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# Feeding performance in broiler chickens fed diets containing DAS-59122-7 maize grain compared to diets containing non-transgenic maize grain

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**Abstract**

Event DAS-59122-7 is a maize (*Zea mays*) genetically modified to contain *cry*34Ab1 and *cry*35Ab1 genes from *Bacillus thuringiensis* (*Bt*) strain PS149B1 and the *pat* (phosphinothricin acetyltransferase) gene from *Streptomyces viridochromogenes*. *In planta*, co-expression of the Cry34Ab1 and Cry35Ab1 proteins confer resistance to corn rootworms (*Diabrotica virgifera virgifera* LeConte and *Diabrotica barberi* Smith and Lawrence, respectively; CRW), a major pest of maize. Expression of the PAT protein confers tolerance to herbicides containing glufosinate-ammonium. The current study was conducted to evaluate the nutritional value of grain containing event DAS-59122-7 (59122) by comparing the growth performance and carcass yield of broiler chickens fed diets prepared with 59122 maize grain as the sole source of corn with that of broiler chickens fed diets containing near isoline maize grain (control) and three non-transgenic reference maize-grain controls (Pioneer hybrids 33P66, 33J56, and 33R77). Diets produced with 59122 or non-transgenic maize grain were fed to broilers ($n = 120$/group) for a period of 42 days in three phases: Starter, Days 0–21 [530 g maize grain per kg of diet], Grower, Days 22–35 [580 g maize grain per kg of diet] and Finisher, Days 36–42 [700 g maize grain per kg

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of diet] in accordance with standard commercial poultry farming practice. Performance and standard carcass yield data were determined at the end of the feeding trial. Differences between 59122 maize and near isoline control maize-grain means were evaluated with statistical significance at $P<0.05$. Performance and carcass traits from broilers consuming diets produced with 59122 and near isoline were compared to tolerance intervals constructed using data from broiler groups fed diets produced with reference maize grains. No statistically significant differences were observed in mortality, weight gain, feed efficiency (corrected for mortalities), and carcass yields between broilers consuming diets produced with 59122 maize and those consuming diets produced with near isoline control grain. Additionally, all response variables evaluated in both groups fell within the tolerance intervals of the values observed in broilers fed diets produced with the reference maize grains. Based on the results from this study, it was concluded that 59122 maize was nutritionally equivalent to non-transgenic control maize.
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*Keywords:* Broiler; Corn; GM crops

## 1. Introduction

Western corn rootworms (CRW; *Diabrotica virgifera virgifera* LeConte and *Diabrotica barberi* Smith and Lawrence) are responsible for more annual crop damage in the US than any other insect (Metcalf, 1986). Although pesticide treatment and crop rotation strategies have historically been used to control the impact of CRW on crop yield, the potential economic and biological benefits of CRW-resistant maize are substantial (see Oehme and Pickrell, 2003).

DAS-59122-7 is a transgenic maize line that was produced by insertion of the *cry*34*Ab*1 and *cry*35*Ab*1 genes from *Bacillus thuringiensis* (*Bt*) Berliner strain PS149B1 and the phosphinothricin acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes*. Co-expression of the Cry34Ab1 and Cry35Ab1 proteins confer *in planta* resistance to CRW and expression of the PAT protein confers tolerance to herbicides containing glufosinate-ammonium (*e.g*., Liberty™).

The Cry34Ab1 and Cry35Ab1 proteins (14 and 44 kDa, respectively) were isolated from the parasporal inclusion bodies of *Bt* PS149B1 (Moellenbeck et al., 2001; Ellis et al., 2002). The toxicity of these proteins toward CRW appears to be attributable to pathologic changes in the CRW midgut epithelium. The efficacy of maize containing *cry*34*Ab*1 and *cry*35*Ab*1 transgenes *in planta* toward controlling damage caused by CRW has been demonstrated (Moellenbeck et al., 2001; Herman et al., 2002).

Previous studies have evaluated the nutritional value of transgenic maize grains by comparing the growth performance and carcass yields of broiler chickens fed diets containing transgenic maize grain with that of broiler chickens fed diets composed of non-transgenic maize with a comparable genetic background (Brake and Vlachos, 1998; Sidhu et al., 2000; Taylor et al., 2003a,b,c, 2005; Brake et al., 2003, 2005). The objective of the current study was to evaluate the nutritional equivalence of 59122 maize grain and non-transgenic controls in growing broilers.

## 2. Materials and methods

### *2.1. Broilers, housing and observations, gross necropsy, and carcass yield evaluation*

Commercial-type broilers (Ross males × Cobb females cross) were obtained at hatch (termed Trial Day 0) from a commercial Maryland hatchery, and transported to Solution BioSciences Inc. (Farm #1, 3141 White Haven Road, Tyaskin, MD, USA). Bird health and the actual number of birds were documented upon receipt. Broilers were evaluated upon receipt for signs of disease or other complications that could affect the outcome of the study. Following examination, broilers were weighed, identified with a wingband, and randomly allocated into pens, assuring five healthy males and five healthy females were placed into each pen. Broilers were housed in a room containing forced air heaters and individual pen heat lamps with a cross-house ventilation system that simulates commercial poultry farming practices. A continuous 24 h lighting program was followed. Broilers were placed in 0.91 m × 1.22 m floor pens at a density of approximately 0.0929 $m^2$ of floor space per broiler. Pens were separated by a wire partition and did not touch other pens from any side, minimizing the potential for cross-contamination. Birds were observed at least three times daily for overall health, behavior and/or evidence of toxicity. No type of medication was administered during the feeding trial. Mortalities were recorded and complete necropsy exab minations were performed on all broilers found dead or moribund. Birds were not replaced during the trial. Feed and drinking water were provided *ad libitum*.

### *2.2. Experimental design*

Sufficient numbers of broilers were obtained prior to study initiation to insure the availability of 600 healthy chicks (vent sexed and added to each pen at 50% males and 50% females) for the conduct of the study. There were 10 broilers per pen (five males and five females) with 12 pens (replicates) per treatment for a total of 120 broilers per treatment. Broilers were fed their respective dietary treatments from time of hatching (Trial Day 0) to 42 days of age. Body weights and feed weights (including amount of feed added and amount remaining) were determined every 7 days. Body weight gain, feed intake, and mortality-corrected feed efficiency (weight of feed consumed per weight of gain) were calculated for Trial Days 0 through 42. A growth curve was prepared from weekly body weights. All birds were humanely euthanized on Trial Day 42 by cervical dislocation, and each bird received a gross necropsy. Carcass and carcass-parts yield data were collected from 480 broilers (four males and four females per pen per treatment). Carcass-parts yield data included: carcass dry yield (post-chill), thighs, breasts, wings, legs, abdominal fat (including fat around gizzard), kidneys and whole liver. Combined total mass was recorded for all parts (*i.e.* legs, thighs, both sides of the breast). Kidney and liver weights were expressed as g/kg of whole live bird weight. Carcass dry yield was expressed as g/kg of whole live bird weight, and part yields were expressed as g/kg of post-chilled dressed carcass weight.

### 2.3. Corn sources

All maize grains were supplied by Pioneer Hi-Bred International Inc., (Pioneer, Johnston, IA, USA) from the same field production trial in 2004 conducted in Santiago, Chile. The 59122 maize grain was sourced from plants that received two sequential applications of glufosinate-ammonium herbicide (Liberty$^{TM}$; Bayer). The non-transgenic control maize with comparable genetic background (Control) and three non-transgenic commercial maize hybrid reference grains (Pioneer hybrids 33P66, 33J56, and 33R77) were produced 201 m from the 59122 maize production plot to avoid cross pollination. Prior to production of the diets, all maize grains were evaluated using event-specific qualitative polymerase chain reaction (PCR) methods to determine if the transgene insert (*cry*34*Ab*1, *cry*35*Ab*1, and *pat*) was present (DuPont Experimental Station, Wilmington, DE). All grains were also evaluated for expression of the Cry34Ab1, Cry35Ab1, and PAT proteins using specific enzyme-linked immunosorbent assay (ELISA) methods (Pioneer). Proximate (moisture, protein, fat, carbohydrate, fiber, and ash), calcium, phosphorous, amino acid composition (Woodson–Tenent Laboratories Inc., Des Moines, IA, USA), gross energy content (Pioneer), and mycotoxin analyses were conducted on all maize grains prior to diet preparation.

### 2.4. Diet preparation and administration

Commercial-type diets were fed in three phases: Starter (Days 0–21), Grower (Days 22–35), and Finisher (Days 36–42). The feeding intervals were intended to mimic standard commercial feeding regimens. All diets were offered as a mash feed *ad libitum*. Starter, Grower, and Finisher diets were formulated to meet, or exceed, the NRC Nutrient Requirements for Poultry (1994). Approximately 362 kg of each maize grain was individually cleaned, milled to an average particle diameter of 650–750 μm using a Bliss Experimental hammer mill (Bliss Manufacturing, Ponca City, OK, USA), and packaged into 22.7 kg bags. Packaged milled grains were shipped to Solution BioSciences for production of experimental feeds. Maize grains were added to the corresponding experimental diet in equal amounts (530 g/kg in Starter, 580 g/kg in Grower, and 700 g/kg in Finisher) and requirements for protein, lysine, methionine, cystine, threonine, tryptophan, arginine, calcium, and phosphorus were met by adjusting other ingredients commonly used in US commercial poultry rations. Diets were mixed in the following order to minimize the potential for cross-contamination of non-transgenic maize grain with transgenic maize grain: Control, 33P66, 33J56, 33R77, and 59122 maize. Diets were sampled for nutritional proximates, calcium, phosphorus, amino acids (Woodson–Tenent Laboratories), and gross energy content (Pioneer). All diets were also evaluated for Cry34Ab1, Cry35Ab1, and PAT protein concentrations using antibody specific ELISA methods (Pioneer). Subsamples of the 59122 diet were collected at the beginning, middle, and end of production and evaluated for the concentrations of the transgenic proteins to determine if the diets were blended homogeneously. Concentrations of the transgenic proteins were also evaluated in samples of the 59,122 diets collected over the course of the feeding trial to determine stability during the study.

### 2.5. Statistical analysis

Data generated during this trial were analyzed using a linear mixed model to obtain the estimates of the variance components (PROC MIXED, SAS® version 8.2 software, SAS Institute Inc., Cary NC, USA) that were used to provide estimates of the treatment means. 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: $Y_{ij} = U + T_i + B_j + T_iB_j + e_{ij}$ where $Y_{ij}$ = observed bird response, $U$ = overall mean, $T_i$ = treatment effect, $B_j$ = random block effect, $T_iB_j$ = random treatment*block effect (referred to as pen) and $e_{ij}$ = residual error. $T_iB_j$ was used as the error term for the fixed effect of treatment ($T_i$), which allowed within-pen variability to become the residual error. The observed P value determined whether the 59122 mean was significantly different from the Control mean for live performance and carcass data analyses; differences between means were considered significant at $P \leq 0.05$. False discovery rate (FDR; Westfall et al., 1999) method was used to account for the numerous comparisons and minimize the number of differences being declared to be significant due to chance (PROC MULTTEST, SAS® version 8.2 software, SAS Institute Inc., Cary NC, USA). Data from commercial reference maize grain groups (33P66, 33J56, and 33R77) were not included in the statistical analysis; instead, these data were used to construct a 95% tolerance interval on 99% of the population for each trait as described by (Graybill, 1976). Data from the 59122 and Control treatment groups were then graphically evaluated to determine whether or not the observed values were contained within this interval. If all observations for a treatment were contained within the interval, the treatment was considered to be consistent with how a broiler would perform when fed a diet containing similar levels of commercial reference maize with respect to the desired response variable. Tolerance intervals for organ and carcass response variables were created by gender due to the expected yield differences between male and female broilers.

## 3. Results

### 3.1. 59122, control, and commercial reference maize grain characterization

The presence of the CRY34Ab1 ($31.3 \pm 1.8$ ng/mg of grain), CRY35Ab1 ($1.50 \pm 0.11$ ng/mg of grain), and PAT proteins ($0.033 \pm 0.018$ ng/mg of grain)[1] were confirmed in the 59122 maize grain with quantitative ELISA but were not detected in control or reference maize grains (data not shown). Fumonisin B1 (FB1) was detected in all maize grain samples, except commercial reference grain 33P66, at concentrations ranging from 0.1 to 0.5 mg/kg. All concentrations were below the US FDA guideline values for incorporation into broiler feed (U.S. FDA, 2000, 2001). The proximate, essential

[1] Two out of 10 values in the PAT assay were below the lower limit of quantification (LLOQ = 0.034 ng/mg grain) and were given a value of zero for calculation purposes. The mean value for PAT with those two points excluded was 0.044 ng/mg of grain.

Table 1
Compositional analysis[a] of Control, 59122, and commercial maize grains[b]

| Component | Control | 59122 | 33P66 | 33J56 | 33R77 |
|---|---|---|---|---|---|
| Dry matter (g/kg) | 874 | 876 | 876 | 871 | 875 |
| Protein (g/kg) | 73.0 | 73.2 | 74.9 | 77.0 | 73.4 |
| Fat (g/kg) | 36.3 | 36.6 | 36.1 | 30.8 | 32.4 |
| Carbohydrates (g/kg) | 734 | 737 | 733 | 735 | 737 |
| Fiber (g/kg) | 18.0 | 18.0 | 19.0 | 16.0 | 19.0 |
| Ash (g/kg) | 12.7 | 11.4 | 12.9 | 12.2 | 13.5 |
| Calcium (g/kg) | 0.04 | 0.03 | 0.02 | 0.02 | 0.02 |
| Phosphorus (g/kg) | 2.2 | 2.3 | 2.6 | 2.3 | 2.6 |
| Gross energy (MJ/kg) | 16.5 | 16.5 | 16.4 | 16.2 | 16.3 |
| Alanine (g/kg) | 6.3 | 6.1 | 6.4 | 6.5 | 6.1 |
| Arginine (g/kg) | 3.4 | 3.5 | 3.4 | 3.5 | 3.4 |
| Aspartic acid (g/kg) | 9.2 | 6.2 | 9.3 | 9.6 | 9.1 |
| Cystine (g/kg) | 1.6 | 1.7 | 1.7 | 1.7 | 1.7 |
| Glutamic acid (g/kg) | 16.6 | 14.7 | 16.1 | 16.4 | 15.6 |
| Glycine (g/kg) | 3.3 | 3.2 | 3.3 | 3.3 | 3.3 |
| Histidine (g/kg) | 2.2 | 2.3 | 2.3 | 2.4 | 2.3 |
| Isoleucine (g/kg) | 2.7 | 2.6 | 2.7 | 2.9 | 2.7 |
| Leucine (g/kg) | 9.5 | 9.3 | 9.8 | 10.2 | 9.4 |
| Lysine (g/kg) | 2.4 | 2.5 | 2.3 | 2.4 | 2.3 |
| Methionine (g/kg) | 1.4 | 1.6 | 1.6 | 1.6 | 1.5 |
| Phenylalanine (g/kg) | 3.9 | 3.8 | 3.8 | 3.9 | 3.6 |
| Proline (g/kg) | 7.9 | 8.0 | 7.2 | 7.2 | 7.3 |
| Serine (g/kg) | 4.4 | 4.0 | 4.2 | 4.3 | 4.2 |
| Threonine (g/kg) | 3.5 | 3.3 | 3.6 | 3.6 | 3.5 |
| Tryptophan (g/kg) | 0.70 | 0.60 | 0.60 | 0.60 | 0.60 |
| Tyrosine (g/kg) | 2.0 | 2.0 | 2.0 | 2.1 | 1.8 |
| Valine (g/kg) | 3.6 | 3.7 | 3.8 | 4.0 | 3.8 |

[a] All analyses, with the exception of gross energy, conducted by Woodson–Tenent Laboratories Inc. Gross energy analysis was conducted by Pioneer Hi-Bred International Inc.
[b] Means reported on an "as-is" basis.

amino acid (lysine, methionine, cystine, threonine, tryptophan, and arginine), calcium, phosphorous, and gross energy content of all maize grains were similar (Table 1).

### 3.2. Diet composition

Nutrient requirements by feeding phase (NRC, 1994) were satisfied by production of diets with maize grains and other nutritional ingredients (Table 2). Compositional analysis of the nutrients in all diets demonstrated that there were no material differences between diets produced with 59122 maize grain compared to diets produced with Control, 33P66, 33J56, or 33R77 maize grains (Table 3).

### 3.3. Growth performance

No statistically significant differences were observed in growth performance metrics between groups of broilers consuming 59122 or Control maize diets (Table 4) and all

Table 2
Ingredient composition of Control, 59122, and commercial maize diets

| Ingredient (g/kg) | Starter phase | | | | | Grower phase | | | | | Finisher phase | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | 59122 | 33P66 | 33J56 | 33R77 | Control | 59122 | 33P66 | 33J56 | 33R77 | Control | 59122 | 33P66 | 33J56 | 33R77 |
| Control | 530 | – | – | – | – | 580 | – | – | – | – | 700 | – | – | – | – |
| 59122 | – | 530 | – | – | – | – | 580 | – | – | – | – | 700 | – | – | – |
| 33P66 | – | – | 530 | – | – | – | – | 580 | – | – | – | – | 700 | – | – |
| 33J56 | – | – | – | 530 | – | – | – | – | 580 | – | – | – | – | 700 | – |
| 33R77 | – | – | – | – | 530 | – | – | – | – | 580 | – | – | – | – | 700 |
| Soybean meal, 48%[a] | 307 | 307 | 305 | 303 | 308 | 256 | 256 | 254 | 252 | 258 | 182 | 182 | 179 | 177 | 184 |
| Soy hulls[b] | 43.5 | 45.2 | 44.9 | 43.1 | 40.2 | 50.8 | 52.6 | 52.3 | 50.4 | 47.2 | 26.6 | 28.9 | 28.5 | 26.1 | 22.3 |
| Protein blend S.E.[c] | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 |
| Animal–vegetable fat | 47.8 | 46.8 | 49.0 | 52.3 | 50.5 | 43.9 | 42.7 | 45.2 | 48.8 | 46.8 | 21.7 | 20.3 | 23.3 | 27.6 | 25.2 |
| D,L-Methionine | 4.7 | 3.9 | 4.0 | 4.0 | 4.0 | 4.2 | 3.4 | 3.4 | 3.4 | 3.5 | 4.3 | 3.4 | 3.4 | 3.4 | 3.4 |
| L-Lysine–HCl | 0.10 | – | 0.23 | 0.25 | 0.16 | 0.80 | 0.69 | 0.93 | 0.96 | 0.86 | 3.13 | 3.00 | 3.29 | 3.32 | 3.21 |
| Limestone | 7.9 | 7.9 | 8.1 | 8.0 | 8.2 | 7.3 | 7.3 | 7.5 | 7.4 | 7.6 | 7.3 | 7.3 | 7.6 | 7.4 | 7.7 |
| Di-Cal 21 | 17.7 | 17.7 | 17.4 | 17.7 | 17.3 | 16.5 | 16.4 | 16.1 | 16.4 | 16.1 | 15.4 | 15.3 | 15.0 | 15.3 | 14.9 |
| Choline chloride, 60% | 0.68 | 0.68 | 0.69 | 0.69 | 0.68 | 0.28 | 0.28 | 0.29 | 0.29 | 0.28 | – | – | – | – | – |
| Salt | 4.6 | 4.6 | 4.6 | 4.6 | 4.6 | 4.1 | 4.1 | 4.1 | 4.1 | 4.1 | 3.7 | 3.7 | 3.7 | 3.7 | 3.7 |
| Trace mineral premix[d] | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Vitamin premix[d] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |

[a] Non-GMO soybean meal (Cargill Inc., Bloomington, IL, USA 61701-4785.

[b] Non-GMO soy hulls, fiber source (Creston Bean Processing, Creston, IA 50801 – 1804, USA.

[c] Protein blend SE is a proprietary blend of blood meal, feather meal, amino acids, and beef protein offal meal.

[d] Vitamin and mineral premix (28–1500; North American Nutrition Companies Inc., Lewisburg, OH 45338, USA).

Table 3
Analyzed nutrient compositions[a] of Control, 59122, and commercial diets ("As-Fed" basis)

| Component | Starter phase | | | | | Grower phase | | | | | Finisher phase | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | 59122 | 33P66 | 33J56 | 33R77 | Control | 59122 | 33P66 | 33J56 | 33R77 | Control | 59122 | 33P66 | 33J56 | 33R77 |
| Dry matter (g/kg) | 883 | 880 | 882 | 879 | 880 | 875 | 881 | 879 | 882 | 882 | 879 | 876 | 881 | 878 | 878 |
| Protein (g/kg) | 230 | 228 | 228 | 225 | 228 | 205 | 210 | 211 | 207 | 206 | 173 | 178 | 170 | 171 | 176 |
| Fat (g/kg) | 70.1 | 69.4 | 69.3 | 72.6 | 68.7 | 48.5 | 59.6 | 57.3 | 59.7 | 58.8 | 52.6 | 49.5 | 52.5 | 49.3 | 51.4 |
| Fiber (g/kg) | 29.0 | 27.0 | 25.0 | 27.0 | 26.0 | 29.0 | 31.0 | 32.0 | 31.0 | 29.0 | 24.0 | 24.0 | 21.0 | 24.0 | 19.0 |
| Ash (g/kg) | 51.4 | 52.0 | 51.3 | 51.9 | 52.0 | 46.7 | 50.1 | 47.4 | 49.8 | 49.7 | 44.2 | 44.5 | 43.0 | 41.7 | 44.9 |
| Calcium (g/kg) | 7.9 | 8.3 | 7.8 | 8.2 | 8.0 | 7.2 | 7.8 | 7.1 | 7.8 | 7.5 | 7.3 | 7.6 | 7.0 | 6.8 | 7.5 |
| Phosphorus (g/kg) | 6.8 | 6.7 | 6.7 | 6.7 | 6.8 | 6.3 | 6.5 | 6.3 | 6.5 | 6.5 | 5.9 | 5.8 | 5.9 | 5.7 | 6.2 |
| Gross energy (MJ/kg) | 17.5 | 17.5 | 17.5 | 17.5 | 17.5 | 16.8 | 17.1 | 17.1 | 17.1 | 17.2 | 16.8 | 16.7 | 16.9 | 16.7 | 16.8 |
| Alanine (g/kg) | 12.1 | 12.1 | 12.6 | 12.2 | 12.2 | 11.6 | 11.8 | 11.9 | 11.6 | 11.5 | 10.0 | 10.0 | 10.1 | 10.2 | 10.1 |
| Arginine (g/kg) | 14.5 | 14.2 | 15.3 | 14.6 | 14.1 | 13.1 | 14.1 | 13.3 | 13.0 | 13.0 | 10.4 | 9.6 | 10.0 | 10.1 | 10.4 |
| Aspartic acid (g/kg) | 27.6 | 27.5 | 27.4 | 28.6 | 28.3 | 25.3 | 23.5 | 26.5 | 25.9 | 25.7 | 18.8 | 16.2 | 19.4 | 17.5 | 20.2 |
| Cystine (g/kg) | 3.8 | 3.9 | 3.9 | 3.9 | 4.0 | 3.6 | 3.8 | 3.7 | 3.6 | 3.8 | 3.1 | 3.3 | 3.2 | 3.3 | 3.3 |
| Glutamic acid (g/kg) | 42.2 | 40.3 | 42.8 | 41.6 | 41.8 | 37.6 | 37.9 | 39.5 | 38.5 | 38.3 | 31.2 | 29.8 | 30.3 | 29.7 | 31.2 |
| Glycine (g/kg) | 10.2 | 10.2 | 10.3 | 10.2 | 10.2 | 9.5 | 9.8 | 9.7 | 9.5 | 9.5 | 7.7 | 7.7 | 7.5 | 7.5 | 7.8 |
| Histidine (g/kg) | 6.7 | 6.6 | 6.8 | 6.7 | 6.8 | 6.2 | 6.4 | 6.3 | 6.3 | 6.4 | 5.0 | 5.1 | 5.1 | 5.3 | 5.3 |
| Isoleucine (g/kg) | 9.5 | 9.3 | 9.6 | 9.7 | 9.4 | 8.6 | 8.8 | 8.8 | 8.7 | 8.7 | 6.8 | 6.8 | 6.6 | 6.7 | 6.8 |
| Leucine (g/kg) | 20.5 | 20.3 | 20.9 | 20.7 | 20.4 | 19.5 | 19.6 | 19.8 | 19.6 | 19.4 | 16.5 | 16.4 | 16.8 | 17.2 | 16.8 |
| Lysine (g/kg) | 13.1 | 13.0 | 13.3 | 13.2 | 12.8 | 12.4 | 12.4 | 12.6 | 12.4 | 12.4 | 11.2 | 11.8 | 11.0 | 11.6 | 11.6 |
| Methionine (g/kg) | 7.1 | 6.7 | 6.5 | 6.8 | 6.5 | 6.0 | 5.9 | 5.8 | 5.9 | 5.8 | 6.4 | 5.3 | 5.3 | 5.3 | 5.7 |
| Phenylalanine (g/kg) | 11.8 | 11.6 | 12.1 | 11.8 | 11.7 | 10.9 | 11.0 | 11.1 | 10.9 | 10.9 | 9.0 | 9.1 | 8.7 | 8.9 | 9.1 |
| Proline (g/kg) | 14.3 | 14.3 | 14.3 | 14.6 | 14.8 | 13.6 | 13.4 | 13.9 | 13.5 | 13.8 | 12.1 | 11.8 | 11.7 | 11.1 | 12.2 |
| Serine (g/kg) | 12.2 | 12.2 | 12.3 | 12.2 | 12.1 | 11.4 | 11.4 | 11.7 | 11.3 | 11.3 | 9.1 | 8.3 | 8.8 | 8.8 | 9.4 |
| Threonine (g/kg) | 9.8 | 9.8 | 10.1 | 9.9 | 10.0 | 9.1 | 9.0 | 9.4 | 9.2 | 9.3 | 7.4 | 6.9 | 7.2 | 6.9 | 7.6 |
| Tryptophan (g/kg) | 2.5 | 2.6 | 2.5 | 2.5 | 2.5 | 2.3 | 2.4 | 2.3 | 2.3 | 2.4 | 1.8 | 1.8 | 1.7 | 1.7 | 1.8 |
| Tyrosine (g/kg) | 7.0 | 6.8 | 7.1 | 6.8 | 6.8 | 6.3 | 6.4 | 6.6 | 6.0 | 6.5 | 5.0 | 5.2 | 4.9 | 4.9 | 5.1 |
| Valine (g/kg) | 11.4 | 11.2 | 11.5 | 11.8 | 11.6 | 10.6 | 11.1 | 10.8 | 10.8 | 10.8 | 8.7 | 8.7 | 8.7 | 8.7 | 8.9 |

[a] All analyses, with the exception of Gross energy, conducted by Woodson-Tenent Laboratories Inc. Gross energy analysis performed by Pioneer Hi-Bred International Inc.

Table 4
Growth performance[a] of broilers fed Control and 59122 diets from Day 0 to Day 42

| Criteria | Control | 59122 | S.E.M. | P value | Adjusted P value[b] | Tolerance interval[c] |
|---|---|---|---|---|---|---|
| Initial weight (g) | 51 | 51 | 0.2 | 0.209 | 0.903 | 48.8–53.1 |
| Final weight (g) | 1918 | 1916 | 21.2 | 0.944 | 1.00 | 1674.7–2144.4 |
| Mortality (%) | 0.83 | 0.83 | 0.83 | 1.000 | 1.00 | −10.65–13.99 |
| Total gain (g) | 1868 | 1866 | 21.0 | 0.953 | 1.00 | 1625.4–2091.7 |
| Feed:gain[d] (g/g) | 1.877 | 1.865 | 0.015 | 0.572 | 0.911 | 1.702–2.027 |

[a] Control and 59122 treatment means not different (P>0.05) for individual performance traits.
[b] P value adjusted using False Discovery Rate.
[c] Lower and upper limits of a 95% tolerance interval on 99% of the population calculated using data from 33P66, 33J56, and 33R77 reference maize treatment groups. All Control and 59122 treatment observations were within tolerance interval for performance traits evaluated.
[d] Feed:gain calculated as gram of feed intake per gram of body weight gain.

treatments groups followed a similar growth pattern (Fig. 1). Additionally, all growth performance values for broilers fed the 59122 maize diet and values for broilers fed the Control maize diet fell within the tolerance range calculated for this study using data obtained from broilers consuming diets produced with 33P66, 33J56, and 33R77 commercial maize grains.

### 3.4. Carcass yields

No significant differences were observed for carcass, individual part yields (post-chill), or kidney weights between the 59122 maize diet group and the Control maize diet group (Table 5). Overall and male liver yields were not significantly different between the 59122

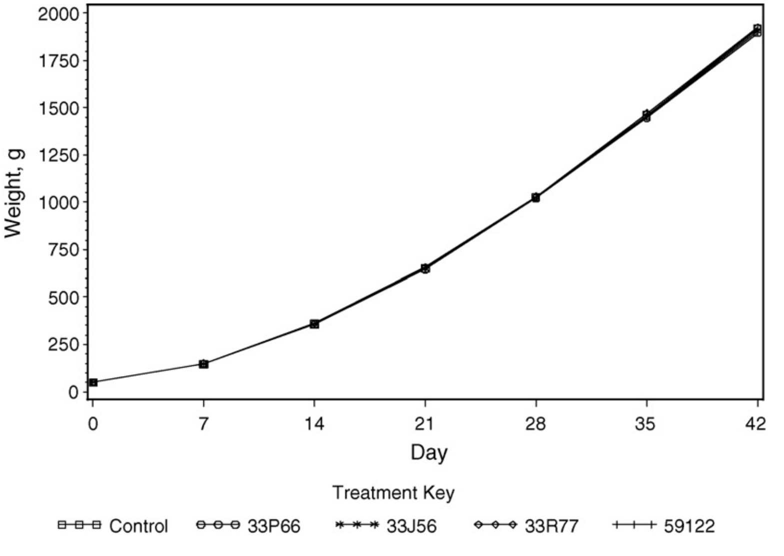


Fig. 1. Weekly body weight (g) of broilers over the 42-day feeding period.

Table 5
Pre-chill organ, carcass, and post-chill parts yields[a,b] of broilers fed Control and 59122 diets

| Criteria | Control | 59122 | S.E.M. | P value | Adjusted P value[c] | Tolerance interval[d] |
|---|---|---|---|---|---|---|
| Pre-chill organ yield kidney (g/kg BW) | | | | | | |
| Overall | 20 | 20 | 0.5 | 0.967 | 1.00 | |
| Males | 20 | 20 | 0.6 | 0.402 | 0.903 | 8.5–33.2 |
| Females | 20 | 21 | 0.6 | 0.436 | 0.903 | 8.2–33.2 |
| Liver (g/kg CCW) | | | | | | |
| Overall | 35 | 36 | 0.6 | 0.154 | 0.903 | |
| Males | 35 | 36 | 0.8 | 0.881 | 1.00 | 20.5–50.6 |
| Females[e] | 34[b] | 37[a] | 0.8 | 0.05 | 0.903 | 19.5–51.0 |
| Post-chill carcass and parts yields carcass (g/kg BW) | | | | | | |
| Overall | 706 | 710 | 3.2 | 0.428 | 0.903 | |
| Males | 708 | 713 | 4.5 | 0.399 | 0.903 | 626–792 |
| Females | 705 | 707 | 4.5 | 0.750 | 1.00 | 622–791 |
| Breast (g/kg CCW) | | | | | | |
| Overall | 269 | 265 | 2.2 | 0.231 | 0.903 | |
| Males | 270 | 265 | 3.0 | 0.206 | 0.903 | 207–324 |
| Females | 267 | 265 | 3.0 | 0.603 | 0.911 | 206–331 |
| Thigh (g/kg CCW) | | | | | | |
| Overall | 159 | 159 | 1.5 | 0.982 | 1.00 | |
| Males | 158 | 156 | 2.0 | 0.629 | 0.911 | 119–202 |
| Females | 160 | 162 | 2.0 | 0.606 | 0.911 | 120–199 |
| Wing (g/kg CCW) | | | | | | |
| Overall | 105 | 105 | 0.8 | 0.935 | 1.00 | |
| Males | 104 | 105 | 1.1 | 0.384 | 0.903 | 85.1–125 |
| Females | 107 | 105 | 1.1 | 0.323 | 0.903 | 86.1–125 |
| Leg (g/kg CCW) | | | | | | |
| Overall | 143 | 143 | 1.2 | 0.984 | 1.00 | |
| Males | 141 | 143 | 1.7 | 0.416 | 0.903 | 113–176 |
| Females | 144 | 142 | 1.7 | 0.432 | 0.903 | 108–181 |
| Abdominal fat (g/kg CCW) | | | | | | |
| Overall | 15 | 15 | 0.3 | 0.528 | 0.911 | |
| Males | 15 | 14 | 0.5 | 0.106 | 0.903 | 5.0–24.1 |
| Females | 15 | 15 | 0.5 | 0.482 | 0.911 | 5.3–24.4 |

[a] Pre-chill organ and carcass yield calculated as g per kg live bird weight (BW). Post-chill parts calculated as g per kg of post-chill carcass weight (CCW).

[b] Control and 59122 overall treatment means not different ($P>0.05$) for individual traits.

[c] Adjusted P value calculated from False Discovery Rate.

[d] Lower and upper limits of a 95% tolerance interval on 99% of the population calculated using data from 33P66, 33J56, and 33R77 reference maize treatment groups. All Control and 59122 treatment observations were within tolerance intervals for organ yield traits evaluated.

[e] Control and 59122 treatment means within a gender lacking a common superscript letter differ ($P\leq0.05$).

maize diet group and the Control maize diet group. Within females, mean liver weight was higher ($P<0.05$) for the 59122 maize diet group compared to the Control maize diet group, however, when the P value was adjusted using False Discovery Rate, the higher mean liver weight was deemed not significant ($P=0.90$).

## 4. Discussion

Differences in study design have been reported in broiler feeding studies conducted to evaluate the nutritional performance of transgenic maize grains (*e.g.*, number of feeding periods, concentration of maize grain within defined periods). However, the general design of these studies was a comparison of predetermined nutritional performance response variables in animals consuming diets prepared with transgenic maize grains to the values observed in broilers consuming near isogenic and reference non-transgenic maize grains. Transgenic maize grains with numerous traits including resistance to European corn borer parasites, herbicide tolerance, and stacked trait products in broiler chickens have been reported (Brake and Vlachos, 1998; Sidhu et al., 2000; Taylor et al., 2003a,b,c, 2005; Brake et al., 2003, 2005). While statistically significant differences in certain response variables of broilers consuming diets prepared with transgenic maize grain were observed in some studies (see Brake and Vlachos, 1998 and Taylor et al., 2003a, 2005), overall these studies demonstrated that the nutritional performance of transgenic maize grains was equivalent to that of non-transgenic maize grains.

The current study was conducted to assess the nutritional performance of a novel transgenic maize grain that expresses CRW selective proteins (CRY34Ab1 and CRY35Ab1 from *Bt* Berliner strain PS149B1) and a protein conferring tolerance to herbicides containing glufosinate ammonium (PAT from *S. viridochromogenes*). The transgenic maize grain in this study is novel in that the target species is a pervasive crop parasite that is not sensitive to other *Bt* toxins such as Cry1Ab.

Analyses conducted prior to initiating the feeding study demonstrated that the transgenic maize grain (59122) and diets prepared from this maize grains was appropriately segregated using qualitative PCR analysis with DAS-59122-7 specific primer sets and quantitative ELISA techniques (data not shown). Nutrient composition analysis did not identify any significant differences among the maize grains or the diets prepared from the maize grains.

No significant differences between broiler chickens consuming diets prepared with 59122 maize grain and the near isogenic or reference maize grains were observed in growth performance or carcass and organ yields. Initial statistical analysis of the liver yield data indicated a significant increase ($P<0.05$) in liver yield of females, but not in the overall or male yields, from broilers consuming diets produced with the 59122 maize grain. Most published studies have not reported gender effects, although in some cases they have been observed such as with increased thigh weight in female broilers consuming Roundup Ready corn (NK603; Taylor et al., 2003a). In that case, it was determined that even though the thigh weight was significantly different from the control group within the study, that it was not a biologically significant increase because the mean value was similar to that reported in historical control female broilers. Previously published nutritional equivalency studies have not reported liver yield data, therefore whether the liver yield in the female broilers consuming the 59122 maize grain the current study was similar to the yields in prior studies can not be determined. Regardless, the increase was not considered significant when adjusting for False Discovery Rate, and it is unlikely to be biologically significant because all of the individual liver weights in the female broilers consuming the 59122 maize grain diets fell within the tolerance interval calculated from the liver weights of female broilers consuming the reference maize grain that were determined to be significant *a priori*.

## 5. Conclusion

Based on the performance of broiler chickens, maize grain derived from transgenic maize line 59122 that expresses the Cry34Ab1, Cry35Ab1, and PAT proteins is nutritionally equivalent to maize grain derived from non-transgenic control maize.

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