was maintained. All meals were evaluated by ELISA for the expressed GAT4621 protein (DuPont Pioneer). Duplicate samples of each canola meal were evaluated for nutrient composition including proximates and minerals (calcium and phosphorous), and mycotoxin profile at Cumberland Valley Analytical Services (Hagerstown, MD). Dry matter (930.15), protein (990.03), fiber (978.10), ash (942.05), and calcium and phosphorus (985.01) analyses were performed according to AOAC International (2000) methods; fat analysis was determined according to AOAC International (2006) method 2003.05. Mycotoxin assay methods were as follows: aflatoxins and fumonisins determined according to AOAC International (2000) methods 994.08 and 995.15, respectively (with modifications); vomitoxin determined according to MacDonald et al. (2005), with modifications; 3- and 15-acetyl deoxynivalenol determined according to Tacke and Casper (1996); T2 toxin screening according to Romer (1986); and zearalenone screening according to AOAC method 976.22 (with modifications). University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) analyzed duplicate samples of each canola meal source for amino acid profile in accordance with AOAC International (2000) methods 988.15, 982.30, and 994.12. Canola meal samples were analyzed for gross energy content using a bomb calorimeter (Parr Instruments Model 1271, Parr Instruments, Moline, IL) at DuPont Pioneer (Urbandale, IA).

Broilers and Housing

DuPont Pioneer's Internal Animal Care and Use Committee approved all animal care, housing, and handling procedures, which conformed to animal care and use practices referenced in Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Commercial broilers (Ross 708) feather-sexed at hatch were obtained at day of hatch (trial d 0) from a commercial Maryland hatchery and transported to AHPharma Inc. (farm #1, Tyaskin, MD) in November 2010. Broilers were obtained in sufficient numbers to ensure availability of 1,080 healthy chicks (50% males and 50% females) for the conduct of the study. Farm personnel evaluated broilers upon receipt for signs of disease or other complications that may affect the outcome of the study; bird health observations and actual number of broilers received were documented. Healthy broilers were weighed, identified with a wingband, and placed randomly into 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; the pen litter consisted of used litter mixed with new pine shavings with a minimal amount of sawdust. Pens were separated by a wire partition and did not touch other pens from any side to minimize potential for cross-contamination. Broilers were housed in a room containing forced-air heaters and individual pen heat lamps with a cross-house ventilation system, and a continuous 24-h lighting program was followed. Farm personnel observed broilers 3 times daily for overall health, behavior, 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. Broilers were provided drinking water for ad libitum consumption.

Experimental Design

This study was designed as a randomized complete block with 7 dietary treatments [control, 73496, 73496(S), 45H72, 45H73, 46A65, and 44A89]; 2 additional treatment groups not related to event DP-Ø73496–4 were also included in this study (referred to below as diet A and diet B), but individual data for these 2 groups are not reported here. Each treatment was assigned 120 broilers with 10 broilers per pen (5 males and 5 females) and 12 pens (replicates) per

treatment. The sexes were penned together in accordance with nutritional equivalency studies conducted at this facility (McNaughton et al., 2007a, 2008, 2011a) and also in alignment with standard industry practice. Broilers were fed their dietary treatments prepared with the respective canola meal sources from trial d 0 to 35 d of age. Diets containing the canola meal sources were fed in 2 phases: starter (d 0 to 21) and grower (d 22 to 35). Due to the presence of seed hulls in canola meal, it is standard commercial practice to remove canola meal from the diet during the last 5 d before harvest to reduce potential contamination of the carcass with the seed hulls during processing (Canola Council of Canada, 2009). A common diet formulated with non-GM corn and non-GM soybean meal that did not contain canola meal from any source was fed across all treatment groups during a 7-d finisher phase (d 36 to 42) to comply with that practice.

Diet Preparation

All diets were offered as a mash feed for ad libitum consumption. Starter, grower, and finisher diets were formulated to meet the nutrient requirements of a typical commercial broiler diet using the NRC (1994) Nutrient Requirements of Poultry as a guideline. Diets were prepared at the DuPont Pioneer Livestock Nutrition Center Feed Mill (Polk City, IA). Canola meal inclusion rates were 10 and 20%, respectively, for starter and grower phase diets, as per Canola Council of Canada (2009) recommendations. Control, test, or reference canola meals were added to the indicated diets in equal amounts within the starter and grower phases; requirements for protein, lysine, methionine, cysteine, calcium, and phosphorus were met by adjusting the concentrations of noncanola meal ingredients. All diets were formulated to the same ME level within the starter (3,135 kcal of ME/kg) and grower (3,164 kcal of ME/kg) phases. The standard finisher diet (formulated to 3,186 kcal of ME/kg, 18.00% protein, 1.08% lysine, and 0.85% methionine + cysteine), composed of 73% corn and 18% soybean meal, with the balance consisting of oil, supplement, vitamins, minerals, and amino acids, was prepared first. Starter and grower diets for each canola meal source were mixed in the order of control, 45H72, 45H73, 46A65, 44A89, 73496, 73496(S), diet A, and diet B. All diets were prepared using a ribbon mixer (Sudenga M750, Sudenga Industries Inc., George, IA) that was flushed with non-GM soybean hulls before diet preparation and cleaned between each diet (finisher, starter, and grower) using compressed air and vacuum; mixing equipment was flushed with non-GM soybean hulls between each canola meal source. Composite samples of each diet were prepared for proximate analysis (including calcium and phosphorus), amino acid analysis, and gross energy analysis, all as previously described. The presence of event DP-Ø73496–4 in the test diets and its absence from control and reference diets was determined in the composite diet samples using event-specific qualitative PCR. Concentration of GAT4621 protein in samples collected at the time of each diet preparation (beginning, middle, and end of diet production) was evaluated using ELISA to determine if the diets were blended homogenously and to confirm that the protein was absent from control, reference, and standard finisher diets. Stability of GAT4621 protein (as determined by concentration at feeding period end relative to concentration at feeding period start) over the duration of the starter and grower feeding phases was evaluated using ELISA on samples collected from 73496 and 73496(S) diets at the start and end of the starter and grower phases.

Measurements

Following determination of BW at d 0, BW and feed weights (including amount of feed added and amount remaining) were determined every 7 d with BW gain, feed intake, and mortality-corrected feed efficiency (feed:gain ratio) calculated for 1) d 0 to 35 and 2) d 0 to 42. All surviving broilers were humanely euthanized on d 42 by cervical dislocation and a gross necropsy was performed. Carcass, carcass parts, and selected organ yield data were collected from 4 males and 4 females per pen (96 broilers/treatment) and included carcass yield (postchilled), thighs, breasts, wings, legs, abdominal fat (including fat around gizzard), kidneys, and whole liver. The combined total mass was recorded for all parts considered as pairs (e.g., legs, thighs, and so on). Carcass, kidney, and liver yields were each expressed as a percentage of whole live bird weight. Individual carcass 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 the control, 73496, and 73496(S) canola meal groups was calculated for each variable. Two sets of hypotheses were evaluated in this study. The primary hypothesis tested for event 73496 was that growth performance, organ yield, and carcass yield would be different between broiler chickens fed diets containing the test canola meal and those fed diets containing non-GM near-isogenic canola meal. The secondary hypothesis tested was that growth performance, organ yield, and carcass yield of broilers fed diets containing test canola meal produced under an herbicide spray regimen [73496(S)] would be different from that of broilers fed diets containing control canola meal. Thus, the true comparisons of interest were control versus 73496 and control versus 73496(S).

Data were analyzed using a mixed model ANOVA (PROC GLIMMIX, SAS version 9.1 software, SAS Institute Inc., Cary, NC). The following model was used for live performance data analysis (with the exception of mortality): $Y_{ij} = U + T_i + B_j + e_{ij}$ where Y_{ij} = ob-

served pen response, U = overall mean, T_i = treatment effect, B_j = random block effect, and e_{ij} = residual error. DIST = Gaussian option was used in the model statement for all growth performance traits with the exception of mortality. The model used for mortality data was: $\text{logit}(Y_{ij}/N_{ij}) = U + T_i + B_j + e_{ij}$, where Y_{ij} = observed number of dead birds within a pen, N_{ij} = total number of birds within a pen, logit = logit link function commonly used for binomial generalized linear mixed model, U = overall mean, T_i = treatment effect, B_j = random block effect, and e_{ij} = residual error. DIST = binomial option with the default logit link was used in the mortality model statement. Carcass and organ data analysis was performed using the model of $Y_{ijk} = U + T_i + B_j + G_k + (TG)_{ik} + e_{ijk}$, where Y_{ijk} = observed bird response, U = overall mean, T_i = treatment effect, B_j = random block effect, G_k = sex effect, $(TG)_{ik}$ = treatment by sex interaction, and e_{ijk} = residual error. A covariance structure of Compound Symmetry was used for the residual error covariance to allow positive as well as negative covariance among individual birds within the same pen. The LSMEANS statement with the DIFF = CONTROL option was used to generate the comparisons of interest [control versus 73496 or 73496(S)] for each live performance, organ, and carcass trait. The false positive rate was controlled through the use of false discovery rate (**FDR**) as described by Benjamini and Hochberg (1995) applied across all response variables analyzed. The FDR-adjusted P-value was reviewed in the event that a statistically significant difference ($P < 0.05$) generated from the estimate comparison statement was observed for a trait.

Reference (45H72, 45H73, 46A65, and 44A89) group data were used in the mixed model analysis to improve experimental variability estimate for each trait; individual reference treatment means were generated, but comparisons between each reference group and control, 73496, or 73496(S) groups were not performed. Reference canola meal sources were included in the study to construct tolerance intervals containing 99% of the observed performance (excluding mortality), organ, and carcass trait values at 95% confidence from broilers fed typical (non-GM commercial) canola meal diets, as described by Graybill (1976). These tolerance intervals were an additional comparison along with 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 control, 73496, or 73496(S) diets. Data from control, 73496, and 73496(S) groups were evaluated to determine whether or not the observed values were contained within the tolerance interval. If an observed response value for a treatment was contained within the tolerance interval, that value was considered to be similar to feeding typical canola meal. Creation of tolerance intervals for organ and carcass response variables was performed by sex due to the expected yield differences between male and female broilers.

RESULTS AND DISCUSSION

Canola Meal Characterization and Nutrient Composition

Qualitative real-time PCR analysis confirmed the 73496 meals (unsprayed or sprayed) contained event DP-Ø73496–4 and that the event was absent from control and reference (45H72, 45H73, 46A65, and 44A89) meals. Analysis by ELISA demonstrated the presence of GAT4621 protein in 73496 and 73496(S) canola meals (0.35 and 0.44 ng/mg of meal, respectively); the protein was absent from all other canola meals [assay lower limit of quantitation (**LLOQ**) = 0.22 ng of GAT4621/mg of meal].

No nutrient deficiencies that would have prevented any meal source from being used in this study were observed (Table 1). Crude protein concentrations of 73496 and 73496(S) meals were approximately 4 to 8 percentage points higher compared with that of the other canola meal sources, and protein values of most meals produced for this study were near the upper range value (44.3%) of typically processed meals (OECD, 2011). Essential amino acid values of the canola meals were above the minimum range value, and in most cases exceeded the maximum range value, of processed meals reflecting the higher protein values of the meals produced for this study. Crude fat, ash, and calcium values were within the typical range of processed canola meals, whereas fiber values of most meals were approximately 1 to 4 percentage points higher than the range of observed values (7.7 to 11.2%; OECD, 2011). Phosphorus values for control, 45H72, 46A65, and 44A89 were also above the upper limit of OECD (2011) range values (0.94 to 1.29%). The variation in phosphorus content between control and 73496 or 73496(S) canola meals was similar to the variation observed with the reference canola meals. Yearly growing and environmental conditions, along with processing conditions, may influence nutrient composition of the processed meal (Bell, 1993), which may account for differences between nutrient composition of canola meals used in this study and published ranges. Gross energy values of the processed meals used in this study were similar to those observed by Bell et al. (1998). Mycotoxin analysis showed the low level presence (0.1 mg/kg) of fumonisin B1 in only 45H72 canola meal; no other mycotoxins were present in measurable concentrations in any canola meal source (data not shown).

Total glucosinolates of all canola meal sources (Table 2) ranged from 6.66 to 9.06 μmol/g, well below the limit of 30 μmol/g, and were within the range of total glucosinolate content of canola meal processed at commercial plants (Newkirk et al., 2003). Concentrations of the other antinutrients did not exceed the upper values of ranges typically observed in processed canola meal (Bell, 1993). Tannin and phytic acid values for 73496 meals (unsprayed and sprayed) and some refer-

Table 1. Analyzed nutrient composition[1] (DM basis) of canola meal sources[2]

Item	Control	73496	73496(S)	45H72	45H73	46A65	44A89
Proximates and minerals, %							
DM	90.7	91.3	92.3	92.2	91.3	91.1	91.2
Protein	44.3	48.7	48.5	43.7	40.8	42.7	44.4
Fat	2.5	1.6	1.7	2.3	2.6	2.3	2.4
Fiber	12.0	10.0	10.2	12.4	13.7	14.7	13.2
Ash	7.8	7.0	7.0	8.1	9.3	8.0	7.6
Calcium	0.74	0.73	0.74	0.65	0.81	0.95	0.72
Phosphorus	1.59	1.19	1.19	1.47	1.07	1.53	1.42
Gross energy, kcal/kg	4,776	4,822	4,805	4,751	4,673	4,743	4,809
Essential amino acid, %							
Arginine	2.82	3.08	3.06	2.88	2.49	2.66	2.72
Histidine	1.30	1.41	1.41	1.37	1.17	1.26	1.28
Isoleucine	1.86	2.00	2.01	1.85	1.65	1.75	1.82
Leucine	3.18	3.47	3.46	3.29	2.85	3.04	3.10
Lysine	2.70	2.89	2.91	2.87	2.39	2.56	2.60
Methionine	0.84	0.93	0.93	0.91	0.77	0.81	0.82
Methionine + cysteine	1.99	2.19	2.18	2.11	1.75	1.94	1.95
Phenylalanine	1.81	1.97	1.96	1.84	1.61	1.71	1.76
Threonine	1.83	1.98	1.96	1.94	1.67	1.83	1.76
Tryptophan	0.50	0.56	0.57	0.51	0.48	0.52	0.52
Valine	2.36	2.54	2.56	2.38	2.12	2.22	2.33

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

[2]Control = canola meal processed from non-genetically modified (GM) canola seed with genetic background comparable with 73496 canola seed; 73496 and 73496(S) = canola meal processed from canola seed containing event DP-Ø73496–4 (glyphosate acetyltransferase, *gat*4621 gene); 45H72, 45H73, 46A65, and 44A89 = canola meal processed from commercially available non-GM DuPont Pioneer varieties. All canola seed was produced by DuPont Pioneer in a 2010 US field production trial, and processed at GLP Technologies (Navasota, TX) under similar conditions to meet specifications of commercial canola meal (with hulls).

ence meals were below the lower values of the respective range (tannins, 1.5 to 3.0%; phytic acid, 3 to 6%). Mailer et al. (2008) observed a similar range of sinapine values (0.52 to 1.62%) as that found between the commercial reference variety meals (0.267 to 0.637%) and the control, 73496, and 73496(S) meals (1.55 to 1.72%) in this study.

Diet Characterization and Nutrient Composition

Results of PCR analysis confirmed 73496 (unsprayed or sprayed) starter and grower diets were positive for event DP-Ø73496–4, whereas starter and grower control and reference (45H72, 45H73, 46A65, 44A89) diets, and the standard finisher diet, were negative for the event. Analysis of the control and reference starter and grower diets and standard finisher diet samples collected for homogeneity confirmed the absence of GAT4621 protein (assay LLOQ = 0.11 ng of GAT4621/mg of diet). The GAT4621 protein was not detectable above the assay LLOQ in 73496 or 73496(S) starter and grower diets. The lack of detection above the assay LLOQ was likely due to the dietary inclusion rates. The calculated GAT4621 values for 73496 and 73496(S) diets were 0.035 and 0.044 ng/mg diet, respectively, for starter di-

Table 2. Analyzed antinutrient composition[1] (as-is basis) of canola meal sources[2]

Antinutrient	Control	73496	73496(S)	45H72	45H73	46A65	44A89
Tannins,[3] %	1.81	1.12	1.05	1.23	2.11	1.49	1.61
Sinapine, %	1.55	1.60	1.72	0.547	0.637	0.500	0.267
Phytic acid, %	3.79	2.70	2.87	3.42	2.52	3.79	3.65
Total glucosinolates, μmol/g	8.45	6.66	7.24	9.06	6.86[4]	7.73	7.81
Aliphatic[5]	3.19	2.14	2.40	3.78	3.67	3.99	3.53
Aromatic and indolic[6]	5.26	4.52	4.84	5.28	3.18	3.74	4.28

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

[2]Control = canola meal processed from non-genetically modified (GM) canola seed with genetic background comparable with 73496 canola seed; 73496 and 73496(S) = canola meal processed from canola seed containing event DP-Ø73496–4 (glyphosate acetyltransferase, *gat*4621 gene); 45H72, 45H73, 46A65, and 44A89 = canola meal processed from commercially available non-GM DuPont Pioneer varieties. All canola seed was produced by DuPont Pioneer in a 2010 US field production trial,and processed at GLP Technologies (Navasota, TX) under similar conditions to meet specifications of commercial canola meal (with hulls).

[3]Total of soluble and insoluble tannins.

[4]The total differs from the sum of the individual glucosinolate groups due to postcalculation rounding.

[5]Aliphatic glucosinolates include progoitrin, gluconapin, glucobrassicanapin, glucoalyssin, and glucoraphanin; glucoiberin, epiprogoitrin, and gluconapoleiferin were not detected in quantifiable amounts.

[6]Aromatic and indolic glucosinolates include gluconasturtin, glucobrassicin, 4-hydroxyglucobrassicin, and neoglucobrassicin; 4-methoxyglucobrassicin was not detected in a quantifiable amount.

ets and 0.070 and 0.088 ng/mg of diet, respectively, for grower diets. The GAT4621 protein was not detected above the assay LLOQ in stability samples collected for 73496 and 73496(S) starter diets or d 22 73496(S) grower diet (data not shown). The GAT4621 protein was detected just above (0.12 ng of GAT4621/mg of diet) the assay LLOQ in 73496(S) grower d 35 samples; however, stability could not be calculated due to the d 22 value being below the assay LLOQ.

Starter and grower diets were formulated based upon the analyzed nutrient concentrations of the canola meals. The diets produced from control, 73496, 73496(S), 45H72, 45H73, 46A65, or 44A89 canola meals were similar in proximate, energy, mineral, and amino acid content within the starter and grower phases (Tables 3 and 4, respectively). Calculated total glucosinolate content was less than 1 μmol/g across all treatment groups for starter phase diets and between 1 and 2 μmol/g across all treatment groups for grower phase diets. These levels were below the 2.5 μmol/g dietary limit recommended by Bjerg et al. (1987). Sinapine is known to impart a bitter taste in canola and rapeseed meals. The calculated dietary sinapine levels observed with the control, 73496, and 73496(S) treatments (up to 0.17 and 0.34% in starter and grower diets, respectively, versus 0.06 and 0.13% for all other treatments) did not affect feed intake. Average daily pen intakes for control, 73496, and 73496(S) groups were 969, 978, and 985 g, respectively, versus 968 to 978 g for all other groups. Qiao and Classen (2003) fed 0.15 to 0.30% dietary sinapine to broilers and similarly observed no negative impact on feed intake. The nutrient composition of the standard finisher diet was sufficient to meet broiler needs during the finisher phase (analyzed values, as-is basis: 18.1% protein, 1.07% lysine, 0.84% methionine + cysteine, and 3,936 kcal/kg of gross energy).

Table 3. Ingredient and analyzed nutrient[1] compositions (as-is basis) of starter diets[2,3]

Item	Control	73496	73496(S)	45H72	45H73	46A65	44A89
Ingredient, %							
Maize	58.6	59.2	59.2	58.5	58.3	58.4	58.6
Soybean meal	24.9	24.7	24.8	25.2	24.7	25.0	25.0
Canola meal	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Protein blend[4]	1.60	1.14	1.04	1.47	2.10	1.71	1.53
Soybean oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sodium chloride	0.43	0.43	0.43	0.43	0.42	0.43	0.43
Limestone	0.93	0.92	0.92	0.95	0.83	0.86	0.92
Dicalcium phosphate	1.62	1.69	1.69	1.63	1.71	1.63	1.65
DL-Methionine	0.24	0.24	0.24	0.23	0.24	0.24	0.24
L-Lysine-HCl	0.034	0.032	0.024	0.002	0.061	0.041	0.046
Vitamin-mineral premix[5]	0.63	0.63	0.63	0.63	0.63	0.63	0.63
Analyzed nutrient composition							
Proximates and minerals, %							
Moisture	10.8	11.0	10.7	10.7	10.8	10.9	10.9
Protein	22.4	22.1	22.1	22.1	22.3	22.2	21.8
Fat	3.6	3.4	3.7	3.8	3.7	3.7	3.7
Fiber	3.6	3.9	4.1	3.6	4.1	4.2	3.8
Ash	5.7	5.7	5.4	5.7	5.6	5.6	5.6
Calcium	0.90	0.80	0.84	0.83	0.88	0.79	0.84
Phosphorus	0.85	0.79	0.78	0.76	0.81	0.79	0.75
Gross energy, kcal/kg	3,956	3,956	3,978	3,972	3,950	3,968	3,970
Essential amino acid							
Arginine	1.39	1.39	1.39	1.37	1.35	1.36	1.31
Histidine	0.57	0.58	0.58	0.57	0.55	0.56	0.55
Isoleucine	0.95	0.96	0.95	0.93	0.92	0.93	0.90
Leucine	1.83	1.83	1.83	1.81	1.84	1.83	1.78
Lysine	1.18	1.19	1.17	1.13	1.11	1.16	1.12
Methionine	0.56	0.55	0.53	0.55	0.54	0.54	0.53
Methionine + cysteine	1.00	0.97	0.94	0.95	0.96	0.94	0.92
Phenylalanine	1.04	1.04	1.03	1.02	1.02	1.02	1.00
Threonine	0.82	0.81	0.83	0.80	0.82	0.82	0.79
Tryptophan	0.23	0.26	0.25	0.25	0.27	0.26	0.26
Valine	1.13	1.13	1.12	1.10	1.10	1.10	1.07

[1]Each value represents 1 determination.

[2]Diets were formulated to contain: ME, 3,135 kcal/kg; protein, 22.00%; lysine, 1.245%; and methionine + cysteine, 1.02%.

[3]Control = canola meal processed from non-genetically modified (GM) canola seed with genetic background comparable with 73496 canola seed; 73496 and 73496(S) = canola meal processed from canola seed containing event DP-Ø73496–4 (glyphosate acetyltransferase, *gat*4621 gene); 45H72, 45H73, 46A65, and 44A89 = canola meal processed from commercially available non-GM DuPont Pioneer varieties.

[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 + cysteine, 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.

Table 4. Ingredient and analyzed nutrient[1] compositions (as-is basis) of grower diets[2]

Item	Control	73496	73496(S)	45H72	45H73	46A65	44A89
Ingredient, %							
Maize	57.5	58.6	58.6	57.2	57.0	57.2	57.5
Soybean meal	15.4	13.5	13.4	15.6	16.6	16.0	15.2
Canola meal	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Soybean oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Solka-Floc[3]	2.60	3.29	3.43	2.80	1.85	2.43	2.71
Sodium chloride	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Limestone	0.95	0.88	0.87	0.96	0.81	0.83	0.92
Dicalcium phosphate	1.33	1.49	1.49	1.36	1.49	1.35	1.39
DL-Methionine	0.16	0.15	0.14	0.13	0.19	0.16	0.17
L-Lysine-HCl	0.067	0.092	0.082	0.011	0.088	0.074	0.095
Vitamin-mineral premix[4]	0.63	0.63	0.63	0.63	0.63	0.63	0.63
Analyzed nutrient composition							
Proximates and minerals, %							
Moisture	11.0	11.0	10.7	10.8	10.6	10.9	11.1
Protein	20.1	19.8	20.2	19.8	20.0	20.3	20.3
Fat	3.5	3.4	3.4	3.7	3.9	3.5	3.6
Fiber	6.3	6.6	7.2	6.6	6.4	6.3	6.3
Ash	5.3	5.2	5.3	5.5	5.6	5.1	5.3
Calcium	0.84	0.87	0.76	0.82	0.75	0.82	0.90
Phosphorus	0.82	0.77	0.76	0.84	0.72	0.83	0.81
Gross energy, kcal/kg	3,955	3,955	3,971	3,960	3,959	3,969	3,959
Essential amino acid							
Arginine	1.31	1.23	1.21	1.25	1.20	1.23	1.17
Histidine	0.56	0.54	0.53	0.55	0.51	0.53	0.51
Isoleucine	0.88	0.83	0.82	0.84	0.81	0.83	0.79
Leucine	1.76	1.63	1.62	1.67	1.60	1.64	1.57
Lysine	1.16	1.14	1.08	1.09	1.08	1.18	1.09
Methionine	0.53	0.48	0.47	0.48	0.50	0.47	0.47
Methionine + cysteine	0.94	0.86	0.86	0.90	0.87	0.86	0.84
Phenylalanine	0.96	0.89	0.88	0.91	0.87	0.90	0.85
Threonine	0.81	0.75	0.74	0.78	0.73	0.76	0.73
Tryptophan	0.22	0.24	0.24	0.23	0.24	0.24	0.25
Valine	1.05	1.01	1.00	1.00	0.97	1.01	0.95

[1]Each value represents one determination.

[2]Diets were formulated to contain: ME, 3,164 kcal/kg; protein, 20.00%; lysine, 1.175%; and methionine + cysteine, 0.92%.

[3]Control = canola meal processed from non-genetically modified (GM) canola seed with genetic background comparable with 73496 canola seed; 73496 and 73496(S) = canola meal processed from canola seed containing event DP-Ø73496–4 (glyphosate acetyltransferase, *gat*4621 gene); 45H72, 45H73, 46A65, and 44A89 = canola meal processed from commercially available non-GM DuPont Pioneer varieties.

[4]Solka-Floc, International Fiber Corporation (North Tonawanda, NY).

[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.

Broiler Response Variables

Growth performance measures of broilers fed control, 73496, and 73496(S) diets from d 0 to 35 are presented in Table 5, along with measures calculated for the full 42-d feeding period; weekly BW are presented in Figure 1. No statistically significant differences for either feeding period (0 to 35 d or 0 to 42 d), were observed between broilers consuming control and broilers consuming 73496 or 73496(S) diets, and all observed values of growth performance measures for broilers fed control, 73496, or 73496(S) diets fell within the respective tolerance interval calculated for this study using the data obtained from broilers consuming diets produced with non-GM reference canola meal.

No statistically significant differences in kidney yields (Table 5) were observed between broilers consuming control and broilers consuming 73496 or 73496(S) diets and all observed values for broilers fed control, 73496, or 73496(S) diets fell within the calculated tolerance interval. Overall liver yield and liver yield for male broilers did not differ ($P > 0.05$) between control and 73496 diet groups. Female broilers fed the 73496 diet had a lower ($P = 0.012$) liver yield than females fed the control diet. However, when the P-value was adjusted using FDR, the within-female difference was not significant ($P = 0.33$) and all observed values were within the tolerance interval calculated for this study using data obtained from broilers consuming diets produced with non-GM reference canola meals. Liver yield was not significantly different between control and 73496(S) diet groups. No statistically significant differences were observed for carcass yield or any individual parts yield between control and 73496 or 73496(S) treatment groups (Table 5). All observed yield values were within the tolerance intervals calculated using data obtained from broilers consuming diets produced with non-GM reference canola meals.

Nutritional equivalency studies have long been conducted using broiler chickens because they are a rapidly growing species and because weight gain and mortality are sensitive indicators of changes in the nutritional

Table 5. Growth performance,[1] prechill organ yields,[2] and postchill carcass and parts yields[3] of broilers fed diets[4] containing meal from nontransgenic near-isogenic control canola or meal from 73496 or 73496(S) canola

					Control vs. 73496		Control vs. 73496(S)			Reference group[6]			
Item	Control	73496	73496(S)	SEM	FDR *P*-value[7]	Raw *P*-value[8]	FDR *P*-value	Raw *P*-value	Tolerance interval[5]	45H72	45H73	46A65	44A89
Growth performance, 0 to 35 d													
Initial weight (g), d 0	50.2	49.9	50.0	0.2	0.91	0.14	0.96	0.43	47.9 to 52.0	49.9	50.3	49.7	49.7
Weight (g), d 35	1,676	1,671	1,687	15	0.99	0.81	0.96	0.61	1,494 to 1,836	1,669	1,677	1,657	1,658
Mortality (%)	3.33	1.67	0.83	1.55	0.99	0.40	0.96	0.19		2.50	1.67	1.67	1.67
Feed:gain[9] (g/g)	1.736	1.736	1.723	0.012	0.99	0.99	0.96	0.46	1.593 to 1.875	1.734	1.732	1.739	1.674
Growth performance, 0 to 42 d													
Final weight (g), d 42	2,298	2,298	2,326	24	1.00	1.00	0.96	0.41	2,027 to 2,557	2,298	2,303	2,285	2,281
Mortality (%)	3.33	1.67	0.83	1.55	1.00	0.40	0.96	0.19		2.50	1.67	1.67	1.67
Feed:gain (g/g)	1.864	1.855	1.832	0.015	1.00	0.66	0.96	0.14	1.690 to 2.023	1.853	1.852	1.857	1.864
Prechill organ yield													
Kidney (%)													
Overall	2.09	2.09	2.10	0.05	1.00	0.97	0.96	0.90		2.09	1.98	2.04	2.05
Males	1.99	2.13	2.07	0.07	0.91	0.14	0.96	0.38	0.75 to 3.31	2.03	1.97	2.08	2.02
Females	2.18	2.05	2.12	0.07	0.91	0.16	0.96	0.49	0.76 to 3.34	2.14	1.99	2.00	2.07
Liver (%)													
Overall	3.62	3.47	3.51	0.06	0.91	0.078	0.96	0.21		3.52	3.49	3.55	3.56
Males	3.55	3.54	3.44	0.08	1.00	0.93	0.96	0.33	1.92 to 5.13	3.60	3.41	3.56	3.52
Females	3.69	3.40	3.59	0.08	0.33	0.012[10]	0.96	0.38	1.98 to 5.08	3.44	3.56	3.55	3.60
Postchill carcass and parts yields													
Carcass (%)													
Overall	71.0	70.8	71.0	0.3	1.00	0.60	0.96	0.96		70.7	71.1	71.4	71.0
Males	71.4	71.0	71.0	0.5	1.00	0.54	0.96	0.56	61.9 to 80.3	71.3	70.8	71.5	70.9
Females	70.6	70.6	70.9	0.5	1.00	0.91	0.96	0.61	61.6 to 80.3	70.1	71.4	71.2	71.0
Breast (%)													
Overall	26.7	26.8	26.8	0.2	1.00	0.66	0.96	0.80		26.8	26.8	26.3	26.5
Males	26.8	26.7	26.7	0.3	1.00	0.85	0.96	0.92	20.5 to 32.5	26.5	26.9	26.2	26.4
Females	26.7	27.0	26.9	0.3	1.00	0.43	0.96	0.66	20.8 to 32.5	27.1	26.7	26.3	26.5
Thigh (%)													
Overall	15.9	15.8	16.0	0.1	1.00	0.76	0.96	0.43		16.0	15.9	15.9	16.0
Males	15.8	15.7	16.0	0.2	1.00	0.61	0.96	0.51	11.8 to 20.1	15.8	16.1	15.9	16.0
Females	16.0	16.0	16.1	0.2	1.00	0.91	0.96	0.71	11.8 to 20.2	16.2	15.8	15.9	16.1
Leg (%)													
Overall	14.4	14.5	14.3	0.1	0.97	0.24	0.96	0.77		14.2	14.5	14.3	14.2
Males	14.3	14.6	14.3	0.2	0.97	0.21	0.96	0.81	10.7 to 17.8	13.9	14.5	14.4	14.3
Females	14.4	14.5	14.3	0.2	1.00	0.70	0.96	0.88	10.8 to 18.0	14.5	14.6	14.3	14.2
Wing (%)													
Overall	10.5	10.6	10.5	0.1	0.97	0.27	0.96	0.83		10.5	10.7	10.4	10.5
Males	10.5	10.6	10.5	0.1	1.00	0.40	0.96	0.88	8.3 to 12.8	10.5	10.7	10.4	10.5
Females	10.5	10.6	10.5	0.1	1.00	0.47	0.96	0.89	8.5 to 12.6	10.6	10.6	10.4	10.6
Abdominal fat (%)													
Overall	1.47	1.49	1.46	0.03	1.00	0.67	0.96	0.91		1.46	1.44	1.43	1.48

Continued

Table 5 (Continued). Growth performance,[1] prechill organ yields,[2] and postchill carcass and parts yields[3] of broilers fed diets[4] containing meal from nontransgenic near-isogenic control canola or meal from 73496 or 73496(S) canola

					Control vs. 73496		Control vs. 73496(S)			Reference group[6]			
Item	Control	73496	73496(S)	SEM	FDR *P*-value[7]	Raw *P*-value[8]	FDR *P*-value	Raw *P*-value	Tolerance interval[5]	45H72	45H73	46A65	44A89
Males	1.46	1.50	1.47	0.05	1.00	0.61	0.96	0.87	0.39 to 2.51	1.46	1.44	1.45	1.44
Females	1.48	1.48	1.45	0.05	1.00	0.96	0.96	0.76	0.43 to 2.48	1.45	1.44	1.42	1.51

[1]Individual treatment growth performance least squares means represent 12 pens per treatment group with 10 broilers/pen.

[2]Prechill organ yields calculated as percent of live bird weight. Individual treatment least squares means represent 12 pens per treatment group with 8 broilers/pen.

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

[4]Control = canola meal processed from non-genetically modified (GM) canola seed with genetic background comparable with 73496 canola seed; 73496 and 73496(S) = canola meal processed from canola seed containing event DP-Ø73496–4 (glyphosate acetyltransferase, *gat4621* gene); 45H72, 45H73, 46A65, and 44A89 = canola meal processed from commercially available non-GM DuPont Pioneer varieties.

[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 broilers fed 45H72, 45H73, 46A65, and 44A89 reference canola meal diets.

[6]Commercial reference canola meal least squares means included for reference purposes only. The comparisons of interest were 1) control versus 73496 and 2) control versus 73496(S).

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

[8]Nonadjusted *P*-value.

[9]Feed:gain calculated as gram of feed intake per gram of BW gain, and was adjusted for mortality by adding mortality BW at removal to the live weight of broilers remaining in a pen.

[10]Statistically significant difference, nonadjusted *P*-value <0.05.

quality of their diet (International Life Sciences Institute, 2003). No significant differences in BW, weight gain, organ yields, or carcass yields were observed between broiler chickens consuming diets prepared with meal processed from 73496 canola plants (unsprayed or sprayed) or canola meal processed from non-GM near-isogenic canola plants, and all observed values of the control and test canola meal groups fell within the tolerance intervals generated from the commercial reference canola meal groups. These results are consistent with previous animal feeding trials conducted with canola meal produced from other glyphosate-tolerant canola events. Stanford et al. (2002) and Brown et al. (2003) concluded that glyphosate-tolerant canola meal was nutritionally equivalent to its non-GM control when fed to lambs or rainbow trout, respectively. Taylor et al. (2004) observed no differences in growth performance or carcass yields between broilers fed diets containing a glyphosate-tolerant canola meal and those fed non-GM control or commercial canola meals.

The presence of antinutritional factors (Bailey et al., 2000; Farran et al., 2005) may affect liver and kidney yields of broilers and antinutritional factors such as glucosinolates, tannins, and phytic acid are present in canola meal (Bell, 1993). Indeed, the observed increase in liver weights of broilers fed higher concentrations of dietary glucosinolates is well established (Janjecic et al., 2002; Maroufyan and Kermanshahi, 2006; Taraz et al., 2006). Kidney and liver yields in this study were similar to yields observed (1.98 to 2.14%, and 3.40 to 3.63%, respectively) for broilers fed standard corn-soybean meal-based diets at the same facility that were prepared from maize grain and soybean meal, separately or in combination, produced from glyphosate-tolerant plants (McNaughton et al., 2007b, 2008, 2011b). No biologically significant differences in organ yields were observed in this study between broilers fed diets prepared with 73496 canola meals (unsprayed or sprayed) and those fed diets with canola meal from a non-GM control. Similarly, no differences in kidney or liver yields were observed between rats fed meal from a glyphosate-tolerant canola event and those fed meals from a parent line or commercial canola or rapeseed (Nickson and Hammond, 2002).

Statistical analysis of all data in this study resulted in the rejection of the hypotheses of expected growth performance, organ yield, and carcass yield differences between broilers fed non-GM near-isogenic control canola meal and broilers fed canola meal from event 73469 (unsprayed or sprayed). The results from this study demonstrated that canola meal produced from 73496 canola seed with the GAT4621 herbicide tolerance trait was nutritionally equivalent to meal produced from non-GM near-isogenic canola seed. These results are also in agreement with those of other input-trait feeding studies conducted with maize, soybean, and cotton where no biologically significant differences were observed between animals within the targeted animal

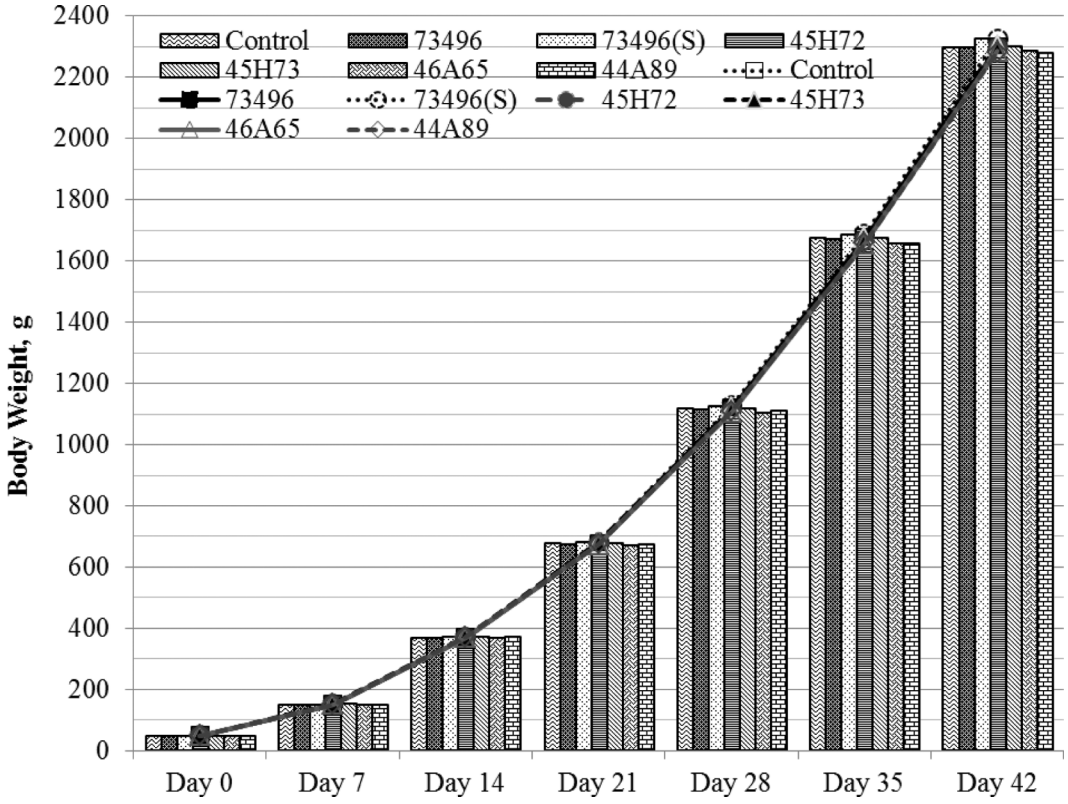

Figure 1. Weekly BW of broilers fed diets containing meal from nontransgenic near-isogenic control canola, 73496 or 73496(S) canola, or commercial varieties (45H72, 45H72, 46A65, and 44A89) from d 0 through 42.

species fed with feedstuffs derived from the GM trait or its isogenic counterpart (Flachowsky et al., 2012).

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